



## **Evaluation of the environmental quality monitoring protocol for Amazonian streams: a systematic review**

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### **ABSTRACT**

The Amazon Rainforest is renowned for its extraordinary biodiversity and the ravages of pervasive deforestation. The removal of natural vegetation significantly affects Amazonian streams, leading to alterations in their environmental conditions. In the United States, the Environmental Protection Agency has devised a comprehensive protocol to monitor changes in the environmental quality of streams. Since 2011, the Sustainable Amazon Network has implemented this protocol in Amazonian streams. Our systematic review aimed to address the following questions: i) How widely has the protocol been employed in Amazonian streams?; ii) Is the utilization of this protocol well-distributed across the Amazon Basin?; iii) Which types of land use have been assessed using this protocol?; iv) Which components of the aquatic biota have been studied in conjunction with the protocol?; and, v) Which metrics of the protocol are most crucial for elucidating aquatic biota distribution? We conducted searches using the Web of Science and Google Scholar databases, identifying 34 studies that directly aligned with our objectives. It was observed that the Eastern Amazon had the highest number of streams evaluated. Additionally, aquatic insects emerged as an effective tool when utilized alongside the protocol for evaluating the impacts of changes in land use. Ultimately, the most important metrics for assessing impacts on aquatic biota were shelter availability in the channels, the preservation status of riparian forests, and water quality. We recommend developing a simplified version of this protocol to facilitate its application for research teams with limited personnel and financial resources available for fieldwork.

**Keywords:** aquatic conservation, biomonitoring, land use change, sampling efficiency, stream ecology.



## Avaliação da eficácia do protocolo de monitoramento da qualidade ambiental para riachos amazônicos: uma revisão sistemática

### RESUMO

A Floresta Amazônica é renomada por sua extraordinária biodiversidade e pelo difundido desmatamento. A remoção da vegetação natural impacta significativamente os riachos amazônicos, levando a alterações em suas condições ambientais. Nos Estados Unidos, a Agência de Proteção Ambiental desenvolveu um protocolo abrangente para monitorar mudanças na qualidade ambiental dos rios. Desde 2011, a Rede Amazônica Sustentável implementou esse protocolo em riachos amazônicos. Nossa revisão sistemática teve como objetivo abordar as seguintes questões: i) Quão amplamente o protocolo foi utilizado em riachos amazônicos? ii) A utilização desse protocolo está bem distribuída na bacia amazônica? iii) Quais tipos de uso da terra foram avaliados com esse protocolo? iv) Quais componentes da biota aquática foram estudados em conjunto com o protocolo? v) Quais métricas do protocolo são mais cruciais para elucidar a distribuição da biota aquática? Conduzimos buscas nas bases de dados Web of Science e Google Scholar, identificando 34 estudos que se alinham diretamente com nossos objetivos. Observou-se que a Amazônia Oriental teve o maior número de rios avaliados. Além disso, insetos aquáticos surgiram como uma ferramenta eficaz quando utilizados junto ao protocolo para avaliar os impactos das mudanças no uso da terra. Por fim, as métricas mais importantes para avaliar os impactos na biota aquática foram a disponibilidade de abrigo nos canais, o estado de preservação das florestas ripárias e a qualidade da água. Recomendamos o desenvolvimento de uma versão simplificada desse protocolo para facilitar sua aplicação em equipes de pesquisa com recursos financeiros limitados e um número restrito de pessoal disponível para trabalho de campo.

**Palavras-chave:** biomonitoramento, conservação aquática, ecologia de riachos, eficiência de amostragem, mudança de uso da terra.

### 1. INTRODUCTION

Amazon has the largest hydrographic basin in the world. Its water network is mainly composed of numerous first- and third-order streams, which harbor a rich aquatic biodiversity (Junk *et al.*, 2007) that depends on the riparian vegetation and allochthonous material (Vannote *et al.*, 1980). However, the expansion of different land use and land covers over Amazonia represents a constant threat to the maintenance of its environmental conditions and biodiversity (Gardner *et al.*, 2013). Some of the main land uses in Amazon are livestock (Barona *et al.*, 2010), monocultures (Juen *et al.*, 2016), selective and non-selective logging (Calvão *et al.*, 2016) and mining (Sonter *et al.*, 2017; Paiva *et al.* 2021). These activities cause the total or partial removal of the riparian vegetation (Teresa and Casatti, 2017), and the transformation of natural forests into agriculture (Leal *et al.*, 2017), pasture (Cederberg *et al.*, 2011), or urban areas (Faria *et al.*, 2017; Brito *et al.*, 2021) potentially impacting biodiversity.

Environmental agencies are aware of these and other potential anthropic impacts. For this reason, they seek to standardize consistent methodologies for laboratory and field activities, to better monitor streams' environmental quality (Buss *et al.*, 2014). In the United States, the Environmental Protection Agency (US-EPA) developed a protocol for field operations, which evaluates the conservation status of streams in forest regions (Peck *et al.*, 2006). This extensive protocol includes around 240 environmental variables that describe characteristics of the physical habitat like: i) stream morphology; ii) substrate types; iii) hydrodynamics; iv) riparian vegetation; v) wood debris; vi) refuge to aquatic biota; vii) anthropogenic disturbance; and viii) water variables (physical-chemical) (Peck *et al.*, 2006). This protocol is relatively cheap to

apply, requiring only a team of approximately four members, a millimeter pipe, a compass and some visual estimates to obtain the vast majority of variables (except for the water variables that are measured with expensive multiparameter probes) (Kaufmann *et al.*, 1999; Peck *et al.*, 2006). This makes the set of variables provided by the US-EPA protocol, associated with different biological groups, a useful tool for ecological and biomonitoring studies.

Several studies have used diversity metrics of biological communities associated with environmental variables from the protocol to produce a complete diagnosis of the environmental and ecological quality of streams (Karr, 1991; Allan, 2004; Ochocka and Pasztaleniec, 2016). In these studies, fish and aquatic insects are among the most relevant components of the aquatic biota in environmental monitoring programs (Plafkin, 1989; Hughes *et al.*, 1998; Buss *et al.*, 2014). Fish live exclusively in aquatic environments and some species are sensitive to changes in the habitat. Structural alterations, for example, may lead to changes in biological communities because fish use stream channels to feed and shelter (Montag *et al.*, 2019). Aquatic insects are also sensitive to changes in the characteristics of streams like riparian vegetation, physical integrity and substrate types, both in their adult (and in some cases terrestrial) (e.g., Oliveira-Junior *et al.*, 2019a; Bastos *et al.*, 2021) or immature phase (generally strictly aquatic) (e.g., Mendes *et al.*, 2020; Cruz *et al.*, 2022). But these groups can also respond to changes including pesticide contamination (Mendes *et al.*, 2020; Sumudumali and Jayawardana, 2021), temperature increase and oxygen depletion (Martins *et al.*, 2017).

The US-EPA protocol has been used in countries of all continents, except Antarctica, (Borja *et al.*, 2008; Karr and Chu, 2000; Xu *et al.*, 2013), and in Brazil it has been used in Cerrado (e.g., Silva *et al.*, 2018; Alvarenga *et al.*, 2021; Silva *et al.*, 2022), and Amazon biomes (e.g., Juen *et al.*, 2016; Faria *et al.*, 2017; Leal *et al.*, 2017). In the Amazon Rainforest, though, until the year 2000 there were no relevant programs for monitoring streams. This contrasts with the importance of this biome, which is the largest tropical forest with the highest biodiversity of the world. Only in 2001, a project named Igarapés was created in Brazil with research teams that started researching small lotic environments. Later, in 2008, a group of scientists from different institutions and nationalities created the Sustainable Amazon Network (RAS - Rede Amazônia Sustentável, in Portuguese). These researchers aimed to assess the sustainability of land-use systems in two regions of the Brazilian Amazon, and for this, they applied the US-EPA protocol to 100 streams in these areas (Gardner *et al.*, 2013). Since then, this protocol has been widely used in scientific research to evaluate the ecological conditions of streams that have been altered by different types of land use in the Amazon (Leitão *et al.*, 2017; Chen *et al.*, 2017; Pereira *et al.*, 2019).

The US-EPA protocol has been used as a predictor of changes in taxonomic (Oliveira-Junior *et al.*, 2015), functional (Pereira *et al.*, 2019; Luiza-Andrade *et al.*, 2017), phylogenetic (Bastos *et al.*, 2021), morphological (Mendes *et al.*, 2019a) and behavioral (Resende *et al.*, 2021) diversity of several aquatic organisms. For this reason, this protocol has been useful to quantify natural variation in Amazonian streams (Benone *et al.*, 2017; Shimano *et al.*, 2021), as well as for monitoring environmental quality of streams in altered regions. The already mentioned land-use and land-cover changes are some of the multiple stressors that impact Amazonian streams (Albert *et al.*, 2021; He *et al.*, 2017) and cause changes in habitat structure and loss of biodiversity (Juen *et al.*, 2016; Leal *et al.*, 2016). As such, evaluating whether and how environmental changes impact Amazonian streams is crucial for ecosystem conservation (Leal *et al.*, 2016; Martins *et al.*, 2018). Tropical countries still need great improvements in freshwater systems' research (Brasil *et al.*, 2020), so it is important to identify the main knowledge gaps and indicate directions for future studies to be efficient (Diniz-Filho *et al.*, 2010).

Considering this scenario, we aimed to answer several questions related to the use of the US-EPA protocol in Amazonian streams: 1) In how many Amazonian streams has the US-EPA

protocol been used?; 2) Is the use of this protocol well distributed across the Amazonian Basin?; 3) Which types of land use have been evaluated using the protocol?; 4) Which components of the aquatic biota have been used together with the protocol to assess environmental quality of Amazonian streams?; and, 5) Which metrics of this protocol were the most important to explain aquatic biota distribution?

## 2. MATERIAL AND METHODS

We used the Web of Science (WoS) database to search for scientific papers related to our objectives. This platform contains about 21,100 studies from different areas of knowledge and a wide database from the global scientific literature. It also has indexed journals of reliable quality and a rigorous selection process to include studies in its database (Clarivate, 2021). We also searched for scientific papers on Google Scholar (GS), which is a web search engine that enables simple access to academic literature. It contains relevant studies from all over the world, published as articles, theses, books, abstracts, among other resources (Google Scholar, 2021).

We searched the studies at WoS using the words and the Boolean operators: (“Physical Habitat” OR “Environmental Protection Agency” OR EPA) AND (Amazon\* OR Brazil\*) AND (stream\* OR river\*). During the selection process, we noticed that most scientific papers cited the studies of Kaufmann *et al.* (1999) and Peck *et al.* (2006). For this reason, we searched for articles that cited these specific studies considering that these works were those that described the calculations of variables and the method of measurement in the field, respectively and fitted our searching terms (Amazon\* OR Brazil\* AND Stream\* OR river\*) at Google Scholar.

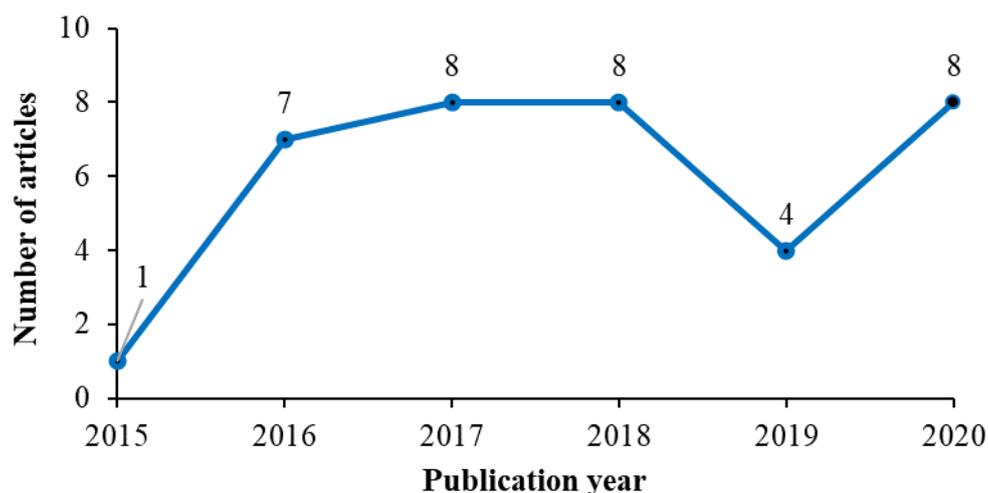
We searched the articles at WoS and GS from April 5th to July 20th, 2021, and selected articles published between 2013 (year when the US-EPA protocol was used in Amazon by Gardner *et al.*, 2013) and 2020 because it corresponded to the last year of our searching process. We only considered documents that effectively used the US-EPA protocol in Amazonian streams. We selected only studies published as “article” type, because these publications are peer reviewed by the editorial group of a scientific journal, and hence, they are not considered gray literature (such as monographies, dissertations, and theses). Finally, we accepted articles published in any language (e.g., English, Portuguese, and Spanish).

We organized the selected studies in a spreadsheet editor software to produce graphs and tables. We inserted the studies as rows and the basic information, such as title, year of publication, journal and first author, as columns. After reading the articles, we included other data as columns in the spreadsheet. These included: number of streams in each study, geographic coordinates of each stream where the US-EPA was applied, main land-use type in the study, the protocol metrics most affected by changes in land use, and the taxonomic groups used together with the US-EPA protocol. Finally, to answer our questions, we quantified the data obtained from the selected studies and used graphs and tables to represent the results. We also produced a map to spatially represent the geographic distribution of the Amazonian streams where the US-EPA protocol was applied. For this, we used the software Quantum GIS.

## 3. RESULTS AND DISCUSSION

### 3.1. General description of the scientometrics

Initially, we obtained 243 articles from the WoS and GS database, but after the selection process, 36 remained, as they were the only studies in accordance with our objectives. The first study that described RAS activities was published in 2013 (Gardner *et al.*, 2013), but the first studies that used the US-EPA protocol in Amazonian streams were only published in 2015 (Oliveira-Junior *et al.*, 2015) (Figure 1).



**Figure 1.** Number of scientific productions that used the US-EPA protocol for environmental monitoring Amazonian streams, and that were indexed to WoS and GS between 2015 and 2020.

### 3.2. In how many Amazonian streams has the US-EPA protocol been used?

In our study, we found that the US-EPA protocol was used in 889 Amazonian streams. However, many of the filtered papers share the same databases, so the actual number of sampled streams identified in our search is 380; but still, this number may be overestimated. When we plotted the coordinates, we found that some points were less than 100 meters apart, which may be related to the accuracy of the coordinates provided by papers that share the same data. In contrast, according to public data from US-EPA, in 2014 this protocol had already been applied to 91,975 North American streams to assess their environmental quality (USEPA, 2021). All these numbers show that the coverage of Amazonian streams where this protocol is still small compared to the US. But considering the period in which it began to be applied and the logistical difficulties in Amazon, especially in relation to access, the US-EPA protocol has been applied successfully.

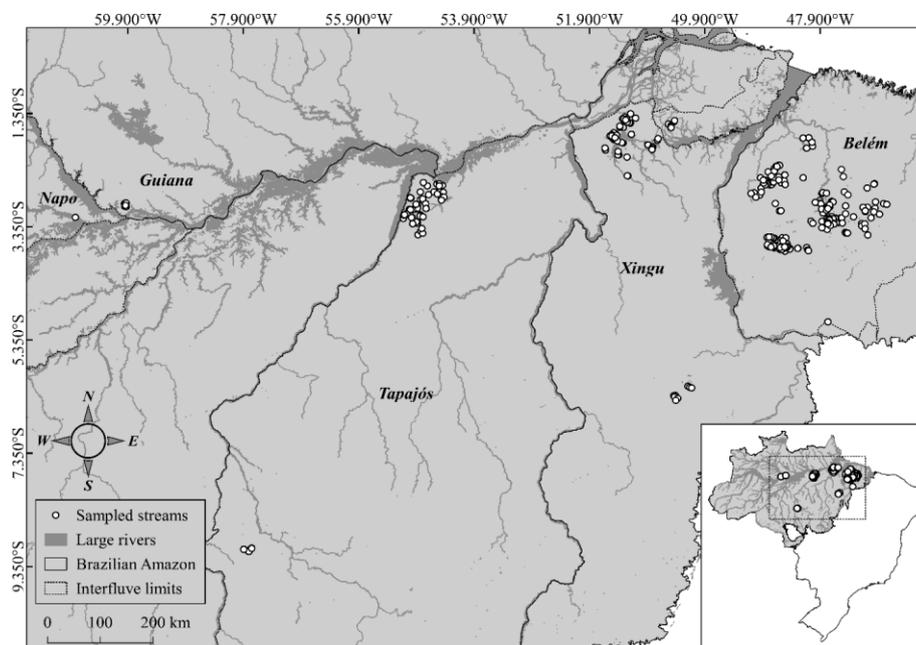
It is important that Brazilian federal and state governments, environmental and funding agencies, and private companies use this tool for monitoring environmental quality of Amazonian streams, as well as to achieve a representative evaluation of these habitats. These institutions can create specific agencies to evaluate aquatic systems, or fund studies, environmental companies, Non-Governmental Organizations (NGOs), universities and research institutions to widen the use of the US-EPA protocol in Amazonian streams. Only with these incentives, we will be able to obtain an expansion of environmental monitoring and have a good projection of the environmental quality of Amazonian streams. Scenarios of fire, deforestation, and use of natural areas for anthropic purposes make this agenda even more urgent.

### 3.3. Is the use of the US-EPA protocol well distributed across the Amazonian Basin?

We found that the use of the US-EPA protocol was restricted to five of the nine Amazon endemism areas: Belém (231), Xingú (75), Tapajós (69), Guiana (10) and Imeri (1) (Figure 2). Most of the streams were in Pará state, and Acará Basin (in Belém endemism area) had the highest concentration of streams evaluated by scientific studies in comparison to other basins.

We do not find application records of the US-EPA protocol to streams in the Northern and Western Amazon or in the states of Roraima and Acre. These areas differ greatly in limnological characteristics, as they present upland forests (Pires and Prance, 1985; Pazin *et al.*, 2006) and floodplains of  $8.4 \times 10^5$  km<sup>2</sup>. During the rainy period, plains become flooded, and lakes get

connected to each other and to the mainstream, producing a diversity of trophic environments (Hess *et al.*, 2015). These regions also differ in climate, soil, and hydrographic characteristics due to the Amazon evolutionary history. Thus, each region has specific physicochemical characteristics, which create an important environmental heterogeneity in the Amazon biome (Shimano *et al.*, 2021).



**Figure 2.** Spatial distribution of the streams with EPA-protocol applications in Amazonian streams between 2015 and 2020.

### 3.4. Which types of land use have been evaluated using the protocol?

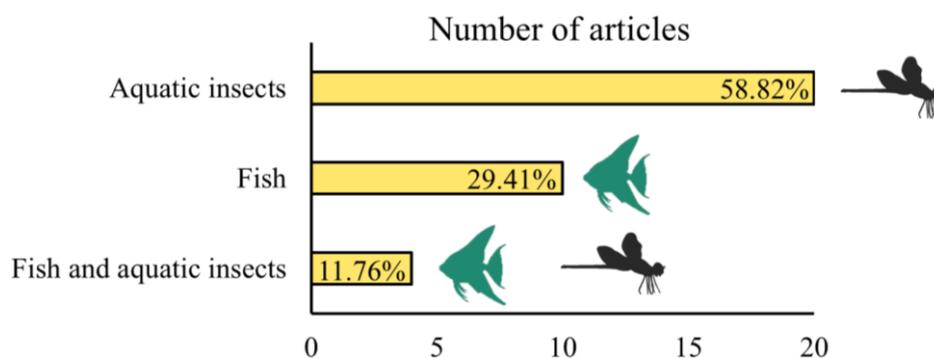
The analyzed streams were surrounded by different types of land use and occupation, which included forest (37.23%), agriculture (27.66%), logging (15.96%), livestock (12.77%) and urbanization (6.38%). The data obtained in forest streams provide a good base to describe the expected characteristics of morphology, structure, and water quality for Amazonian streams in the sampled area. Streams in preserved regions present considerable natural variation between plain areas with low-flow water bodies and upland areas, with fast-flowing waters (Benone *et al.*, 2017; Shimano *et al.*, 2021). Such environmental variation contributes to the diversity of fish (Benone *et al.*, 2017) and aquatic insects of the Order Ephemeroptera (Shimano *et al.*, 2021) in Amazon, in an evolutionary history context.

In 2020, the MapBiomas platform registered that 15% of Amazonian areas had been transformed into other types of land use, among which livestock and agriculture were the most common activities (Souza *et al.*, 2020). Evidence shows that the expansion of soybean crops and beef production in Brazil cause the decrease of rainfall in these areas (Leite-Filho *et al.* 2021). As such, measuring and monitoring the effect of anthropic disturbances in forest streams, especially those in different Amazonian regions, can be challenging, but they are crucial to protect the remaining natural vegetation and to maintain rainfall patterns.

### 3.5. Which components of the aquatic biota have been used together with the protocol to assess environmental quality of Amazonian streams?

We found that most studies (n=20) used aquatic insects from the orders Ephemeroptera, Plecoptera, Trichoptera, Hemiptera and Odonata to complement the US-EPA protocol, followed by studies that used fish (n=10), and a minority of studies that used aquatic insects and fish together (n=4) (Figure 3). Many aquatic insect groups are sensitive to environmental

changes or are bioindicators from some specific impact and, in addition, occupy different microhabitats on stream ecosystems, which allows them to be used to assess impacts on different aspects of the physical structure of Amazonian streams. For example, Odonata (adults) are associated to riparian vegetation, while Heteroptera and Trichoptera (larvae) are associated with surface and substrates, respectively (Juen *et al.*, 2016; Cunha *et al.*, 2022; Cruz *et al.*, 2022), so they are widely used for monitoring environmental impacts. This explains why we found a high number of articles using these organisms (Oliveira-Junior and Juen, 2019a; Mendes *et al.*, 2019b; Brasil *et al.*, 2020). The low number of studies using fish and aquatic insects together is likely due to the long time necessary to sort and identify species in the laboratory, in addition to difficulties of finding specialists in the taxonomy of these groups. For these reasons, it is more usual to find studies that work with these groups separately. Recent studies have used the environmental variables analyzed in the US-protocol together with the diversity and distribution of aquatic macrophytes (Fares *et al.*, 2020; Carmo *et al.*, 2023).



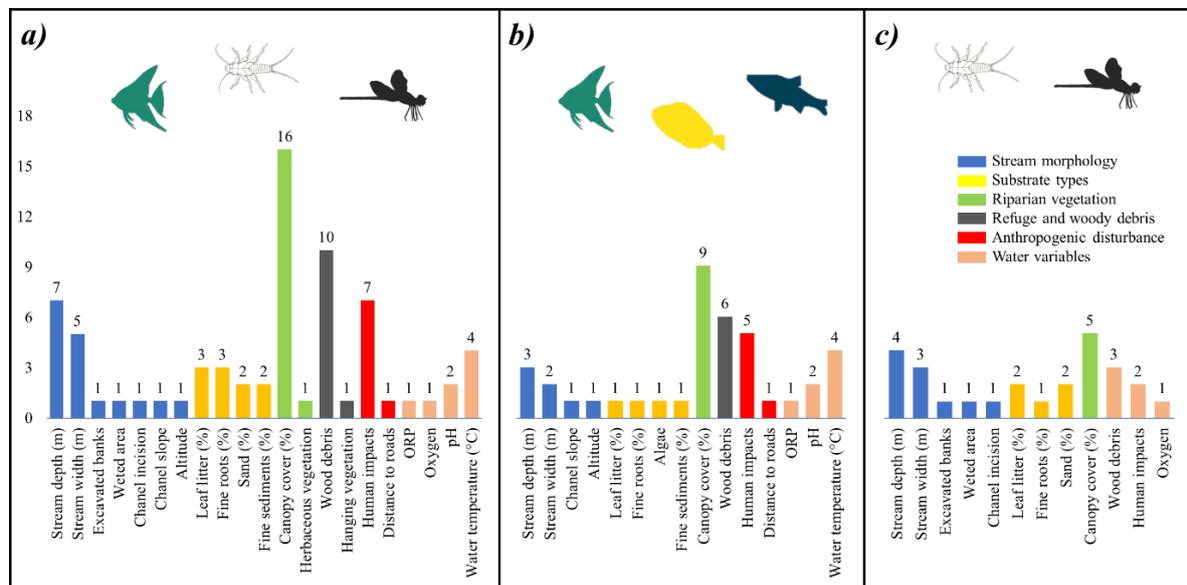
**Figure 3.** Quantity of studies and biological groups that were used together with the US-EPA protocol for monitoring Amazonian streams.

### 3.6. Which metrics of the protocol were the most important to explain aquatic biota distribution?

Among the 239 metrics analyzed in the US-EPA protocol, five repeatedly presented significant statistical relationships with fish and aquatic insect communities in the studies. These were: canopy cover or density (1), presence of wood in the channel (2), anthropic impacts in the riparian zone or in the landscape (3), width and depth of the channel (4), and water temperature (5). We also observed other variables in the studies, such as type of substrate, which included sand, leaves and roots in the stream channel, and physicochemical characteristics of the water such as pH, oxidation/reduction potential (ORP) and dissolved oxygen in the water (Figure 4 A, B and C).

Such relationships between the metrics of the protocol with fish and aquatic insects have some specificities. For both, the most important metric found repeatedly in the studies was the canopy cover. Low percentages of canopy cover reflect deforestation surrounding the stream, which is composed of forests in the case of Amazon. For fish, canopy cover can positively affect the species richness, as observed by Montag *et al.* (2019) in the surroundings of Conservation Units. However, canopy cover loss negatively affects the composition of fish communities (Prudente *et al.*, 2016). Considering aquatic insects, especially Odonata adults, both loss of canopy cover and anthropic impacts in the riparian zone increase the entrance of sunlight into forest streams (Juen *et al.*, 2016). The species richness of Zygoptera and Anisoptera, for example, is restricted to sunlight availability, which determines their environmental gradient of distribution (Calvão *et al.*, 2016; Oliveira-Junior and Juen, 2019b). Anisoptera species occur mainly in deforested areas, where Zygoptera corresponds to the minority of species. As such, these groups are commonly used as bioindicators of environmental quality in Amazonian streams (Oliveira-Junior and Juen, 2019b). This pattern is

so consistent that the Brazilian Environmental Agency (Chico Mendes Institute for Biodiversity Conservation, ICMBio) uses it as a protocol for monitoring environmental conditions of streams within Amazonian Conservation Units (Brasil *et al.*, 2020).



**Figure 4.** Most important metrics of the US-EPA protocol for the aquatic biota, represented by (A) fish and/or aquatic insects, (B) fish, and (C) aquatic insects. The colors represent the block of variables.

Stream morphology variables, like width and depth, are also important for fish and aquatic insect communities, but anthropic changes in the landscape can change these characteristics. Consequently, local populations of specialist species can be made extinct or replaced by generalist species. This may not affect species richness but can change the composition of fish in these habitats (Prudente *et al.*, 2016; Ilha *et al.*, 2019). Differently from this pattern, Leão *et al.* (2020) found a higher diversity of fish in streams of pasture areas than in those of forest areas. However, their results were associated with different levels of environmental heterogeneity, and riparian vegetation around the sampled streams.

In addition to the incidence of light, the removal of riparian vegetation also causes the increase of water temperature in the stream, and the decrease of available oxygen for immature insect communities, such as Ephemeroptera, Plecoptera and Trichoptera (Juen *et al.*, 2016; Shimano and Juen, 2016; Mendes *et al.*, 2019b). As such, we can expect that alterations in the riparian zone reduce species richness of sensitive aquatic macroinvertebrates. Consequently, generalist species richness increases, whereas populations of specialist species, which depend on high environmental quality, decrease or are extinct (Martins *et al.*, 2017). This makes it clear that the riparian vegetation is an important aspect of the stream's physical habitat.

Additionally, the presence of wood and other organic substrates in the stream channel can be important for fish and insect communities by providing refuge, food resources and habitat heterogeneity. Fish of the genus *Characidium*, for example, use leaves, stems, and branches to hide and capture prey (Montag *et al.*, 2019). Insects from the Trichoptera order, like *Phylloicus* and *Triplectides*, depend on these substrates for refuge construction and feeding and are excluded from the streams under high levels of deforestation (Lima *et al.*, 2022). Furthermore, the reduction in the availability of allochthonous organic resources affects not only the organisms that directly benefit from them, but also the entire food chain in stream ecosystems (Vannote *et al.*, 1980; Tereza *et al.*, 2015; Lima *et al.*, 2022).

## 4. CONCLUSIONS

Our study shows that the US-EPA protocol is an important tool for characterizing streams, monitoring environmental impacts, and predicting the aquatic biota. However, this protocol is not homogeneously used across the Amazon Basin. The Western, Northern and Southern Amazon Basin still need a greater sampling effort using this protocol so we can obtain a complete environmental characterization of Amazonian streams. The use of the US-EPA protocol for monitoring the environmental quality of streams should be expanded to enable the evaluation of different types of land-use impacts. Also, whenever possible, it should be used together with a component of the aquatic biota, especially fish and aquatic insects, which have proven to be efficient tools to detect impacts and for monitoring the aquatic environment.

In Brazil, scientific research still lacks financial resources and environmental changes in the Amazon Rainforest are accelerated. As such, we suggest the development of a simplified version of the US-EPA protocol, based on the most important metrics for the aquatic biota, to be used in Amazonian streams. This would enable the evaluation of a greater number of Amazonian streams, even those of difficult access. It would also be an important initiative for research teams with low funding, which cannot gather enough fieldworkers to apply the complete protocol.

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