

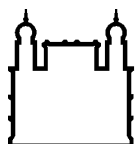
MINISTÉRIO DA SAÚDE  
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INSTITUTO OSWALDO CRUZ

Programa de Pós-Graduação em Biodiversidade e Saúde

APLICAÇÃO DE FERRAMENTAS PARA BIOMONITORAMENTO  
UTILIZANDO MACROINVERTEBRADOS EM RIACHOS NO ESTADO  
DO RIO DE JANEIRO, BRASIL

PRISCILLA DA SILVA PEREIRA

Rio de Janeiro  
Novembro/2020



Ministério da Saúde

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## **INSTITUTO OSWALDO CRUZ**

**Programa de Pós-Graduação em Biodiversidade e Saúde**

**PRISCILLA DA SILVA PEREIRA**

Aplicação de ferramentas para biomonitoramento utilizando macroinvertebrados em riachos no estado do Rio de Janeiro, Brasil

Tese apresentada ao Instituto Oswaldo Cruz  
como parte dos requisitos para obtenção do título  
de Doutor (a) em Ciências

**Orientador:** Prof. Dr. Daniel Forsin Buss

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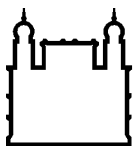
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JANEIRO, BRASIL

**ORIENTADOR: Prof. Dr. Daniel Forsin Buss**

**Aprovada em: 17/ 11/ 2020**

### **EXAMINADORES:**

**Prof. Dr. Felipe Ferraz Figueiredo Moreira - Presidente (IOC/FIOCRUZ)**

**Prof. Dr. Vinicius Neres de Lima (IBRAG/UERJ)**

**Prof. Dr. Ronaldo Figueiró (CENTRO UNIVERSITÁRIO ESTADUAL ZONA OESTE/UEZO)**

**Prof. Dra. Rachel Ann Hauser Davis (IOC/FIOCRUZ)**

**Prof. Dr. Elidiomar Ribeiro da Silva (Instituto de Biociências/UNIRIO)**

Rio de Janeiro, 17 de Novembro de 2020

Dedico este trabalho à minha avó  
Marinete e aos macroinvertebrados.

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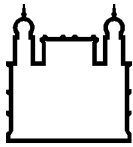
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“O caminho de volta é pertencer-se. Não há rio que siga seu curso sem que se preencha todas as depressões que estão sempre um passo à frente. Aprender e agir é o curso para o conhecimento. Pertencer-se é nossa busca.”

Orunmila



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### APLICAÇÃO DE FERRAMENTAS PARA BIOMONITORAMENTO UTILIZANDO MACROINVERTEBRADOS EM RIACHOS NO ESTADO DO RIO DE JANEIRO, BRASIL

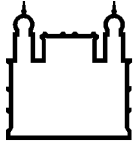
#### RESUMO

#### TESE DE DOUTORADO EM BIODIVERSIDADE E SAÚDE

**Priscilla da Silva Pereira**

O levantamento biológico da macrofauna constitui uma metodologia bastante utilizada para a avaliação da integridade ecológica de ecossistemas aquáticos. O primeiro capítulo teve como objetivo avaliar a abundância e riqueza de cada Grupo Funcional de Alimentação (GFA) e utilizar razões de GFA como ferramenta para caracterizar a integridade ecológica dos ecossistemas aquáticos. As áreas de coletas no estado do Rio de Janeiro foram classificadas *a priori* como referência, intermediária ou impactada, a partir de critérios preestabelecidos. Foram ajustados diferentes modelos de efeito misto para cada GFA. Os modelos mostraram que os GFA diferiam em suas respostas às variáveis abióticas (Stream Width, Altitude, DO, pH, Cond, NH<sub>3</sub>, Ch, TH, TA, Ca and, HAP). As razões de GFA apresentaram diferenças significativas entre as classes de impacto. A análise da razão de GFA mostrou-se uma ferramenta rápida e barata com potencial para ser utilizada no biomonitoramento do bioma da Mata Atlântica. O segundo capítulo avaliou o desempenho de seis sistemas de classificações de rios, observando os efeitos da resolução taxonômica (gênero e família), tipo de dados (abundância e riqueza) e o valor de espécie indicador. ANOSIM e a Força de Classificação foram utilizadas para avaliar a performance de cada sistemas de classificação. A Geomorfologia obteve o melhor desempenho em discriminar a variação nos padrões biológicos. Táxons semelhantes foram encontrados na análise IndVal entre os tipos de dados, sendo representativos de áreas de referência. A classificação de riachos é essencial para projetar programas de gestão e vigilância ambiental. Esses capítulos contribuem para a implantação de programas de biomonitoramento e para a gestão de rios de pequeno porte no Estado do Rio de Janeiro, Brasil.





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### BIOMONITORING TOOLS USING MACROINVERTEBRATES IN STREAMS IN THE STATE OF RIO DE JANEIRO, BRASIL

#### ABSTRACT

#### PHD THESIS IN BIODIVERSITY AND HEALTH

**Priscilla da Silva Pereira**

The biological survey of the macrofauna is a widely used methodology for assessing the ecological integrity of aquatic ecosystems. The first chapter of this thesis aimed to evaluate the effects of abiotic variables on the abundance and richness of each Functional Feeding Group (FFG) and use attributes of the ecosystem (FFG ratios) as a tool to characterize the ecological integrity of the water. We classified sampling areas *a priori* as reference, intermediate or impaired using pre-established criteria. The mixed-effect models showed that the FFG differed in their responses to abiotic variables (Stream Width, Altitude, DO, pH, Cond, NH<sub>3</sub>, Ch, TH, TA, Ca and, HAP). The FFG ratios presented significant differences between classes of impairment. The analysis of the FFG ratio proved to be a fast and cheaper tool, with the potential to be used in the biomonitoring of the Atlantic Forest biome. The second chapter aimed to evaluate the performance of seis stream classification systems, observing the effects of taxonomic resolution (genera and family), type of data (abundance and richness) and the indicator species value. Our evaluation indicated that the Geomorphology is one of the most capable of discriminating variation in biologic patterns. Similar taxa were found in the IndVal analysis between data types, being representative of reference areas. Stream classification is essential for designing sampling programs and environmental monitoring. These chapters contribute to the implementation of biomonitoring programs and to the management of streams in Rio de Janeiro, Brasil.

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## LISTA DE ABREVIATURAS E SIGLAS

<b>AIC</b>	Akaike Information Criterion
<b>ANOSIM</b>	Analysis of similarities
<b>AQEM</b>	Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates
<b>CERH-MG</b>	Conselho Estadual de Recursos Hídricos do Estado de Minas Gerais
<b>CETESB</b>	Companhia Ambiental do Estado de São Paulo
<b>CONAMA</b>	Conselho Nacional do Meio Ambiente
<b>COPAM</b>	Conselho Estadual de Política Ambiental
<b>CS</b>	Classification Strength
<b>EPA</b>	Environmental Protection Agency
<b>FFG</b>	Functional Feeding Group
<b>HAP</b>	Habitat Assessment Protocol
<b>IndVal</b>	Indicator value
<b>INEA</b>	Instituto Estadual do Ambiente
<b>RBP</b>	Rapid Bioassessment Protocols
<b>WFD</b>	Water Framework Directive

## 1. INTRODUÇÃO

A poluição das águas decorre da adição de substâncias químicas e/ou de microrganismos que, direta ou indiretamente, alteram as suas características. Uma das principais causas é a presença excedente de matéria orgânica e de nutrientes, como carbono, nitrogênio e fósforo, oriundos do despejo de esgoto doméstico e industrial (Vasco et al. 2011). Elevadas concentrações de outros poluentes como óleos, graxas, agrotóxicos, metais, hormônios, entre outros podem depreciar ainda mais a qualidade da água, reduzindo a possibilidade de prestação de serviços ambientais pelos corpos hídricos, como a utilização da água para abastecimento público ou manutenção do equilíbrio da vida aquática (Von Sperling 2007).

Atualmente, cerca de 2,1 bilhões de pessoas no mundo não têm acesso a água potável, 4,5 bilhões não possuem acesso a serviços de saneamento, e mais de 3 milhões de pessoas morrem todos os anos vítimas de doenças causadas por água de má qualidade (ONU 2003). A saúde da população humana depende principalmente da manutenção da qualidade da água e, para atingir esse padrão, é necessário o investimento em sistemas de gestão mais integrados. A dificuldade em suprir as necessidades de qualidade e quantidade de água é uma realidade em países desenvolvidos ou em desenvolvimento. Vale ressaltar que esse cenário é ainda mais cruel em países subdesenvolvidos.

O Brasil é um país que possui uma vasta e densa rede hidrográfica e detém cerca de 10% de toda a água doce da superfície da Terra (cerca de 1.488.000 m<sup>3</sup>/s), com muitos de seus rios destacando-se pela extensão, largura e profundidade (Rebouças, 2002). Entretanto, o crescimento da população urbana promoveu um aumento considerável nas demandas hídricas associadas à degradação dos mananciais, contaminação e poluição (Tundisi & Tundisi 2011).

Este perfil de degradação pode ser observado nos rios do Estado do Rio de Janeiro principalmente a partir da segunda metade do século XX. Neste período, a integridade dos ambientes aquáticos começou a ser afetada pelo aumento da extração de madeira, avanço das fronteiras agrícolas e intensificação de áreas de pastagem. Tais formas inadequadas de uso e ocupação do solo favoreceram o desmatamento das áreas marginais dos rios e contribuíram com outras fontes poluidoras (contaminação química na agricultura). Estas perturbações vêm reduzindo significativamente a biodiversidade e provocando alterações na mata ciliar, diminuindo conseqüentemente a

oferta de água e comprometendo a manutenção dos ecossistemas aquáticos (Bergallo et al. 2009).

A área de drenagem do Estado do Rio de Janeiro é formada por nove regiões hidrográficas: Ilha Grande, Guandu, Médio Paraíba do Sul, Piabanha, Baía da Guanabara, Lagos São João, Rio Dois Rios, Macaé e Rio das Ostras e Baixo Paraíba do Sul e Itabapoana (Figura 1).



**Figura 1.** Mapa com as Regiões Hidrográficas do estado do Rio de Janeiro. Fonte INEA.

Essas regiões estabelecem comunicação com uma rede de pequenos riachos e rios de pequeno, médio e grande porte (Bergallo et al. 2009). Ao redor dessa malha hídrica podem ser encontradas diversas unidades industriais, áreas de atividade agropecuárias, além de vilarejos e pequenas cidades em constante expansão imobiliária. Essas atividades integradas e sem um adequado sistema de tratamento dos seus efluentes podem provocar alteração nos corpos hídricos.

### 1.1 Legislação ambiental

Foi promulgada no Congresso dos EUA em 1972, a primeira legislação nacional abrangente sobre a água, “Clean Water Act” em resposta à crescente preocupação com a saúde pública devido à poluição. Trata-se da principal lei federal daquele país que protege a saúde dos ecossistemas aquáticos, incluindo lagos, rios e áreas



costeiras. O objetivo principal dessa lei é restaurar e manter a integridade física, química e biológica (Karr 1999) dos corpos d'água. Seus regulamentos são administrados pela Environmental Protection Agency (EPA), em coordenação com os governos estaduais. Foi então que se criou um esforço no sentido de coletar, analisar e interpretar dados biológicos para permitir ações de monitoramento, controle e mitigação de impacto. Em 1985, foi realizado um grande levantamento para verificar quais estados já possuíam algum sistema de monitoramento e os métodos mais utilizados (Carter & Resh 2001). Com isso, surgiram Rapid Bioassessment Protocols - RBP (Protocolos de Bioavaliação Rápida), que tinham como base desenvolver procedimentos rápidos para levantamento biológico que permitissem o entendimento por leigos e gestores, gerando resultados que viabilizassem as decisões de manejo. O RBP I (Plafkin et al. 1989) foi criado com base nos procedimentos de algumas agências estaduais. Atualmente, existe o RBP III, que agrega informações e métodos de mais agências (Barbour et al. 1999).

Na Europa, em 2000 foi publicada uma diretiva, Water Framework Directive (WFD), que orienta os estados membros da União Europeia a alcançar um bom status qualitativo e quantitativo da água, visando normatizar os aspectos da política de gestão de recursos hídricos na Europa. O grande avanço desta iniciativa foi a obrigatoriedade de implementação do monitoramento biológico que deve guiar as medidas de restauração e manejo em ecossistemas aquáticos. Para atender á diretiva, foi desenvolvido o projeto Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates (AQEM). Trata-se de um método abrangente de avaliação de rios que busca a integração e o estabelecimento de normas de proteção e uso sustentável da água (AQEM 2002).

No Brasil, a avaliação da qualidade da água tem sido realizada somente quanto aos parâmetros físico-químicos e microbiológicos, onde são estabelecidos níveis aceitáveis de concentrações para componentes considerados importantes para o diagnóstico. Os princípios básicos para a gestão dos recursos hídricos estão pautados na Constituição Federal de 1988; na Política Nacional de Recursos Hídricos (instituída pela Lei nº 9.433, de 8 de janeiro de 1997, e que inaugurou uma nova perspectiva definindo o gerenciamento de forma descentralizada, participativa e integrada); na CONAMA nº 274 de 2000 (que define os critérios com relação aos padrões de balneabilidade em águas brasileiras); na CONAMA nº357 de 2005 (que dispõe sobre os padrões de classificação dos corpos hídricos e diretrizes para o enquadramento segundo o seu uso, além de estabelecer as condições e padrões de lançamento de

efluentes em cada classe, sendo a primeira legislação que cita o uso de instrumentos de avaliação biológica, Artigo 8º, parágrafo 3º); na CONAMA nº 430 de 2011 (que complementa a resolução CONAMA nº 357 de 2005, designando condições, padrões, parâmetros e diretrizes para o lançamento de efluentes em corpos de água receptores, indicando a utilização da ecotoxicologia como ferramenta para o monitoramento da água, Artigo 18º caput; parágrafo 1º); e na Portaria do Ministério da Saúde nº 2.914 de 2011 (que define a qualidade e os padrões de potabilidade da água para o consumo humano). Apesar da promulgação desses dispositivos legais, poucos avanços referentes ao estabelecimento de ferramentas e diretrizes para a avaliação e conservação dos ecossistemas aquáticos foram definidos (Buss et al. 2008).

Em nível estadual, existem iniciativas que utilizam a biota na avaliação de rios, como em Minas Gerais, que possui a Deliberação Normativa Conjunta COPAM/CERH-MG nº 01, de 05 de maio de 2008, a qual tornou obrigatória a avaliação das águas continentais por meio de indicadores biológicos. No Estado de São Paulo, foi elaborado um protocolo para biomonitoramento baseado nas comunidades bentônicas de rios e reservatórios, o qual incentiva a bioavaliação e facilita sua padronização (CETESB 2012). Entretanto, avaliando o cenário nacional, observa-se uma flexibilização do governo acerca da legislação ambiental, como a mudança do código florestal, o projeto de lei nº 1.876, de 1999, que prevê a redução de áreas de reserva legal e a flexibilização das normas com relação às áreas de preservação permanente, passando a exigir uma menor área de mata ciliar em torno de corpos d'água.

Apesar das reformas legais ocorridas no setor de recursos hídricos no Brasil vislumbrarem o uso sustentável e a gestão adequada dos ecossistemas aquáticos, a política ambiental ainda se mostra incipiente para manter a integridade ecológica dos ecossistemas aquáticos. O arcabouço legal brasileiro ainda não ressalta questões essenciais para a gestão, como a adoção de critérios mais adequados e rigorosos de monitoramento e a padronização das metodologias aplicadas (Santos & Kuwajima 2019).

A necessidade de gerir adequadamente os recursos hídricos torna necessário o uso de mecanismos de caracterização de rios, para o planejamento de políticas públicas. Funcionando como ferramenta de suporte, o uso de mecanismos de caracterização de rios permite a definição de estratégias para a conservação e manejo de recursos naturais, podendo facilitar a comunicação entre os diferentes níveis de gestão (nacional, estadual e municipal).

## 1.2 Biomonitoramento

Um determinado estresse nos ecossistemas aquáticos deve ser avaliado através de análises convencionais físico-químicas, microbiológicas e pelo monitoramento biológico (Kebede et al. 2020). Isso porque os parâmetros físico-químicos mostram alterações pontuais nas propriedades naturais associadas à qualidade da água, enquanto modificações na dinâmica das populações de organismos aquáticos podem refletir em alterações estruturais e funcionais das comunidades nos sistemas ecológicos, em particular, na diversidade e abundância da biota. O uso combinado destas ferramentas aumenta o potencial de detecção das causas e de avaliação dos efeitos de estressores sobre os ecossistemas aquáticos.

O biomonitoramento é uma das metodologias utilizadas para avaliar a “saúde” ou integridade biológica dos ecossistemas aquáticos. Esta abordagem consiste na utilização de respostas biológicas para avaliar as mudanças ambientais (Matthews et al. 1982, Barbour et al. 1999, Baptista et al. 2007, Medupin 2020). Neste contexto, a definição de biomonitoramento mais aceita é o uso sistemático das respostas de organismos vivos para avaliar as mudanças ocorridas no ambiente, geralmente causadas por ações antropogênicas (Matthews et al. 1982).

A primeira abordagem visando a determinação de indicadores biológicos da qualidade das águas, com bases científicas, foi realizada com bactérias, fungos e protozoários na Alemanha, por Kolkwitz & Marsson (1908, 1909). Como praticamente qualquer grupo pode ser utilizado em programas de monitoramento, já existem metodologias de avaliação para macrófitas, peixes, macroinvertebrados, entre outros (Barbour et al. 1999).

Recentemente, tem havido um interesse no biomonitoramento da qualidade da água em países em desenvolvimento é observado devido ao fato destas metodologias de coleta simples e de baixo custo (Cantonati et al. 2020). Para isso é imperativa a realização de estudos que estabeleçam a taxonomia e a ecologia das espécies.

No Brasil, existem diversos grupos de pesquisa trabalhando no desenvolvimento de protocolos de biomonitoramento (Junqueira et al. 2000, Ferreira et al. 2011, Pereira et al. 2016, Ruaro et al. 2019). Porém, se faz necessário maior investimento em todas as regiões e ecossistemas do país, como a definição de protocolos de campo, laboratoriais, análises de dados, estudos da taxonomia dos bioindicadores com o desenvolvimento de chaves taxonômicas para facilitar o processo de identificação e o desenvolvimento de softwares para o cálculo das métricas e índices bióticos.

### 1.2.1 Macroinvertebrados como bioindicadores

Ao se aplicar o biomonitoramento, é preciso realizar uma seleção criteriosa de quais organismos serão úteis e poderão responder a uma determinada condição ambiental, ou seja, escolher adequadamente os bioindicadores (Bonada et al. 2006). Os bioindicadores são grupos de organismos ou espécies que podem ser resistentes ou sensíveis, alterando sua estrutura ou funcionalidade em resposta a determinados tipos de mudanças ambientais (Junqueira et al. 2000, Mugnai et al. 2008, Ferreira et al. 2011). Um mesmo grupo pode ser sensível a algumas mudanças e resistente a outras, dessa maneira, é muito importante estudar esta relação. Ao longo dos anos, grupos específicos de organismos têm sido selecionados como bioindicadores, tais como, protozoários, algas, macroinvertebrados bentônicos, rotíferos e peixes (Rosenberg & Resh 1993, Li et al. 2010, Debastiani et al. 2016). Os macroinvertebrados estão entre os organismos bioindicadores mais utilizados em programas de biomonitoramento (AQEM 2002, Bonada et al. 2006, Baptista 2008, Buss et al. 2015).

Os macroinvertebrados são animais maiores que 0,5 mm que vivem associados ao fundo de rios, lagos, lagoas e reservatórios em pelo menos uma fase do ciclo vida. Este grupo é composto por crustáceos, moluscos e insetos que em sua maioria ficam situados em uma posição intermediária na cadeia alimentar, se alimentando de algas, microrganismos e detritos de origem vegetal terrestre. Segundo Bonada et al. (2006), o uso dos macroinvertebrados como bioindicadores é baseado em sua capacidade de serem aplicados para monitorar fontes não pontuais de poluição, auxiliando os métodos químicos de diagnóstico. Por apresentarem uma distribuição geográfica ampla, os mesmos podem responder a diferentes tipos de impactos, o que justifica o seu potencial uso em programas de biomonitoramento. Além disso, existe um grande número de espécies que responde a diferentes tipos de agentes estressores e a maioria desses organismos possuem baixa migração a longas distâncias e um ciclo de vida longo, permitindo uma análise de mudanças temporais causadas por diferentes tipos de impacto e apresentam baixo custo amostral e de triagem (Barbour et al. 1999, Hering et al. 2010, Alemneh et al. 2019).

### 1.3 FFG e razões de FFG

Segundo Doledec et al. (2011), em rios e riachos o funcionamento do ecossistema pode ser observado através das características ou traços biológicos que relacionam a história de vida dos organismos (tamanho máximo, ciclos reprodutivos por

ano) com características biológicas e fisiológicas (por exemplo, hábitos de alimentação e respiração, resistência e resiliência).

Atualmente, duas abordagens com macroinvertebrados têm sido utilizadas em programas de biomonitoramento para avaliar a qualidade das águas de rios. Na primeira, a comunidade é caracterizada conforme sua composição taxonômica. Já na segunda, a comunidade é caracterizada de acordo com seus atributos funcionais, considerando sua morfologia e comportamento. Com os resultados obtidos, podem-se caracterizar os atributos de um ecossistema (Cummins et al. 2005, Couceiro et al. 2011). Essa metodologia não ignora a taxonomia, mas demanda um menor detalhamento da morfologia e/ou papel funcional das espécies e também elimina o efeito da regionalidade.

O conceito de guilda trófica foi proposto por Root (1967) e definido como um grupo de espécies, independente da afiliação taxonômica, que compartilham a mesma classe de recursos alimentares e hábitos semelhantes. Quando os macroinvertebrados são divididos em guildas tróficas, essas categorias são conhecidas como Grupos Funcionais Alimentares (Functional Feeding Groups – FFG Cummins, 1973, Cummins & Klug 1979, Cummins et al. 2008).

De acordo com a classificação proposta por Cummins (1973), os insetos aquáticos podem ser classificados como coletores-catadores, coletores-filtradores, fragmentadores, predadores ou raspadores, agrupados de acordo com o seu modo de alimentação: (1) coletores-catadores - alimentam-se de pequenas partículas de matéria orgânica por coleta nos depósitos de sedimento; (2) coletores-filtradores – capturam, por filtração, pequenas partículas de matéria orgânica em suspensão na coluna d'água; (3) fragmentadores - mastigam folhas ou tecido de planta vascular vivo ou escavam madeira; (4) predadores - engolem a presa inteira ou ingerem os fluidos do tecido corporal; (5) raspadores - adaptados a raspar superfícies duras, alimentam-se de algas, bactérias, fungos e matéria orgânica morta sedimentados nos substratos (Cummins 1973).

Estudos que envolvem o conceito de guilda trófica possibilitam entender a distribuição da energia dentro da comunidade, do ponto de vista da complexidade e da diversidade. Dessa forma, a avaliação dos atributos relacionados a funções ecossistêmicas nos fornece indícios sobre a condição geral dos ecossistemas aquáticos.

Existem poucos estudos ecológicos acerca de insetos aquáticos em ecossistemas tropicais e geralmente se determinam as categorias tróficas dos taxons

com base em classificações desenvolvidas para regiões temperadas (Baptista et al. 2006, Ramírez & Guitiérrez-Fonseca 2014). Segundo Merritt & Cummins (1986), foi demonstrado em vários estudos que esta aproximação pode ser imprecisa, uma vez que os grupos tróficos classificados em determinados ecossistemas temperados não têm necessariamente os mesmos hábitos alimentares nos trópicos. Além disso, o grupo predominantemente encontrado nas regiões temperadas é fragmentador, diferente do que ocorre nos trópicos, onde os organismos coletores são mais abundantes (Tomanova et al. 2006).

Os atributos relacionados à estrutura e função fornecem como indicadores das condições do ecossistema aquático (Hawkins & Sedell 1981, Ceneviva-Bastos et al 2017, Fugère et al. 2018). De acordo com Vannote et al. (1980), as interações tróficas podem refletir processos ecológicos, influenciando diretamente os fluxos da distribuição de energia e recursos dentro da comunidade. Essa metodologia não ignora a taxonomia, mas fornece um ganho rápido a um custo menor e uma visão eficiente da composição de macroinvertebrados bentônicos (Cummins et al. 2005).

De acordo com Merritt & Cummins (1986), o uso de Razões de FFG como substitutos de medidas diretas de atributos de rios podem ser utilizados em qualquer ecossistema aquático. Esses atributos incluem a relação de produção/consumo (autotrófico/heterotrófico), relação entre a qualidade da cobertura da vegetação ripária e a disponibilidade de liteira que é utilizada pelos fragmentadores, o grau de carga de partículas em suspensão, a estabilidade do habitat e o controle ascendente de predadores.

As características dos ecossistemas aquáticos muitas vezes são alteradas como resposta às perturbações provocadas por ações humanas (Cummins 1973). Informações sobre a organização, estrutura e função trófica podem revelar propriedades fundamentais dos sistemas aquáticos, contribuindo para a compreensão das relações e dinâmicas nestes sistemas (Motta & Uieda 2005).

Desta forma, é possível analisar o funcionamento do ecossistema de maneira mais ampla, suas alterações diante de possíveis impactos e disponibilizar aos comitês de Gestão de Bacias Hidrográficas os resultados do estudo para futuras avaliações em programas de biomonitoramento.

#### 1.4 Sistemas de classificações de rios

Os sistemas de classificação de rios são utilizados para apoiar ações de planejamento relacionadas à gestão e conservação dos recursos aquáticos, por meio do agrupamento de locais com características similares (Leathwick et al. 2011, Rinaldi et al. 2013, Troia & McManamay 2020). Em geral, são determinados através de variáveis ambientais, tais como: geologia, vegetação, clima, solo e uso da terra (e.g. Omernik & Griffith 2014) ou fatores bióticos (e.g. Abell et al. 2008). Podem ser apresentados como um arranjo de características morfológicas como, por exemplo, as ecorregiões aquáticas e as regiões hidrográficas.

As ecorregiões aquáticas são definidas como áreas relativamente homogêneas que têm condições ambientais semelhantes (Omernik 1987, 1995). Podem ser determinadas em diferentes escalas espaciais, e destinam-se a servir como um território para a investigação, avaliação e gestão dos ecossistemas. O delineamento de ecorregiões aquáticas pode ser realizado sob duas óticas. A primeira utiliza grupos biológicos (Abell et al. 2008), como no mapeamento mundial realizado entre 2006 e 2008, pela WWF e a TNC em conjunto com outras organizações de pesquisa, que identificou 426 ecorregiões aquáticas (Freshwater Ecoregions of the World – FEOW), sendo 25 no Brasil. O mapa de ecorregiões de água doce foi baseado nas distribuições e composições de espécies de peixes de água doce e incorpora os principais padrões ecológicos e evolutivos (Abell et al. 2008). A segunda utiliza variáveis ambientais que influenciam os processos ecossistêmicos dos ambientes de água doce (Soranno 2010). Esta abordagem foi utilizada no projeto RADAMBRASIL (Brasil, 1983), que realizou um amplo estudo integrado sobre geologia, geomorfologia, pedologia, vegetação, uso potencial da terra e capacidade de uso dos recursos naturais renováveis (Brasil 1983).

Outro sistema de classificação de rios muito utilizado especialmente no Brasil tem como base as características físicas e de desenvolvimento regional, as regiões hidrográficas. As mesmas são divisões administrativas, sendo compostas por bacias hidrográficas e águas subterrâneas, tendo em vista uma melhor gestão dos recursos hídricos.

Existe divergência na literatura quanto à escala dos fatores ambientais que mais influenciam na distribuição dos macroinvertebrados. Alguns autores encontraram relações entre a fauna e variáveis ambientais ao nível ecorregional (Barbour et al. 1996, Pinto et al. 2009). No entanto, outros estudos verificaram que a fauna parece ser

mais fortemente associada com as escalas micro/local, como substrato e microhabitat (Costa & Melo 2008, Ligeiro et al. 2013).

Diversos estudos avaliaram diferentes sistemas de classificações de rios baseados em comunidades aquáticas em ambientes temperados (Waite et al. 2000, Verdonschot & Nijoeer 2004, Verdonschot 2006, Sánchez-Montoya et al. 2007). No entanto, ainda são escassos estudos realizados no Brasil. Pinto et al. (2009) observaram que ecorregião foi o melhor preditor para a comunidade de peixes no rio Paraíba do Sul e seus tributários. Além disso, Vasconcelos et al. (2013), no Rio Grande do Sul, verificaram que a comunidade de macroinvertebrados estava mais associada ao agrupamento de variáveis ambientais do que outros sistemas de classificação.



## 2 OBJETIVO GERAL

Aplicar ferramentas de biomonitoramento utilizando macroinvertebrados bentônicos em riachos no Estado do Rio de Janeiro.

### 2.1 Objetivos específicos (por capítulo)

#### **Capítulo 1 “Functional Feeding group composition and attributes: evaluation of freshwater ecosystems in the Atlantic Forest, Brazil”**

- Classificar as áreas de estudo em referência, intermediárias e impactadas através de critérios preestabelecidos.

- Calcular as categorias FFG com relação à abundância e à riqueza para os riachos amostrados.

- Avaliar os efeitos das variáveis abióticas com relação à abundância e à riqueza de cada FFG.

- Calcular e aplicar as razões de FFG para caracterizar a integridade ecológica dos riachos amostrados.

#### **Capítulo 2 “Performance of the top-down and bottom-up approaches: stream classification using macroinvertebrates in the Atlantic Forest, Brazil”**

- Realizar um levantamento da fauna de macroinvertebrados bentônicos.

- Testar e avaliar o desempenho de diferentes sistemas de classificações de rios utilizando macroinvertebrados.

- Calcular o valor de indicador dos táxons principais para os sistemas de classificações.

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## Capítulo 1

### **Functional Feeding Group composition and attributes: evaluation of freshwater ecosystems in the Atlantic Forest, Brazil**

***Priscilla da Silva Pereira<sup>1\*</sup>, Natalia Freitas de Souza<sup>1,4</sup>, Darcílio Fernandes Baptista<sup>1</sup>, Marcelo Ribeiro-Alves<sup>3</sup>, Helena Lucia Carneiro Santos<sup>2</sup> & Daniel Forsin Buss<sup>1</sup>***

*<sup>1</sup>Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Avaliação e Promoção da Saúde Ambiental, Rio de Janeiro, RJ, Brasil*

*<sup>2</sup>Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Estudos Integrados em Protozoologia, Rio de Janeiro, RJ, Brasil*

*<sup>3</sup>Fundação Oswaldo Cruz, Instituto Nacional de Infectologia Evandro Chagas, Laboratório de Pesquisa Clínica em DST / AIDS, Rio de Janeiro, RJ, Brasil*

*<sup>4</sup>Universidade Federal do Rio de Janeiro, Departamento de Ecologia, Rio de Janeiro, RJ, Brasil*

**Aceito no periódico Biota Neotropica**

## **Functional Feeding Group composition and attributes: evaluation of freshwater ecosystems in the Atlantic Forest, Brazil**

**Abstract:** Benthic macroinvertebrates Functional Feeding Groups (FFG) have been used to determine aquatic assemblage dynamics and as a biomonitoring tool. The main goals of this study were to assess the effects of stream variables on the abundance and richness of FFGs and evaluate ecosystem attributes (FFG ratios) as a tool to assess ecological conditions of Atlantic Rainforest streams. We sampled 146 sites with different impairment conditions in Rio de Janeiro state, Brazil. Richness was significantly different among impairment conditions for all FFGs. Mixed-effect models show that aquatic macroinvertebrate FFGs differed in their responses to abiotic variables for abundance and richness. Also, they were reduced in the impaired sites when compared to intermediate and reference sites. The FFG ratio indicated significant differences. The FFG ratio analysis was shown to be a fast and cheap tool that can be used for monitoring aquatic ecosystems in the Atlantic Forest biome. However, further studies are required to calibrate the method specifically for the Atlantic Forest region.

**Keywords:** Ecosystem attributes, impairment, macroinvertebrates, streams

## **Composição e atributos de Grupos Funcionais de Alimentação: avaliação de ecossistemas de água doce na Mata Atlântica, Brasil**

**Resumo:** Os Grupos Funcionais de Alimentação (GFA) são utilizados para determinar a dinâmica da comunidade de macroinvertebrados bentônicos e como uma ferramenta de biomonitoramento. Os principais objetivos deste estudo foram: avaliar os efeitos de variáveis de riacho na abundância e riqueza de GFAs e os atributos do ecossistema (razão GFA) como uma ferramenta para avaliar as condições ecológicas dos córregos da Mata Atlântica. Foram amostrados 146 locais com diferentes condições de impacto no Estado do Rio de Janeiro, Brasil. A riqueza foi significativamente diferente com as condições de impacto entre todos os GFA. Os modelos de efeito misto mostraram que os GFA diferiam em suas respostas às variáveis abióticas quanto à abundância e à riqueza. Além disso, eles diferem nas áreas impactadas quando comparados com as áreas intermediária e de referência. A razão de GFA apresentou diferenças significativas ao longo do gradiente de impacto. A análise da razão de GFA evidenciou-se uma ferramenta rápida e barata, com potencial para ser utilizada no monitoramento de ecossistemas aquáticos no bioma Mata Atlântica. No entanto, mais estudos serão necessários para calibrar o método especificamente para a região da Mata Atlântica.

**Palavras-chave:** Água doce, atributos do ecossistema, impacto, macroinvertebrados

## Introduction

Streams and rivers exhibit a high biological diversity and provide critical ecological functions and services. However, they are among the most threatened ecosystems due to anthropogenic activities, such as human settlements, industrial pollution, and agriculture, which have led to increased habitat loss, higher pollution levels, invasions of exotic species, and the changing climate (Allan & Castilho 2007, Ceneviva-Bastos et al. 2017). Climate change tends to exacerbate anthropogenic stress due to increased water temperature and, salinity, and changes in hydrological cycles, which results in shifting rainfall patterns and flow fluctuations (Durance 2007).

Biomonitoring has long been recognized as a tool to screen environmental health changes taking place in the environment (Barbour et al. 1999). Benthic macroinvertebrates are among the most used organisms to assess ecological conditions. Macroinvertebrates are a primary food source for fishes and other organisms (Rosenberg & Resh 1993). They are abundant in most streams, even small ones, have species at different trophic levels, with a wide range of pollution tolerance, and sampling is relatively easy at a low cost (Barbour et al. 1999, Bonada et al. 2006, Henriques-Oliveira & Nessimian 2010, Gieswein et al. 2019).

Two main approaches have been used in biomonitoring programs to assess freshwater macroinvertebrates: one uses richness and diversity indices. and the other uses functional attributes based on morphology and feeding behavior (Cummins 1973, Cummins & Klug 1979, Merritt et al. 1999, Merritt et al. 2002, Cummins 2018). According to Dedieu et al. (2015), biological traits of freshwater organisms, such as feeding behavior, are useful tools for detecting change along gradients of anthropogenic disturbance. In freshwater ecology, macroinvertebrates Functional Feeding Groups (FFGs) have been used to conceptualize community dynamics and assessing ecological status (Vannote et al. 1980). The attributes related to the structure and function give indicators of aquatic ecosystem conditions (Hawkins & Sedell 1981, Ceneviva-Bastos et al. 2017, Fugère et al. 2018). According to Vannote et al. (1980), trophic interactions can affect ecological processes by directly influencing flows of the distribution of energy and resources within the assemblage. Thus, functional analysis focuses on the type of food and food acquisition. FFGs are defined by the way organisms feed: (1) gathering collectors – feed on small organic matter particles deposited in the river bed; (2) filtering collectors – capture, by filtration, small organic matter particles suspended in the water column; (3) scrapers – scrape hard surfaces

and feed on algae, bacteria, fungi, and dead organic matter adsorbed on substrates, (4) predators – swallow whole prey or body tissue fluids and (5) shredders – chew leaves or tissue from living vascular plant or dead wood and debris (Cummins 1973).

According to Merritt et al. (1996), the use of FFG ratios can estimate attributes related to the stream ecosystem. The FFG ratio serves as a surrogate for stream ecosystem attributes. These attributes include a trophic state (autotrophy/heterotrophy), the linkage between to functioning the riparian vegetation and the shredder, relative amounts of coarse and organic particles (transported and stored in the environment), the stability of the habitat, and ascendant control for predators to be driven by prey availability. The FFG ratio is a rapid and integrating technique used to establish a protocol for characterizing ecological condition. This approach has been used to assess river conditions in Brazil. Cummins et al. (2005) used FFG ratios to evaluate sites ecological conditions in Southern Brazil, and Couceiro et al. (2011) assessed streams located in Brazil's Amazon forest. Multimetric and predictive indices for larger-scale protocols also used FFG components in South America (Baptista et al. 2007, Buss et al. 2015, Macedo et al. 2016, Oliveira et al. 2019, Souza et al. 2019).

This study's main goals were to evaluate all FFG categories and the effects of abiotic variables on abundance, richness, and FFG ratios to assess the ecological conditions of Atlantic Forest streams. In this context, this study used FFG and their ratios to assess the ecological conditions of Atlantic Forest streams.

## **Material and Methods**

### *1. Study area*

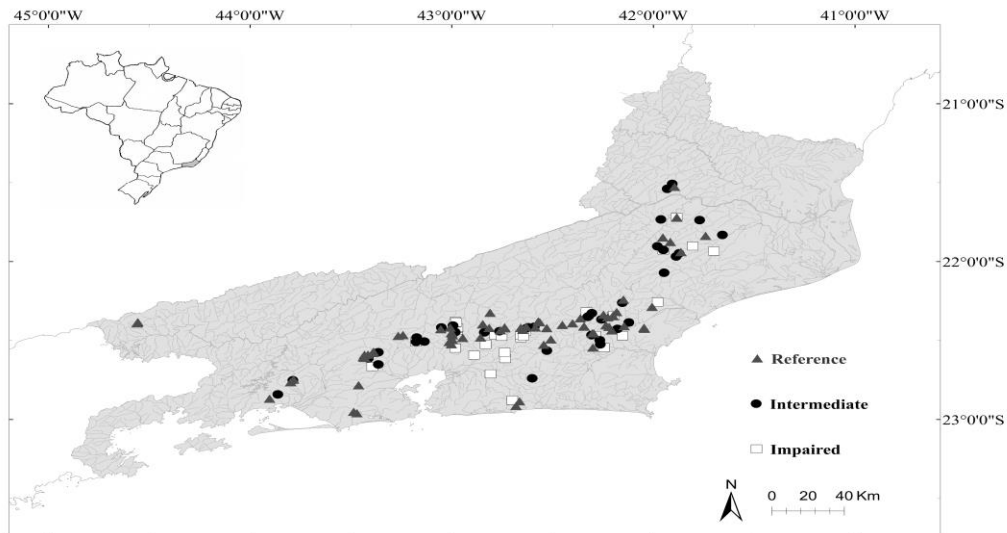
The Atlantic Forest region in Rio de Janeiro state is classified as the tropical with a rainy summer season, with the most mountainous areas classified as humid subtropical, with a hot summer and without a dry season or a dry winter (Alvares et al. 2013). Temperatures oscillate between 15°C and 28°C, and annual rainfall is around 1,000–1,500 mm.

Rio de Janeiro state is composed of a group of coastal plains separated by hills and two mountain chains that run parallel to the ocean (Serra do Mar, ranging from altitudes 0–2000 m a.s.l. and Serra da Mantiqueira, ranging from 800 to 2500 m a.s.l.). The coastal plains are located at the piedmont of Serra do Mar mountain range, with altitudes of about 200 m a.s.l.. It is a depositional zone formed by marine, lacustrine, and fluvial sedimentation processes (Brasil 1983). This region is affected by high impact

by urban areas or agriculture and livestock grazing, making minimally impacted areas (reference) scarce. The mountain chains are located at higher altitudes (from >200 m a.s.l. to around 1,800 m a.s.l.), with high slope and steep scarps. Most sites were sampled within or near protected areas (conservation units), which had low to moderate impact on agricultural activities. For this reason, this region presents the most extensive riparian vegetation and forest fragments.

The Neotropical Atlantic Forest is one of the biodiversity hotspots worldwide. However, the biome has lost 88% of its original extent, and remnants are mostly spread throughout the higher parts of mountains, interspersed with agriculture and pasture (Ribeiro et al. 2011).

We selected sites based on the ad hoc indication and previous knowledge of the area to represent sites classified, *a posteriori*, as a reference, intermediate, or impaired. Sites classified as "reference" should meet all the following criteria: "optimal" or "good" environmental conditions according to the Habitat Assessment Protocol (HAP) (Barbour et al. 1999 – see rationale below); dissolved oxygen concentration  $\geq 6$  mg/l, pH between 6 and 8, absence of channelization, and <40% of the upstream area affected by urban areas. For a site to be classified as "impaired," the following criteria should be met: "poor" classification according to the HAP; dissolved oxygen <6 mg/l. Intermediate sites had characteristics between these two classes. We sampled 146 sites of the Atlantic Forest region in Rio de Janeiro state (74 references, 38 intermediates, and 34 impaireds). Sampled sites ranged from 1st to 5th order according to Strahler classification (Figure 1). The sampling campaigns were carried out between 2010 and 2016 (during the dry season) using the same protocol.



**Figure 1.** Map of the study area, showing the sampling sites in Rio de Janeiro state, Brazil, and indicating the gradient of impairment (reference, intermediate and impaired).

## 2. Sample site evaluation

In each sampling site, the following physicochemical variables were recorded in the field: dissolved oxygen (DO; YSI 550A equipment), pH (LabConte MPA 210p), and Conductivity (Cond; using a LabConte MCA 150p). Water samples were preserved in sterile plastic bags (whirl-pak), according to APHA (2000). In the laboratory, the concentration of Ammonia ( $\text{NH}_3$ ) was determined using a HACH (DR 2500). Chloride ( $\text{Cl}^-$ ), total alkalinity (TA), total hardness (TH), and calcium (Ca) were determined by the titrimetric method following APHA (2000). Sampling sites were also classified in the field according to the HAP (Barbour et al. 1999). The HAP has ten environmental parameters, such as substrate availability for colonization by benthic fauna, water velocity, embeddedness (pool variability for low-gradient streams), channel condition (sinuosity for low-gradient streams), sediment deposition, margin stability, and riparian vegetation. For each variable, a score between 0 and 20 was assigned. Sites were classified conforming to the mean score obtained, as follows: 0–5 "Poor," 5.1–9.9 "Regular," 10–14.9 "Good," and 15–20 "Excellent" environmental condition (Barbour et al. 1999).

## 3. Biological samples

Macroinvertebrates were sampled by using a kick-net with a mesh size of 500  $\mu\text{m}$ . For this, 20 samples (20  $\text{m}^2$ ) were taken proportionally to the substrates available at each site, according to the multi-habitat method (Barbour et al. 1999). Samples were



conserved in the field in 80% ethanol and taken to the laboratory. In the lab, samples were washed to remove coarse organic matter, such as leaves and twigs. The remaining material was deposited into a sampler (64×36 cm), divided into 24 quadrants, each measuring 10.5×8.5 cm (Fiocruz, Patent application number PCT/BR2011/000144). This method is used to assure the randomness of biological assessments, as it is less subject to the variability of team members (Oliveira et al. 2011).

#### 4. Functional Feeding Group classification and ratios

Fauna and FFGs were attributed to each taxon based on keys from regional entomofauna studies (Nessimian 1997, Baptista et al. 2006, Henriques-Oliveira & Nessimian 2010, Fernandes 2015) in Neotropical studies (Velásques & Miserendino 2003, Tomanova et al. 2006, Brasil et al. 2014) and the USA reference (Merritt & Cummins 1996). Also, five FFG ratios, adapted from Merritt et al. (1996), were used (Table 1). The autotrophy and heterotrophy index (A/H) relates primary productivity to total community respiration. The A/H serves to indicate whether a stream is autotrophic (autochthonous organic matter derived algae or rooted vascular aquatic plants) or heterotrophic (allochthonous organic matter resultant from the riparian zone). The ratio between coarse particulate organic matter and fine particulate organic matter (CPOM/FPOM) provides insights into the quality of the riparian zone cover and the availability of litter used by shredders. The ratio of transported fine particulate organic matter and benthic fine particulate organic matter (TFPOM/BFPOM) measures the availability of relative amounts of fine coarse and organic particles (transported and stored in the environment). The habitat stability index (HSI) indicates the abundance of bottom substrates for the colonization of macroinvertebrates such as stones, wood, and aquatic plants. The predator-prey index (Predator) reflects top-down control by predators.

**Table 1.** Functional Feeding Group (FFG) ratios modified from Merritt & Cummins (1996).

Ecosystem attributes	Symbols	FFG ratios	Criteria ratio levels
Autotrophy/Heterotrophy index	A/H	Scraper/ (shredder + total collector)	Autotrophic >0.75
Shredder index	CPOM/FPOM	Shredder/total collector	Shredder availability >0.25
Filtering-collector index	TFPOM/BFPOM	Filtering collector/gathering collector	TFPOM higher than normal >0.50
Habitat Stability index	HIS	(Scraper + filtering collector)/ (shredder + gathering collector)	Stable substrates >0.50
Predator-prey index	Predator	Predator/ (total collector + scraper + shredder)	Predator to prey balance 0.10–0.20

## 5. Data analysis

Abundance and richness of the FFG in each sampled site were calculated to characterize the differences in community trophic structure along the gradient of impairment. Taxa that could be assigned to more than one FFG were equally divided among the possible groups (Mendes et al. 2017). Differences among these groups were estimated by contrasts of the expected mean marginal values obtained from multivariate mixed linear models fitted using the maximum likelihood estimator.

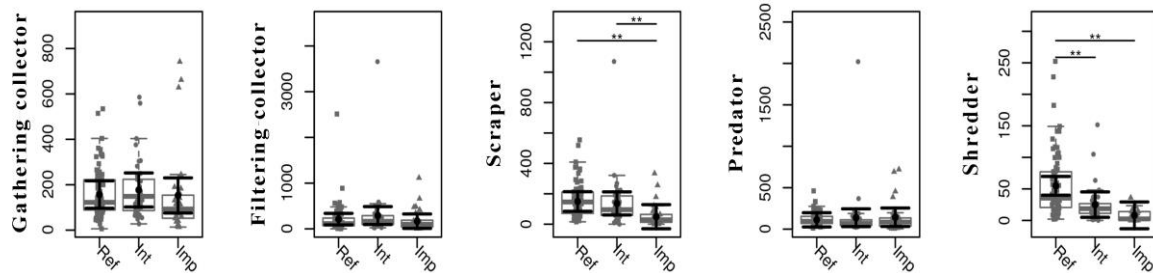
The fixed/systematic component of models included the impairment gradient, while the random component included the river basin of each sampled stream. Also, to eliminate the dependence among the closest sampled stream, a Gaussian spatial correlation structure was considered. For the adjusted models, a graphical analysis of residuals was performed to confirm their randomness. In analyses of model marginal mean estimates contrasts, adjustments of the confidence level were made by Sidak's method, and p-value adjustments were made by multiple comparisons using Tukey's method. Stepwise searches based on the minimization of the Akaike Information Criterion (AIC), in both forward and backward directions, were used to select the optimal, non-redundant, mixed-effect model (similar to the one described above) of abiotic variables (i.e., Stream Width, Altitude, DO, pH, Cond, NH<sub>3</sub>, Cl<sup>-</sup>, TH, TA, Ca and, HAP) on the abundance and richness of each FFG. The level of significance, alpha = 0.05, was used in the analyses. Analyses were performed in R software version 3.6.1 (R Core Team, 2018) with functionalities augmented by the packages 'emmeans' (Russell & Lenth 2020), used in the obtainment of estimated marginal means of the fitted mixed models, and 'nlme' (Pinheiro et al. 2020), used in the fitting of those models.

## Results

A total of 108,282 aquatic benthic macroinvertebrates distributed in 176 taxa were collected during the study (Supplementary Material: Appendix 1). In general, contrasts after the multivariate mixed linear model estimated marginal means showed significant differences along with the impairment gradient sites (a, reference - intermediate; b, reference - impaired; and, c, intermediate - impaired).

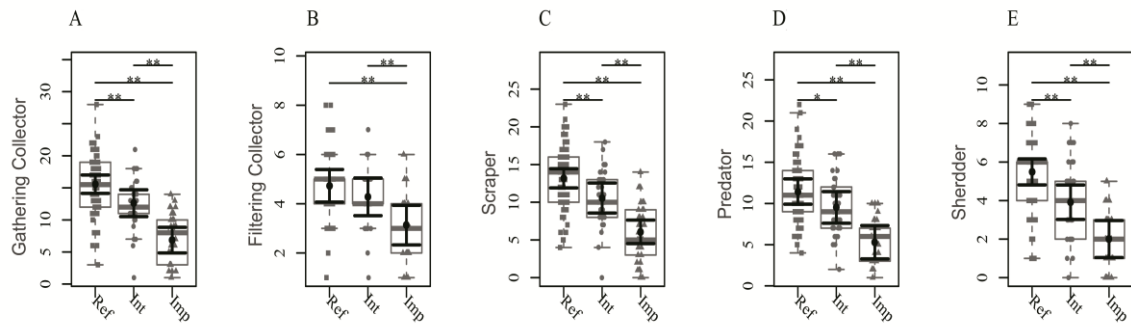
Filtering collector was the most abundant FFG regardless of impairment classes, and *Simuliidae* was the dominant taxa along the impairment gradient (reference,

intermediate, and impaired sites). Figure 2 shows that estimated marginal mean abundance differed along the impairment gradient for scrapers ( $b = 99.97$ ,  $p < 0.000001$ ; and,  $c = 87.35$ ,  $p = 0.006$ ) and shredders ( $a = 29.83$ ,  $p = 0.0005$ ; and,  $b = 46.86$ ,  $p < 0.000001$ ). Gathering and filtering collectors, and predators had their highest mean values at intermediate sites, while scrapers and shredders had higher mean values at reference sites.



**Figure 2.** Distributions of samples and estimates for FFG abundance along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Box-and-whisker and strip plots (gray) representing samples distribution of FFG abundance. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.

A significant decrease for marginal mean estimates of all FFG richness was observed along the impairment gradient (Figure 3). All FFGs showed differences along the impairment gradient: gathering collector ( $a = 2.97$ ,  $p = 0.001$ ;  $b = 8.72$ ,  $p < 0.000001$ ; and,  $c = 5.76$ ,  $p < 0.000001$ ); filtering collector ( $b = 1.60$ ,  $p < 0.000001$ ; and,  $c = 1.15$ ,  $p = 0.0001$ ); scraper ( $a = 2.62$ ,  $p = 0.002$ ;  $b = 7.10$ ,  $p < 0.000001$ ; and,  $c = 4.49$ ,  $p < 0.000001$ ); predator ( $a = 1.93$ ,  $p = 0.01$ ;  $b = 6.15$ ,  $p < 0.000001$ ; and,  $c = 4.22$ ,  $p < 0.000001$ ); and shredders ( $a = 1.57$ ,  $p < 0.000001$ ;  $b = 3.49$ ,  $p < 0.00001$ ; and,  $c = 1.91$ ,  $p < 0.000001$ ). Similarly, mean richness numbers of all FFGs decreased along the impairment gradient.



**Figure 3.** Distributions of samples and estimatives for FFG richness along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Box-and-whisker and strip plots (gray) representing samples distribution of FFG richness. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.

As expected, abiotic variables, i.e., DO, pH, Cond, TH, TA, Ca, and HAP, were significantly different along the impairment gradient (Supplementary Material: Appendix 2). Stepwise searches showed the most relevant among these abiotic variables related to the abundance and richness variance of aquatic macroinvertebrate FFG (Table 2). Overall, the coefficients of determination ( $R^2$ ) of the optimal models selected were low for the abundance of macroinvertebrate FFG, ranging from 0.14 to 0.34 ( $p < 0.001$  for all), suggesting lower importance than anticipated of these abiotic variables on the FFG abundance variation among sites.

Estimated marginal means based on regression analyses (mixed effect models) showed that the altitude was a significant abiotic variable for all FFG abundance (except for filtering collectors). Nonetheless, for the filtering collector and the predator groups, we observed a positive linear correlation between abundance along the impairment gradient (e.g.,  $\text{NH}_3$  and Calcium).

**Table 2.** Stepwise searches based on the Akaike information criterion (AIC) minimization, in both forward and backward directions, of abiotic variables on the abundance of macroinvertebrate Functional Feeding Groups (FFG).

FFG	Variables	B	Std.Error	DF	t.value	p.value
<b>Gathering-collector</b> AIC= 1828.64; R <sup>2</sup> = 0.17; L. Ratio= 22.99; p<0.001	(Intercept)	372.957	90.637	134	4.115	<0.001
	Width (m)	2.225	1.145	134	1.944	0.054
	Altitude (m)	0.117	0.027	134	4.300	<0.001
	Ph	-31.791	12.112	134	-2.625	0.010
	Log10_Cl <sup>-</sup> (mg/L)	-190.588	76.806	134	-2.481	0.014
<b>Filtering-collector</b> AIC= 2109.13; R <sup>2</sup> = 0.24; L. Ratio= 63.75; p<0.001	(Intercept)	-280.649	169.998	131	-1.651	0.101
	Width (m)	8.631	2.217	131	3.894	<0.001
	Log10_NH <sub>3</sub> (mg/L)	1945.715	370.471	131	5.252	<0.001
	DO (mg/L)	44.067	13.699	131	3.217	0.002
	Log10_Ca (mg/L)	1276.571	269.336	131	4.740	<0.001
	Log10_TA	-287.848	198.065	131	-1.453	0.149
	HAP	-14.916	2.813	131	-5.302	<0.001
Log10_Cond (S/cm)	468.123	127.368	131	3.675	<0.001	
<b>Scraper</b> AIC= 1801.01; R <sup>2</sup> = 0.14; L. Ratio= 52.07; p<0.001	(Intercept)	286.510	79.610	131	3.599	<0.001
	Width (m)	1.671	0.698	131	2.394	0.018
	Altitude (m)	0.092	0.033	131	2.766	0.006
	DO (mg/L)	-13.712	4.358	131	-3.146	0.002
	pH	-24.231	9.417	131	-2.573	0.011
	Log10_Cl <sup>-</sup>	-117.153	51.677	131	-2.267	0.025
	HAP	3.897	1.091	131	3.571	<0.001
	Log10_Cond (S/cm)	91.922	51.444	131	1.787	0.076
<b>Predator</b> AIC= 1929.17; R <sup>2</sup> = 0.21; L. Ratio= 33.52; p<0.001	(Intercept)	328.199	122.679	133	2.675	0.008
	Altitude (m)	0.087	0.044	133	1.978	0.050
	Log10_NH <sub>3</sub> (mg/L)	712.903	360.219	133	1.979	0.050
	pH	-34.548	16.472	133	-2.097	0.038
	Log10_Ca (mg/L)	479.868	121.618	133	3.946	<0.001
	Log10_Cond (S/cm)	-142.137	69.245	133	-2.053	0.042
<b>Shredder</b> AIC= 1466.86; R <sup>2</sup> = 0.34; L. Ratio= 69.46; p<0.001	(Intercept)	-59.243	17.263	132	-3.432	0.001
	Altitude (m)	0.030	0.009	132	3.504	0.001
	Log10_NH <sub>3</sub> (mg/L)	-132.232	67.989	132	-1.945	0.054
	DO (mg/L)	3.375	1.461	132	2.311	0.022
	Log10_Ca (mg/L)	-46.292	29.530	132	-1.568	0.119
	Log10_TA (mg/L)	60.436	17.327	132	3.488	0.001
	HAP	2.898	0.609	132	4.758	<0.001

AIC- Akaike Information Criterion, R<sup>2</sup>- Coefficient of Determination, L.Ratio- Likelihood Ratio, p-value- p-value after Likelihood Ratio Test, DF - Degrees of Freedom. Log10\_NH<sub>3</sub> - Ammonia; Log10\_TH- Total Hardness, Log10\_Ch- Chloride, HAP- Habitat Assessment Protocol; DO- Dissolved Oxygen and Log10\_TA- Total Alkalinity and Log10\_Ca- Calcium.

Different results were found for abiotic variables and richness of FFGs (Table 3). Optimal models selected for the richness of FFG the R<sup>2</sup> were moderate, ranging from 0.36 to 0.52 (p<0.001 for all), suggesting higher importance of abiotic variables on the variation of FFG richness than for abundance. Reductions were correlated to the increase of NH<sub>3</sub> for all FFG. Calcium (Ca) was also negatively correlated with richness among predators and shredders. Better tendencies were also observed for Total Hardness (TH) for gathering collectors, filtering collectors, and scrapers, and for Chloride (Ch) for gathering collectors, filtering collectors, scrapers, and predators. As expected, these reductions in the richness of FFG were significant between intermediate and impaired sites, and the reference sites (Figure 3).

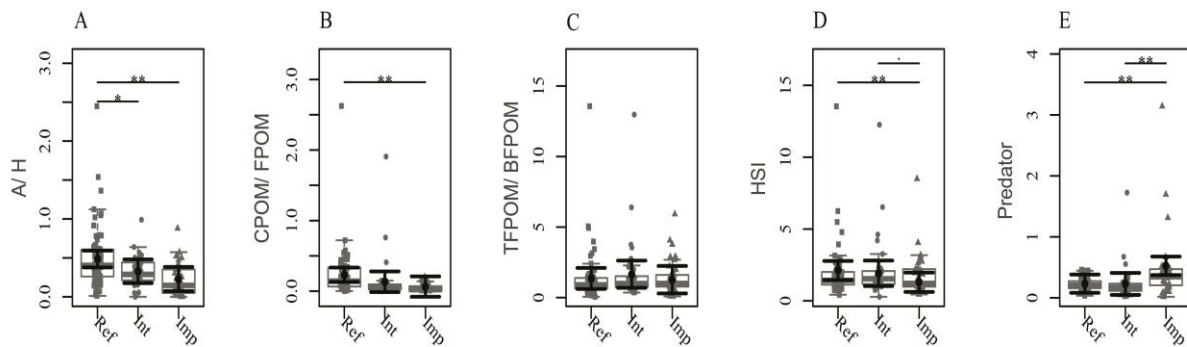
**Table 3.** Stepwise searches based on the Akaike information criterion (AIC) minimization, in both forward and backward directions, of abiotic variables on the richness of macroinvertebrate Functional Feeding Groups (FFG).

FFG	Variables	B	Std.Error	DF	t.value	p.value
<b>Gathering-collector</b> AIC= 804.63; R <sup>2</sup> = 0.52; L. Ratio= 111; p<0.001	(Intercept)	9.743	1.078	133	9.034	<0.001
	Altitude (m)	0.002	0.001	133	2.325	0.022
	log10_NH <sub>3</sub> (mg/L)	-22.917	7.174	133	-3.194	0.002
	log10_TH (mg/L)	-4.755	0.991	133	-4.797	<0.001
	log10_Cl <sup>-</sup> (mg/L)	-4.530	2.188	133	-2.070	0.040
<b>Filtering-collector</b> AIC= 440.02; R <sup>2</sup> = 0.36; L. Ratio= 81.26; p<0.001	(Intercept)	3.998	0.375	133	10.657	<0.001
	Width (m)	-0.013	0.004	133	-3.263	0.001
	log10_NH <sub>3</sub> (mg/L)	-8.742	2.004	133	-4.362	<0.001
	log10_TH (mg/L)	-0.627	0.397	133	-1.579	0.117
	log10_Cl <sup>-</sup> (mg/L)	-1.409	0.646	133	-2.180	0.031
<b>Scraper</b> AIC= 788.85; R <sup>2</sup> = 0.52; L. Ratio= 107.12; p<0.001	(Intercept)	7.896	1.625	132	4.861	<0.001
	log10_NH <sub>3</sub> (mg/L)	-30.211	6.164	132	-4.901	<0.001
	DO (mg/L)	-0.232	0.125	132	-1.860	0.065
	log10_TH (mg/L)	-6.407	1.490	132	-4.300	<0.001
	log10_Cl <sup>-</sup> (mg/L)	-6.062	2.112	132	-2.870	0.005
	log10_TA (mg/L)	4.935	1.897	132	2.602	0.010
<b>Predator</b> AIC= 772.52; R <sup>2</sup> = 0.42; L. Ratio= 75.08; p<0.001	(Intercept)	7.116	0.939	133	7.578	<0.001
	Altitude (m)	0.003	0.001	133	3.366	0.001
	log10_NH <sub>3</sub> (mg/L)	-12.633	6.259	133	-2.018	0.046
	log10_Ca (mg/L)	-7.705	1.705	133	-4.520	<0.001
	log10_Cl <sup>-</sup> (mg/L)	-4.168	2.128	133	-1.959	0.052
<b>Shredder</b> AIC= 557.39; R <sup>2</sup> = 0.51; L. Ratio=104.05; p<0.001	(Intercept)	1.876	0.391	134	4.803	<0.001
	Altitude (m)	0.001	0.000	134	2.188	0.030
	log10_NH <sub>3</sub> (mg/L)	-14.241	3.124	134	-4.558	<0.001
	log10_Ca (mg/L)	-2.305	1.397	134	-1.649	0.101
	HAP	0.216	0.025	134	8.739	<0.001

AIC- Akaike Information Criterion, R<sup>2</sup>- Coefficient of Determination, L.Ratio- Likelihood Ratio, p-value- p-value after Likelihood Ratio Test, , DF – Degrees of Freedom. Log10\_NH<sub>3</sub> - Ammonia; Log10\_TH- Total Hardness, Log10\_Ch- Chloride, HAP- Habitat Assessment Protocol, DO- Dissolved Oxygen, Log10\_TA- Total Alkalinity and Log10\_Ca- Calcium.

In general, estimated marginal means for FFG ratios showed significant differences among the impairment gradient sites. Significant differences along the impairment gradient were found for the autotrophic/heterotrophic index (A/H) (Figure 4A; a = 0.16, p=0.02; b = 0.26, p<0.001). Regardless of the position along the impairment gradient, most sites were below the A/H level of 0.75 being Heterotrophic (91.1%), indicating the dependence of the stream food web on the availability of allochthonous riparian organic matter. A/H numbers for reference, intermediate and impaired sites were 0.49, 0.33, and 0.23, respectively. Coarse Particulate Organic Matter/Fine Particulate Organic Matter index (CPOM/FPOM) is an indicator of the availability of food resources for shredders. For this index (Figure 4B), we found a significant difference along the impairment gradient (b = 0.17, p=0.006). Reference sites had a mean CPOM/FPOM ration of 0.23, close to the ratio level cut (> 0.25). Intermediate sites showed lower shredder interaction with the riparian vegetation (mean = 0.13), and, as expected, impaired sites had shredders very underrepresented (mean = 0.06). For the Transport of Fine Particles Organic Matter/Benthic Fine Particles Organic Matter index (TFPOM/BFPOM) no differences were found along the impairment gradient (Figure 4C). TFPOM/BFPOM indexes were of good quality independently of

the impairment gradient (according to the criteria ratio level > 0.50), with estimated mean marginal values of 1.36, 1.66, and 1.27, for reference, intermediate, and impaired sites, respectively. For the Habitat Stability index (HSI) (Figure 4D), a significant difference along the impairment gradient was found ( $b = 0.82$ ,  $p < 0.001$ ), with estimated mean values of 2.11 and 1.92 for reference, and intermediate sites, respectively, which indicates an abundance of stable substrates. The estimated marginal mean value was 1.29 for impaired sites above the ratio level cut (> 0.50) for a stable substrate. Finally, for the predator-prey index (Predator) (Figure 4E) significant differences were found along the impairment gradient ( $b = -0.29$ ,  $p < 0.000001$ ;  $c = -0.30$ ,  $p < 0.000001$ ). Impaired sites showed the highest estimated marginal mean value, 0.52, compared to the reference (0.23) and intermediate sites (0.23). Most ratios were within the range of criteria levels for reference and intermediate sites and an overabundance of predators at impaired sites.



**Figure 4.** Distributions of samples and estimatives for FFG ratios along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Box-and-whisker and strip plots (gray) representing samples distribution of A/H (Autotrophy and Heterotrophy index), CPOM/FPOM (Coarse Particulate Organic Matter and Fine Particulate Organic Matter index), TFPOB/BFPOB (Transport of Fine Particles Organic Matter, and Benthic Fine Particles Organic Matter index), HSI (Habitat Stability index), and Predator (Predator-prey index) along the impairment gradient. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.

## Discussion

Anthropogenic activities may impact stream ecosystems by causing habitat fragmentation, degradation, sedimentation, which consequently increase the abundance of tolerant species and decrease that of sensitive species (Mangadze et al. 2019). In our study, the abundance of gathering and filtering collectors and predators were higher at intermediate sites. In contrast, abundance and richness of scrapers and shredders were negatively correlated with the impairment gradient. Gathering collectors are often recognized as generalists and are considered dominant along the impairment gradient (Leslie & Lamp 2017). They can also transform the decomposition of Fine Particulate Organic Matter (FPOM) and carbon availability within the sediment (Leslie & Lamp 2017). In our study, filtering collectors were the most abundant FFG along the impairment gradient, due to large numbers of Simuliidae in urban areas. These organisms are reasonably resistant to disturbance (Feld et al. 2002). Furthermore, significant differences were found for scraper abundance between reference, intermediate, and impaired sites. According to Jun et al. (2011), scrapers are sensitive to impairment because they mainly consume algae that grow attached to cobbles and pebbles of undisturbed streams. As for predators, we found lower mean abundance at impaired sites than at intermediate sites. Predator abundance appears to be driven mainly by prey availability and studies have found an increased abundance in streams affected by human activities (Rawer-Jost et al. 2000). As expected, the abundance of shredders was reduced along the stream impairment gradient because of reduced quantities of riparian vegetation. Similar results were found by Oliveira & Nessimian (2010), who reported lower relative participation of this FFG on impaired sites. Although shredder abundance was very low, we found significant differences between reference and impaired sites.

Richness numbers displayed the best response and highest sensitivity to detect impairment. Richness numbers of all FFG were significantly reduced along the impairment gradient stream. Richness can have an essential function in characterizing stream ecosystem condition (Kaboré et al. 2016, Couceiro et al. 2011, Drover et al. 2020).

Estimated marginal means based on regression analyses (mixed-effect models) showed that the altitude was a significant abiotic variable for all FFG abundance, except for the filtering collectors. According to Jacobsen (2008), one possible explanation is that at higher altitudes, streams tend to have lower temperatures and higher slopes,



contributing to higher concentrations of DO. Tomanova et al. (2007) showed that altitude combined with position along the longitudinal gradient is an important factor controlling FFG assemblages of stream macroinvertebrates in neotropical streams. In this study, the richness of all FFGs was reduced along the impairment gradient. These reductions were correlated to the increase of  $\text{NH}_3$  for all FFG. According to Camargo (2019), physicochemical alterations, such as the increase of ammonia ( $\text{NH}_3$ ), can be toxic to sensitive macroinvertebrate taxa. Also, it is well known that large-scale agriculture and urbanization may decrease water quality leading to alterations as the loss of riparian vegetation with a significant effect on the FFG structure and function (Gieswein et al. 2019). Streams in this region are subject to different pressures, including intensive urbanization and untreated sewage discharges. Most sites suffered the influence of multiple chemical and physical anthropogenic stressors. Agriculture and urban land-use practices reduce water quality due to inputs of fine sediments, nutrients, and pesticides. Alterations to the river channel's physical structure may cause a loss in riparian vegetation, which would be expected to produce a significant effect on FFG structure (Fu et al. 2016).

FFG ratios showed a variable response along the impairment gradient. The Autotrophic/Heterotrophic index (A/H) serves as a surrogate of Production/Respiration (P/R), which was significantly different along the impairment gradient. P/R has been used as the relative importance of energy fixed by primary producers (Vannote et al. 1980), i.e., P/R ratios among ecosystems are proxies of autochthonous/allochthonous organic matter ratio. According to this criterium, even though reference sites had higher estimated marginal mean values, almost sites in our study were classified as heterotrophic, independent of their position along the impairment gradient. These results follow Cummins et al. (2005), who also found that all sampled sites on their study of the Atlantic Forest stream of Southern Brazil could be characterized as heterotrophic. Other tropical/subtropical streams in Kenya also classified close-canopy streams as heterotrophic (Masese et al. 2014). According to the Coarse Particulate Organic Matter/Fine Particulate Organic Matter index (CPOM/FPOM), a shallow shredder interaction with riparian vegetation was found in impaired sites. According to Cummins et al. (1989), this decline is most probably related to the removal of riparian vegetation from agricultural and urban areas. We also found a TFPOM/BFPOM index unresponsive to the impairment gradient, which agrees with Couceiro et al. (2011). Moreover, the Habitat Stability index (HSI) indicated stable substrates that were more abundant in intermediate sites. One explanation for these results would be the

intermediate disturbance hypothesis (Ward & Stanford 1983, Ward et al. 1999). Intermediate sites were submitted to constant sewage discharges. It seems to generate moderate mortality in the species not in such numbers that a recovery is impossible, but at the same time, sufficient to limit the growth of competitive species. For the Predator index, it was observed a low top-down control in reference and intermediate sites. Almost all FFG ratios showed significant differences along the impairment gradient. This observation does not agree with Kaboré et al. (2016), which found inconsistent results in different land use in West Africa.

FFG ratios as a surrogate of the ecosystem attributes may reduce the time and costs of the evaluation being fast, cheaper, and an integrated tool based on morphological and behavioural mechanisms of food acquisition. Moreover, this study evaluated marginal mean estimates for FFG ratio, abundance, and mostly for richness, as a useful tool to assess the ecological conditions of Atlantic Forest streams. Despite the almost FFG ratio being able to discriminate along the impairment gradient, further studies would be necessary to calibrate the method specifically for the Atlantic Forest region.

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**Appendix 1. Functional Feeding Groups (FFG) for Atlantic Forest taxa**

Order	Family	Genera	FFG
Acarina	Hydracarina	nd	Predator
Amphipoda	Hyalellidae	<i>Hyalella</i>	Gathering-collector
Annelida (Filo)	Hirudinea	nd	Predator
Annelida (Filo)	Oligochaeta	nd	Gathering-collector
Blattodea	Blattidae	<i>Blattaria</i>	Gathering-collector
Coleoptera	Curculionidae	nd	Shredder
	Dryopidae	nd	Shredder
	Dytiscidae	nd	Predator
	Elmidae	<i>Heterelmis</i>	Gathering-collector – Scraper – Shredder
		<i>Hexacylloepus</i>	Gathering-collector – Scraper
		<i>Hexanchorus</i>	Gathering-collector – Scraper
		<i>Macrelmis</i>	Gathering-collector – Scraper
		<i>Microcyllloepus</i>	Gathering-collector – Scraper
		<i>Neoelmis</i>	Gathering-collector – Scraper
		<i>Phanocerus</i>	Scraper
		<i>Promoresia</i>	Gathering-collector
		<i>Xenelmis</i>	Gathering-collector – Scraper
	Gyrinidae	<i>Gyrinus</i>	Predator
	Hydraenidae	nd	Predator
	Hydrophilidae	<i>Hydroblus</i>	Predator
	Lutrochidae	Nd	Gathering-collector – Scraper
	Noteridae	Nd	Predator
	Psephenidae	Nd	Scraper
	Ptilidae	Nd	Gathering-collector
	Ptilodactylidae	Nd	Shredder
	Scirtidae	<i>Elodes</i>	Gathering-collector
	Staphilinidae	Nd	Predator
	Torridicondolidae	Nd	Scraper
Collembola	nd	Nd	Gathering-collector
Decapoda	Atyidae	<i>A. scabra</i>	Shredder
	Palaemonidae	<i>P. brasiliانا</i>	Shredder
		<i>P. pandaliformis</i>	Shredder
		<i>M. acanthurus</i>	Shredder
		<i>M. iheringi</i>	Shredder
		<i>M. potiuna</i>	Shredder
		<i>M. olfersii</i>	Shredder
	Tricodactylidae	<i>T. dentatus</i>	Shredder
		<i>T. petropolitanus</i>	Shredder
		<i>T. fluviatillis</i>	Shredder
Diptera	Blephariceridae	Nd	Scraper
	Ceratopogonidae	Nd	Gathering-collector - Predator
	Chironomidae	Nd	Gathering-collector – Filtering-collector – Predator – Shredder
	Culicidae	Nd	Gathering-collector – Filtering-collector
	Dixidae	Nd	Gathering-collector
	Empididae	Nd	Gathering-collector
	Ephrydidae	Nd	Gathering-collector
	Psychodidae	Nd	Gathering-collector – Scraper
	Simuliidae	Nd	Filtering-collector
	Stratiomyidae	Nd	Gathering-collector
	Syrphidae	Nd	Filtering-collector
	Tabanidae	Nd	Gathering-collector - Predator
	Tipulidae	Nd	Predator - Shredder
Ephemeroptera	Baetidae	<i>Americabaetis</i>	Scraper
		<i>Baetodes</i>	Gathering-collector – Scraper
		<i>Callibaetis</i>	Gathering-collector
		<i>Camelobaetidius</i>	Scraper
		<i>Cloeodes</i>	Gathering-collector
		<i>Cryptonympha</i>	Gathering-collector
		<i>Paracloeodes</i>	Scraper
		<i>Tupia</i>	Gathering-collector
		<i>Waltzoyphius</i>	Gathering-collector
		<i>Zelusia</i>	Gathering-collector
	Caenidae	<i>Caenis</i>	Gathering-collector – Scraper
	Euthyplociidae	<i>Campylocia</i>	Filtering-collector
	Leptohyphidae	<i>Leptohyphes</i>	Scraper
		<i>Leptohyphodes</i>	Gathering-collector
		<i>Thricorythopsis</i>	Scraper
		<i>Traverhyphes</i>	Gathering-collector
		<i>Tricorythodes</i>	Scraper
	Leptophlebiidae	<i>Askola</i>	Scraper
		<i>Farrodes</i>	Scraper
		<i>Hagenulopsis</i>	Scraper
		<i>Hermanella</i>	Gathering-collector – Scraper

		<i>Hylister</i>	Gathering-collector – Scraper
		<i>Massartela</i>	Gathering-collector – Scraper
		<i>Melaemerella</i>	Gathering-collector – Scraper
		<i>Thraulodes</i>	Scraper
		<i>Ulmeritoides</i>	Gathering-collector – Scraper
		<i>Homathraulus</i>	Gathering-collector – Scraper
		<i>Microphlebia</i>	Gathering-collector – Scraper
		<i>Miroculus</i>	Scraper
		<i>Needhamella</i>	Gathering-collector – Scraper
		<i>Lachlania</i>	Filtering-collector
Hemiptera	Oligoneuriidae		
	Belastomatidae	Nd	Predator
	Corixidae	Nd	Predator
	Gelastocoridae	Nd	Predator
	Gerridae	Nd	Predator
	Hebridae	Nd	Predator
	Helotrephidae	Nd	Predator
	Mesoveliidae	Nd	Predator
	Naucoridae	Nd	Predator
	Nepidae	Nd	Predator
	Notonectidae	Nd	Predator
	Pleidae	Nd	Predator
	Saldidae	Nd	Predator
	Vellidae	Nd	Predator
Isopoda	nd	Nd	Shredder
Lepidoptera	Pyrilidae	Nd	Shredder
Megaloptera	Corydalidae	<i>Corydalus</i>	Predator
Mollusca (Filo)	Ancilidae	Nd	Scraper
	Hydrobiidae	Nd	Scraper
	Lymnaeidae	<i>Lymnaea</i>	Scraper
	Physidae	<i>Physa</i>	Scraper
	Pilidae	<i>Pomacea</i>	Scraper
	Planorbidae	<i>Antillorbis</i>	Scraper
		<i>Biomphalaria</i>	Scraper
		<i>Melanooides</i>	Scraper
Odonata	Thiaridae	<i>Castoraeschna</i>	Predator
	Aeshnidae	<i>Limnetron</i>	Predator
		<i>Rhionaeschna</i>	Predator
	Calopterygidae	<i>Calopteryx</i>	Predator
		<i>Haeterina</i>	Predator
	Coenagrionidae	<i>Argia</i>	Predator
	Cordullidae	<i>Neocordulia</i>	Predator
	Dictyodidae	Nd	Predator
	Gomphidae	<i>Aphylla</i>	Predator
		<i>Archaegomphus</i>	Predator
		<i>Cyanogomphus</i>	Predator
		<i>Epigomphus</i>	Predator
		<i>Gomphoides</i>	Predator
		<i>Phyllocycla</i>	Predator
		<i>Phyllogomphoides</i>	Predator
		<i>Progomphus</i>	Predator
	Libellulidae	<i>Brechmorhoga</i>	Predator
		<i>Dythemis</i>	Predator
		<i>Elasmothermis</i>	Predator
		<i>Elga</i>	Predator
		<i>Erythrodiplax</i>	Predator
		<i>Gynothemis</i>	Predator
		<i>Idiataphe</i>	Predator
		<i>Libellula</i>	Predator
		<i>Macrothemis</i>	Predator
		<i>Planiplax</i>	Predator
		<i>Zenithoptera</i>	Predator
	Megapodagrionidae	<i>Heteragrion</i>	Predator
	Perilestidae	<i>Perilestes</i>	Predator
	Protoneuridae	Nd	Predator
	Grypopterygidae	<i>Grypopteryx</i>	Gathering-collector – Scraper
		<i>Guaranyperla</i>	Gathering-collector
		<i>Paragrypopteryx</i>	Gathering-collector - Shredder
		<i>Tupiperla</i>	Gathering-collector
	Perlidae	<i>Anacroneuria</i>	Predator
		<i>Kempnyia</i>	Predator
		<i>Macrogynoplax</i>	Predator
Trichoptera	Calamoceratidae	<i>Phylloicus</i>	Shredder
	Ecnomidae	Nd	Filtering-collector
	Glossosomatidae	<i>Mortoniella</i>	Scraper
		<i>Protoptila</i>	Scraper
	Helicopsychidae	<i>Helicopsyche</i>	Scraper
	Hydrobiosidae	<i>Atopsyche</i>	Predator
	Hydropsychidae	<i>Blepharopus</i>	Filtering-collector
		<i>Leptonema</i>	Filtering-collector

	<i>Macronema</i>	Filtering- collector
	<i>Smicridea</i>	Filtering-collector
	<i>Synoestrpsis</i>	Filtering-collector
Hydroptilidae	<i>Alisotrichia</i>	Predator
	<i>Hydroptila</i>	Scraper
	<i>Leucotrichia</i>	Scraper
	<i>Metrichia</i>	Gathering-collector – Scraper
	<i>Ochrotrichia</i>	Gathering-collector
Leptoceridae	<i>Atanotica</i>	Scraper
	<i>Grumichella</i>	Scraper
	<i>Nectopsyche</i>	Shredder
	<i>Notalina</i>	Shredder
	<i>Oecetis</i>	Predator - Shredder
	<i>Triplectides</i>	Shredder
Odontoceridae	<i>Barypenthus</i>	Predator
	<i>Marilia</i>	Scraper - Predator
Philopotamidae	<i>Chimarra</i>	Filtering-collector
	<i>Wormaldia</i>	Filtering-collector
Polycentropodidae	<i>Cyrnellus</i>	Filtering-collector
	<i>Polycentropus</i>	Filtering-collector – Predator
	<i>Polyplectropus</i>	Filtering-collector
Sericostomatidae	<i>Grumicha</i>	Scraper
Xyphocetronidae	<i>Xyphocetron</i>	Filtering-collector

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**Appendix 2.** Summary of the abiotic variables (mean value  $\pm$  standard error) investigated along the impairment gradient.

<b>Abiotic variables</b>	<b>Reference (74)</b>	<b>Intermediate (38)</b>	<b>Impaired (34)</b>
pH	6.68 ( $\pm$ 0.19)	7.06 ( $\pm$ 0.21)	7.04 ( $\pm$ 0.20)
Log10_Cond ( $\mu$ S/cm)	0.51 ( $\pm$ 0.14)	0.54 ( $\pm$ 0.15)	0.68 ( $\pm$ 0.22)
Log10_Ch (mg/L)	0.35 ( $\pm$ 0.03)	0.32 ( $\pm$ 0.03)	0.35 ( $\pm$ 0.03)
Log10_TA (mg/L)	0.44 ( $\pm$ 0.07)	0.45 ( $\pm$ 0.08)	0.61 ( $\pm$ 0.08)
Log10_TH (mg/L)	0.41 ( $\pm$ 0.11)	0.43 ( $\pm$ 0.11)	0.51 ( $\pm$ 0.11)
DO (mg/L)	8.18 ( $\pm$ 0.34)	7.35 ( $\pm$ 0.38)	6.04 ( $\pm$ 0.41)
Log10_Ca (mg/L)	0.12 ( $\pm$ 0.03)	0.14 ( $\pm$ 0.03)	0.18 ( $\pm$ 0.03)
Log10_NH <sub>3</sub> (mg/L)	0.01 ( $\pm$ 0.01)	0.01 ( $\pm$ 0.01)	0.02 ( $\pm$ 0.01)
Altitude (m)	515.99 ( $\pm$ 104.45)	386.97 ( $\pm$ 108.54)	342.77 ( $\pm$ 110.87)
Width (m)	8.97 ( $\pm$ 1.07)	11.79 ( $\pm$ 1.49)	9.12 ( $\pm$ 1.57)
HAP	16.27 ( $\pm$ 0.26)	10.10 ( $\pm$ 0.37)	3.62 ( $\pm$ 0.39)
Class (HAP)	Excellent-Good	Good-Regular	Poor

Log10\_NH<sub>3</sub> - Ammonia; Log10\_TH- Total Hardness, Log10\_Ch- Chloride, HAP- Habitat Assessment Protocol DO- Dissolved Oxygen and Log10\_TA- Total Alkalinity and Log10\_Ca- Calcium.

## Capítulo 2

### **Performance of the top-down and bottom-up approaches: stream classification using macroinvertebrates in Atlantic Rainforest streams, Brazil**

***Priscilla da Silva Pereira<sup>1\*</sup>, Darcílio Fernandes Baptista<sup>1</sup>, Marcelo Ribeiro-Alves<sup>2</sup>, & Daniel Forsin Buss<sup>1</sup>***

*<sup>1</sup>Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Avaliação e Promoção da Saúde Ambiental, Rio de Janeiro, RJ, Brasil*

*<sup>2</sup>Fundação Oswaldo Cruz, Instituto Nacional de Infectologia Evandro Chagas, Laboratório de Pesquisa Clínica em DST / AIDS, Rio de Janeiro, RJ, Brasil*

# **Performance of the top-down and bottom-up approaches: stream classification using macroinvertebrates in Atlantic Rainforest streams, Brazil**

## **Abstract**

Stream classifications have an important role in conservation and management, identifying areas with relatively homogenous ecological characteristics. Top-down and bottom-up approaches have been used to classify stream sites. The top-down approach utilizes environmental variables that are chosen based on the previous knowledge of the landscape, and the bottom-up approach uses biological data and statistical analysis for grouping streams. This study aims to compare and validate the performance of six stream classifications throughout the Atlantic Rainforest and also calculate the ecological indicator value (IndVal) for all stream classifications. An ANOSIM test was used to verify any significant differences in macroinvertebrate assemblages between stream classifications. Most ANOSIM R values were significant at the 5% level, despite their taxonomic resolution (genera and family), and the data type (abundance and richness). Our results indicate that the bottom-up approaches to classify stream sites obtained the best performance. Cluster of abiotic variables (Cluster4) had the best performance, despite their taxonomic resolution and the data type. However, presented a scarcity of sites in two classes that limits the interpretation of results. Therefore, our evaluation of the classification performance selected that a geomorphology was the most capable of discriminating variation in biological patterns. Slightly similar number taxa were found in the indicator analysis (IndVal) in taxonomic resolution and data type. These taxa have high values and, thus, some are representative of reference sites such as Ephemeroptera, Plecoptera, and Trichoptera. Stream classification is essential for designing sampling programs and environmental monitoring. It also has the potential for the management of aquatic resources by providing a framework within which the bioassessment is undertaken.

Keywords: Biomonitoring, landscape, management, stream classification

## Introduction

Stream classification is a factor in biomonitoring used to characterize how ecosystems differ in ecological attributes and conditions. It can be characterized as an arrangement by grouping sites with similar characteristics (Omenik & Griffith 2014). These characteristics include environmental variables, e.g., geology, vegetation, climate, soil, and land use. Stream classification is used to support planning actions related to the management and conservation of aquatic resources (Leathwick et al. 2010, Rinaldi et al. 2013).

Top-down and bottom-up approaches have been used to classify stream sites. A top-down approach is based on a conceptual model of environmental characteristics such as ecoregion and watershed (e.g., Omernik 1987, Omernik & Bailey 1997). Ecoregions are defined by relatively homogeneous areas that have similar environmental conditions (Omernik 1995). The latter include physiographic features, like climate, geology, soils, and vegetation (Omernik 1987). Ecoregions can have different spatial scales and are intended to serve as a research territory for the evaluation and management of ecosystems. Ecoregion may also be used after freshwater taxa distribution data (Abell et al. 2008). Watersheds are topographic territories that have apparent surface water, which flows to a stream or to another watershed. These have been essential landscape frameworks used to understand the effects of anthropogenic disturbance on water quality. The bottom-up is another approach used to classify streams. This method uses taxonomic information and/or environmental variables to choose category criteria that maximize the distinctiveness of classes. Leathwick et al. (2010) used Generalized Dissimilarity Modeling (GDM), which models rates of the taxonomic composition as a function of the environment. Snelder et al. (2012) assigned classes at hierarchical levels based on cluster analyses.

Several studies evaluated the performance of different stream classifications in temperate regions (Van Sickle & Hughes 2000, Waite et al. 2000, Verdonschot & Nijoeer 2004, Verdonschot 2006, Sánchez-Montoya et al. 2007, Omenik & Griffith 2014, McManamay et al. 2018). However, studies that develop and assess the different stream classification schemes in tropical regions are still scarce (Vasconcelos et al. 2013, Agra et al. 2019, Pero et al. 2019).

Studies showed that the bottom-up classification performed better than the top-down methods, because they incorporate a broader range of important variables



(Leathwick et al. 2011, Snelder et al. 2012). In this context, we hypothesized that the bottom-up stream classification would perform better than the top-down. This study's main objectives were to (1) compare and validate the performance of six different stream classifications to discriminate patterns of macroinvertebrate assemblages at sites throughout the Atlantic Forest; and, (2) identify the taxa that most contribute to each stream classifications.

## **Material and Methods**

### **1. Study area**

The Atlantic Rainforest region of Rio de Janeiro state is classified as tropical with a rainy summer season, with the most elevated areas classified as humid subtropical, with a hot summer and without a dry season or a dry winter. According to Alvares et al. (2013) review of Köppen's climate classification for Brazil, most of Rio de Janeiro mid-to-lowland portions (44%) is classified as tropical with a summer rainy season (Aw type), and the mountainous regions and plateaus classified as humid subtropical zones with a hot summer, without dry seasons (Cfa type) or with dry winter (Cwa type) (Alvares et al. 2013). Temperatures oscillate between 15°C and 28°C, and annual rainfall is around 1,000–1,500 mm.

Rio de Janeiro state is composed of a group of coastal plains separated by hills and two mountain chains that run parallel to the ocean (Serra do Mar, ranging from altitudes 0–2000 m a.s.l. and Serra da Mantiqueira, ranging from 800 to 2500 m a.s.l.). The coastal plains are located at the piedmont of Serra do Mar mountain range, with altitudes of about 200 m a.s.l.. It is a depositional zone formed by marine, lacustrine, and fluvial sedimentation processes (Brasil 1983). This region is affected by high impact by urban areas or agriculture and livestock grazing, making minimally impacted areas (reference) scarce. The mountain chains are located at higher altitudes (from >200 m a.s.l. to around 1,800 m a.s.l.) with high slope and steep scarps. Most sites were sampled within or near protected areas (conservation units), which had low to moderate impact on agricultural activities. Therefore, this region presents the most extensive riparian vegetation and forest fragments.

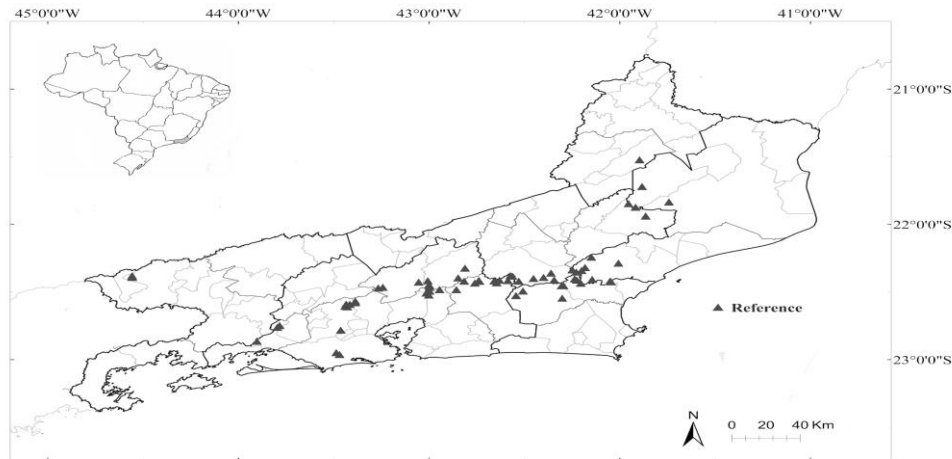
The Atlantic Forest is considered a global biodiversity hotspot (Myers et al. 2000). Despite its biome having lost 88% of its original extent, remnants are mostly spread throughout the higher parts of mountains, interspersed with agriculture and

pasture (Ribeiro et al. 2011). Most of the fragments are located on the steep slopes of mountains composed of small and isolated habitat patches (Ribeiro et al. 2011).

## 2. Survey design

In each sampling site, the following physicochemical variables were recorded in the field: dissolved oxygen (DO; YSI 550A equipment), pH (LabConte MPA 210p), and conductivity (Cond; using a LabConte MCA 150p). Water samples were preserved in sterile plastic bags (whirl-pak), according to APHA (2000). In the laboratory, the concentration of Ammonia (NH<sub>3</sub>) was determined by using a HACH (DR 2500). Chloride (Cl<sup>-</sup>), total alkalinity (TA), total hardness (TH), and calcium (Ca) were determined by the titrimetric method following APHA (2000). Sampling sites were also classified in the field according to the Habitat Assessment Protocol (HAP; Barbour et al. 1999). The HAP has ten environmental parameters, such as substrate availability for colonization by the benthic fauna, water velocity, embeddedness (pool variability for low-gradient streams), channel condition (sinuosity for low-gradient streams), sediment deposition, margin stability, and riparian vegetation. For each variable, a score between 0 and 20 was assigned. Sites were classified conforming to the mean score obtained, as follows: 0–5 "Poor," 5.1–9.9 "Regular," 10–14.9 "Good," and 15–20 "Excellent" environmental condition (Barbour et al. 1999).

We sampled 165 sites based on ad hoc indication and by previous knowledge of the Atlantic Forest region in Rio de Janeiro state. The sampling campaigns were carried out between 2011 and 2017 (during the dry season) using the same protocol. These sites were exposed to anthropogenic impact, and the criteria used to assess the level of disturbance included a qualitative assessment at each site of land-use, water quality, flow modifications, and physical alterations to the channel. Sites were *a priori* classified as "reference" had "Good" or "Excellent" environmental conditions according to the HAP, DO ≥ 6 mg/l, pH between 6 and 8, absence of channelization, and 40% of the upstream area affected by urban areas. After this step, we selected 78 sites to represent reference conditions (Figure 1). All reference sites were located within or near protected areas (conservation units), which were classified as "minimally disturbed areas" or "best attainable" (Reference Condition Approach; Stoddard et al. 2006). The latter occurred in areas outside conservation units, but that had low to moderate impact by agricultural activities, and full or partial riparian vegetation and forest fragments.



**Figure 1.** Map of the study area, showing the sampling sites in the Rio de Janeiro State, Brazil.

### 3. Biological samples

Macroinvertebrates were sampled by using a kick-net with a mesh size of 500  $\mu\text{m}$ . For this, 20 samples ( $1 \text{ m}^2$ ) were taken proportionally to the substrates available at each site, according to the multi-habitat method (Barbour et al. 1999). Samples were conserved in the field in 80% ethanol and taken to the laboratory. In the lab, samples were washed to remove coarse organic matter, such as leaves and twigs. The remaining material was deposited into a sampler ( $64 \times 36 \text{ cm}$ ), divided into 24 quadrants, each measuring  $10.5 \times 8.5 \text{ cm}$  (Fiocruz, Patent application number PCT/BR2011/000144). This method is used to assure the randomness of biological assessments, as it is less subject to the variability of team members (Oliveira et al. 2011). Fauna identification of organisms was performed to the lowest taxonomic level possible, except for Diptera and Hemiptera (identified at the family level), with the aid of keys and taxonomic descriptions (Angrisano 1995, Merritt & Cummins 1996, Nieser & Melo 1997, Carvalho & Nessimian 1998, Carvalho & Calil, 2000, Da-Silva et al. 2003, Olifiers et al. 2004, Salles et al. 2004, Da-Silva et al. 2010) and taxonomic specialists.

### 4. Stream classification

We classified sites according to six different classifications: five top-down approaches, Ecoregion, Watershed, Geomorphology, Altitude, and Stream Order. The methodology used for ecoregions was the Freshwater Ecoregions of the World (FEOW). The map of freshwater ecoregion was based on the distributions and compositions of

freshwater fish species, incorporated the main ecological and evolutionary patterns (Abell et al. 2008) and was developed in the global mapping conducted between 2006 and 2008 by WWF and TNC in conjunction with other research organizations that identified 426 freshwater ecoregions of the world. The FEOW recognizes 25 freshwater ecoregions in Brazil, three of which are present in Rio de Janeiro state: Fluminense, Paraíba do Sul, and Ribeira de Iguape.

Watershed is a land area that channels rainfall streams, rivers, and eventually to outflow points such as reservoirs, bays, and the ocean. While some watersheds are relatively small, others encompass thousands of square miles and may contain streams, rivers, lakes, reservoirs, and underlying inland groundwater. This stream classification was delimited by the Environment Secretary - INEA of Rio de Janeiro (Resolution CERHI-RJ nº 107, 22<sup>nd</sup> of May, 2013). According to the INEA, the state has nine hidrografic regions (RH) for management purposes: RH-1 Ilha Grande, RH-2 Guandu, RH-3 Médio Paraíba do Sul, RH-4 Piabanha, RH-5 Baía de Guanabara, RH-6 Lagos São João, RH-7 Rio Dois Rios, RH-8 Macaé e das Ostras and RH-9 Baixo Paraíba do Sul.

Geomorphology was based on the project RADAMBRASIL (BRASIL 1983) by which considers morphostructural units. The project was carried out by the Brazilian government aiming to undertake a reconnaissance survey of the country. The platform used was a twin-jet Caravelle flying at an altitude of 12 km and approximately 690 km/h. On board, was the side-looking radar Goodyear Mapping System 1000. The project worked with available material, organization of radar strips, high-resolution image scanning, the composition of final images, and its publication (Brasil 1983). We evaluated streams from two morphostructural units presented in Rio de Janeiro state: sedimentary deposits and steep scarps. The sedimentary deposit is located at the piedmont of Serra do Mar mountain range, with low altitudes (about 200 m a.s.l.). It is a depositional zone formed by marine, lacustrine, and fluvial sedimentation processes. The mountainous region is located at an altitude range from >200 m a.s.l. to around 1,800 m a.s.l, with high slope and steep scarps. Streams in steep scarps have a > 80% predominance of rocky substrates (see more details in Pereira et al. 2016).

Classes at three levels were defined by altitude (lowland <200 m, mid-altitude 200–800 m, and high >800 m). These classes were reported in this region as maintaining different aquatic macroinvertebrate assemblages (Henriques-Oliveira & Nessimian 2010, Souza et al. 2019). The distribution of biological diversity from low to

high altitudes, whether decreasing or showing a mid-altitudinal maximum, has been described for many groups, including aquatic macroinvertebrate.

We also classified sites by order, according to Strahler (1957) stream order because this feature is often correlated with biotic assemblage (Van Sickle & Hughes 2000). In Strahler's classification, streams are given an 'order' according to the number of additional tributaries associated with each waterway (Strahler 1957). Aquatic ecologists commonly use Strahler stream order as a surrogate for stream size, and as a basis for allocating field effort over the network of streams in a region. We sampled streams from 1<sup>st</sup> to 5<sup>th</sup> order (1:50,000 scale maps). Stream classification was obtained for each site by a geographic information system (GIS) that comprised layers (freshwater ecoregion, watershed, elevation, and geomorphology) of Rio de Janeiro state. The data were extracted using ArcGIS 10.3 software (ESRI, Redlands, CA, USA).

For the bottom-up approaches, agglomerative hierarchical clusters were tested as stream classification which was defined by abiotic variables and their categorical subdivisions.

## 5. Data analysis

For the description of the samples included in the study, we classified sites using the top-down and the bottom-up approaches.

Agglomerative hierarchical clusters with two to eight groups were obtained by using the Euclidean distance dissimilarity matrix among nine abiotic variables (NH<sub>3</sub>, DO, pH, TH, Ca, Ch, TA, HAP, and Cond) measured at the sampled streams and the complete linkage method for finding similar clusters. Similarly, clustering analyses with complete linkage by using dissimilarity matrices produced by Bray-Curtis or Sorensen distances were used respectively for data type (abundance and richness) analyses based on the aquatic macroinvertebrates. The dendrograms represent the cluster analysis results.

In order to test for a significant difference between two or more groups in the taxonomic resolution (genus and family) of sampling units, we used analysis of similarities (ANOSIM) either the data type with dissimilarity matrix produced by Bray-Curtis or Sorensen distances.

Compositional dissimilarities between the groups ( $r_B$ ) and within groups ( $r_W$ ) of sampling units, and the statistic  $R$ ,  $R = (r_B - r_W) / (N(N-1) / 4)$ , were calculated, where  $R$ -value 0 indicates completely random grouping, alongside the statistical

significance of observed  $R$  assessed by permuting the grouping vector to obtain the empirical distribution of  $R$  under null-model ( $B = 999$ ). Likewise, we also used the Multiple Response Permutation Procedure (MRPP), where differences in the within-group distances (spreads), i.e., Bray-Curtis and Sorensen distances, were tested. In the overall weighted mean of within-group means of the pairwise dissimilarities among sampling units, weights were calculated as the number of unique distances calculated among  $n$  sampling units ( $n(n-1)/2$ ) (Warton et al. 2012).

Classification Strength (CS), defined for dissimilarities as  $Bbar-Wbar$ , where  $Bbar$  is the mean between cluster dissimilarity and  $Wbar$  is the mean within-cluster dissimilarity were tabulated and reported alongside dendrograms with the change-corrected within-group agreement  $A = 1 - \delta/E(\delta)$ , where the statistic  $\delta$  is the overall weighted mean of within-group means of the pairwise dissimilarities among sampling units and  $E(\delta)$  is the expected  $\delta$  assessed as the average of dissimilarities. Then  $\delta$  was recalculated based on permuted ( $B = 999$ ) sampling units and their associated pairwise distances (Van Sickle & Hughes 2000).

Also, the Indicator Value (IndVal, Duf rene & Legendre 1997) method was carried out to determine the most representative macroinvertebrate taxa in each stream classifications.

Taxa present at less than 5% of sites were considered rare taxa and were excluded from the classifications (Mendes et al. 2017). The level of significance,  $\alpha = 0.05$ , was used in the analyses, and all analyses were performed in R software version 3.6.1., with packages 'vegan' and 'cluster'. All analyses were performed in R software version 3.6.1 (R Core Team, 2018).

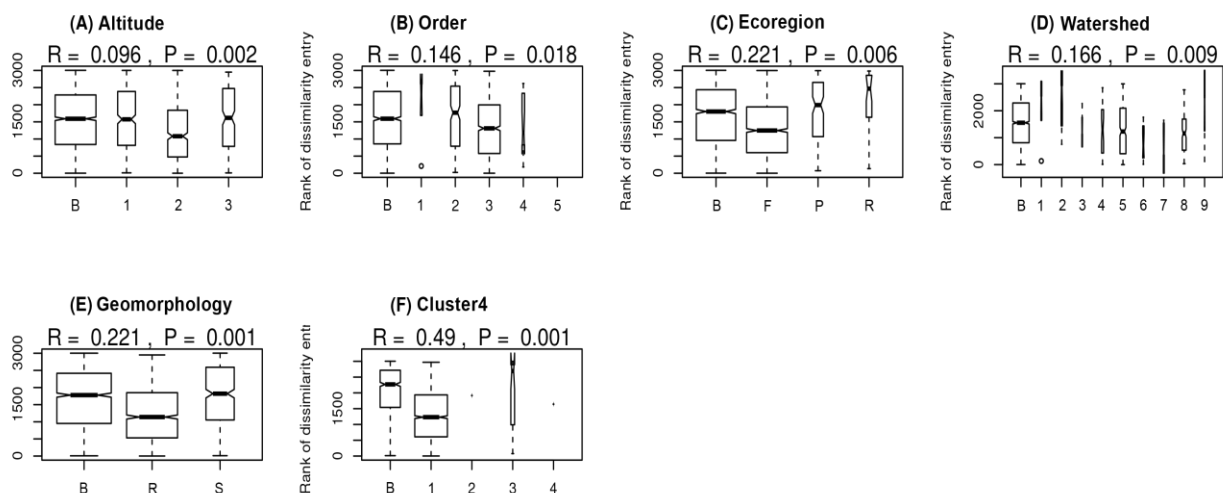
## Results

A total of 59,659 aquatic benthic macroinvertebrates distributed in 95 families and 128 genera were identified in 78 reference sample sites during the study.

Agglomerative hierarchical clusters of the abiotic variables with two to eight groups among nine abiotic variables ( $NH_3$ , DO, pH, TH, Ca, Ch, TA, HAP, and Cond) were assessed to choose the best performance. The cluster of abiotic variables with four groups (Cluster 4) was chosen as the stream classification systems because it presents the best results when compared to the other groups (Cluster 2, 6, 8 and 10). The summary of these variables was found in the Supplementary Material (Appendix 1).

Most of the ANOSIM R values were significant at the 5% level, despite their taxonomic resolution and the data type. The ANOSIM analysis for abundance showed that Cluster 4 had the highest R-value followed by Geomorphology and Ecoregion at both genera (R=0.45,  $p < 0.001$ ; R=0.24,  $p < 0.001$ ; R=0.22,  $p = 0.003$ ) and family levels (R=0.49,  $p < 0.001$ ; R=0.22,  $p < 0.001$ ; R=0.22,  $p = 0.006$ ). Figure 2 showed that Cluster 4 was the most successful at increasing within-class similarity, and decreasing between-class similarity with all sites for the first class (<0.5) were significantly different from the sites of second class (>0.5). However, the scarcity of sites in two classes limits the interpretation of results.

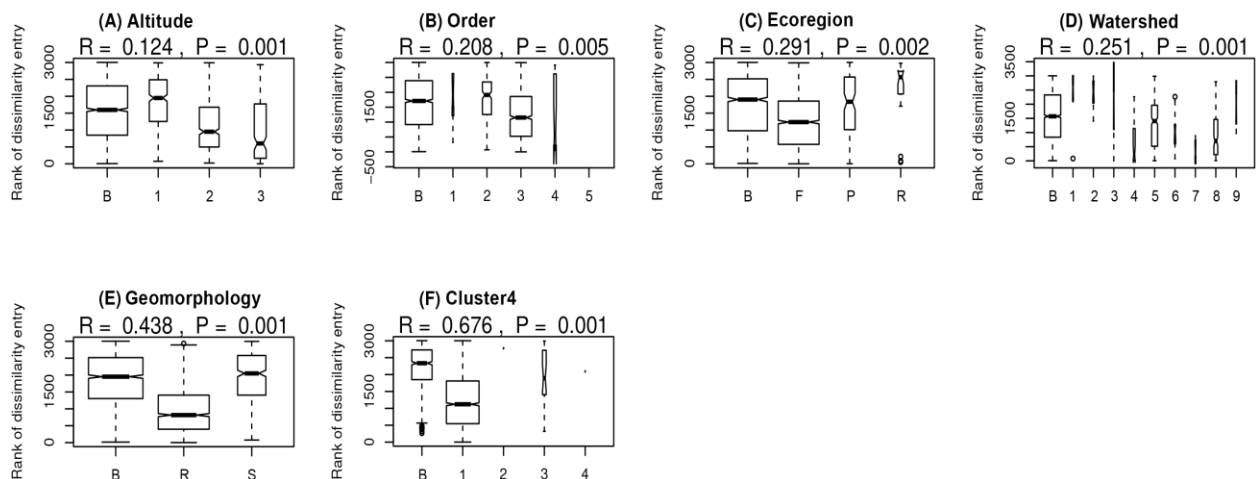
In general, the abundance data were significantly different for streams classification, as indicated by the R-values for both genus and family levels. The analyses showed higher and all positive R values for the stream classifications in the genera ranging from (0.11 to 0.45) and family levels (0.09 to 0.49). These suggest that several classifications had higher within-class similarity than between-class similarity. All of the stream classifications showed a significant result for abundance but the R values were generally low. These results suggest that the classification can provide a general representation of the spatial patterns in macroinvertebrate assemblages.



**Figure 2.** Box-and-whisker plots for macroinvertebrate abundance to test whether there is a significant difference between groups in the genera taxonomic resolution of sampling units using ANOSIM with dissimilarity matrix produced by Bray-Curtis distance (B – Between; Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).

Figure 3 shows the richness of Cluster 4 which was better than other stream classifications followed by Geomorphology and Ecoregion at both genera (R=0.68,

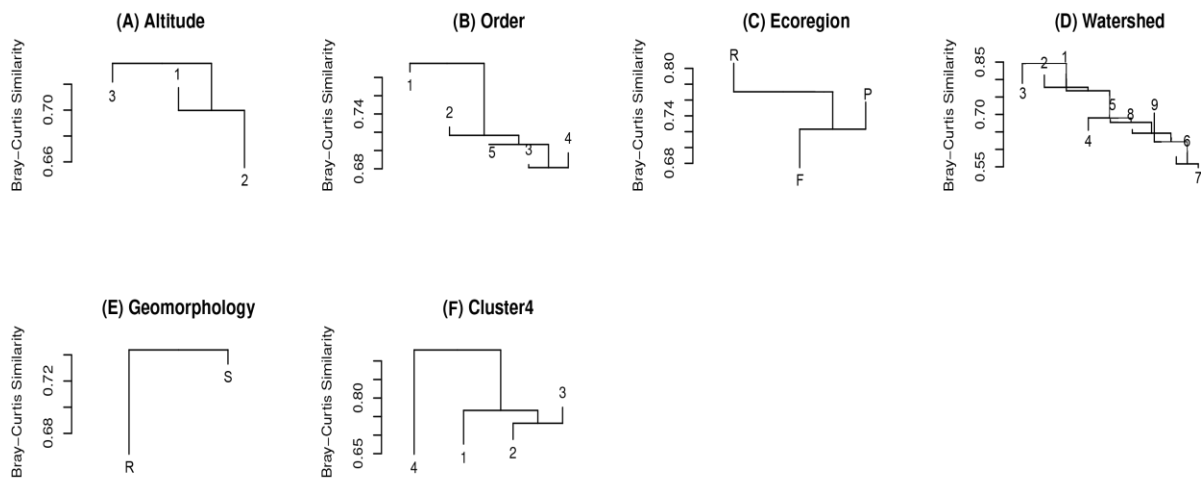
$p > 0.001$ ;  $R = 0.44$ ,  $p < 0.001$ ;  $R = 0.29$ ,  $p = 0.002$ ) and family levels ( $R = 0.59$ ,  $p < 0.001$ ;  $R = 0.38$ ,  $p < 0.001$ ;  $R = 0.27$ ,  $p < 0.001$ ). Cluster 4 had the highest R-value. An examination of pairwise results suggests that it was largely a reflection of differences, with all sites for the classes, they were significantly different. For richness, stream classifications also showed significant differences, as indicated by the R-values. Richness data showed R-values for genera ranging from 0.12 to 0.68 at the genera and from 0.11 to 0.59 at the family levels.



**Figure 3.** Box-and-whisker plots for macroinvertebrate richness to test whether there is a significant difference between groups in the genera taxonomic resolution of sampling units using ANOSIM with dissimilarity matrix produced by Sorensen distance (B – Between; Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).

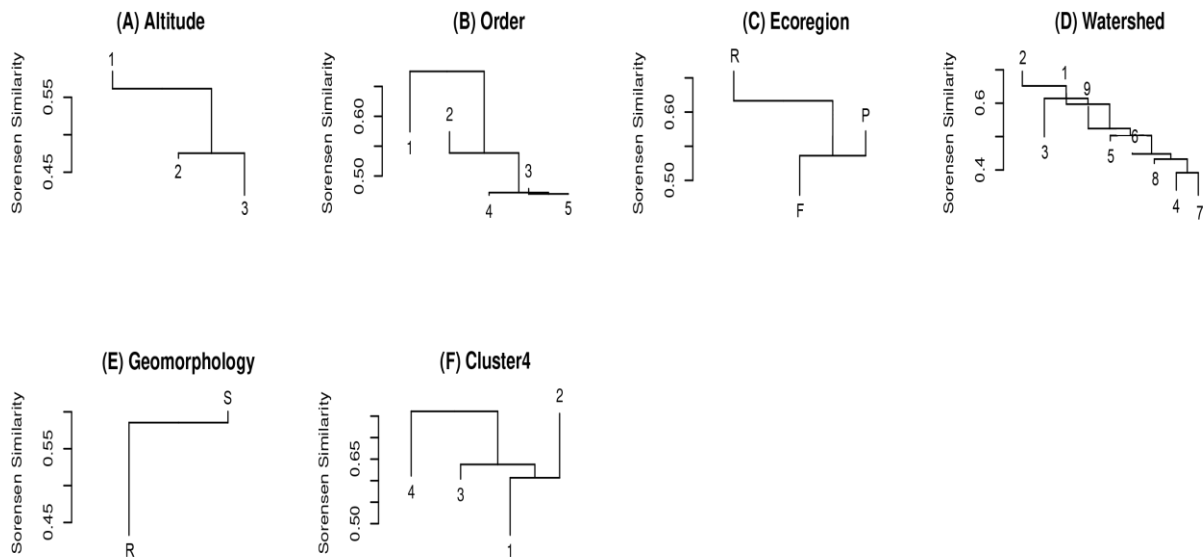
The mean taxonomic dendrograms showed the highest Classification Strength (CS) for abundance was Cluster 4 followed by Geomorphology and Ecoregion at both genera (CS=0.11, B=0.80, W=0.69; CS=0.05, B=0.74, W=0.69; CS=0.04, B=0.74, W=0.70) and at family levels (CS=0.12, B=0.72, W=0.60; CS=0.05, B=0.65, W=0.60; CS=0.04, B=-0.65, W=0.61). Figure 4 shows the CS value for the stream classifications ranging from 0.02 to 0.11 at the genera and from 0.02 to 0.12 at the family levels. CS showed that, among the classifications, even Cluster 4 had the highest CS, although it was weak.





**Figure 4.** Mean similarity dendrograms to the genera level for the abundance of six stream classifications for macroinvertebrate assemblages in the Atlantic Forest. The vertical lines represent the mean between-class similarity (B), and the horizontal lines terminate at the mean within-class similarity (W). (Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).

Figure 5 shows CS stream classifications based on richness. Cluster 4 had the highest CS, followed by Geomorphology and Watershed at genera (CS=0.16, B=0.66, W=0.50; CS=0.09, B=0.59, W=0.49; CS=0.07, B=0.54, W=0.47) and at family levels (CS=0.11, B=0.51, W=0.40; CS=0.06, B=0.46, W=0.40; CS=0.05, B=0.43, W=0.38). CS showed lower values for richness when compared to abundance at both genera and family levels, ranging from 0.04 to 0.17 at the genera and from 0.03 to 0.11 family. All streams classifications showed positive CS values for both genera and family levels.



**Figure 5.** Mean similarity dendrograms to the genera level for the richness of six classifications streams for macroinvertebrate assemblages in the Atlantic Forest. The vertical lines represent the mean between-class similarity (B), and the horizontal lines terminate at the mean within-class similarity (W). (Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).

Overall, we found that the significant associations (IndVal,  $p < 0.05$ ) shared taxonomic resolution (genera and family) and the data type (abundance and richness) in several stream classifications. Geomorphology was chosen because although Cluster 4 had the highest value in relation to abundance, ANOSIM and Classification Strength (CS), the scarcity of sites in two classes limits the interpretation of results.

The indicator analysis (indVal) found some differences between genera and family level (38 and 28, respectively) in the Geomorphology abundance. The orders were the same, however, the taxa found at the genera were different from that of the family. Furthermore, the order Ephemeroptera (*Leptohyphes sp.*, *Traverhyphes sp.*, *Hagenulopsis sp.*, *Thraulodes sp.*) was a significant indicator exclusively associated with genera.

Table 1. Indicator value (IndVal) abundance of significant macroinvertebrate taxa (mostly genera) obtained by Geomorphology.

Taxa	Rocky	Sedimentary	index	IndVal	p.value
<i>Oligochaeta</i>	0	1	2	0.480	0.007
<i>Blattaria</i>	0	1	2	0.649	0.006
<i>Hexanchorus</i>	1	0	1	0.831	0.001
<i>Phanocerus</i>	1	0	1	0.762	0.025
<i>Promoresia</i>	1	0	1	0.633	0.018
<i>Xenelmis</i>	1	0	1	0.756	0.025
<i>Gyrinus</i>	1	0	1	0.447	0.019
<i>Macrobrachium</i>	0	1	2	0.423	0.004
<i>T.fluviatillis</i>	0	1	2	0.533	0.006
Chironomidae	0	1	2	0.784	0.049
Culicidae	0	1	2	0.386	0.039
Psychodidae	0	1	2	0.544	0.006
Simuliidae	0	1	2	0.822	0.028
<i>Americabaetis</i>	0	1	2	0.822	0.028
<i>Baetodes</i>	1	0	1	0.897	0.001
<i>Leptohyphes</i>	1	0	1	0.815	0.001
<i>Traverhyphes</i>	0	1	2	0.696	0.001
<i>Hagenulopsis</i>	0	1	2	0.681	0.001
<i>Thraulodes</i>	1	0	1	0.400	0.047
Belastomatidae	1	0	1	0.490	0.001
Gerridae	0	1	2	0.354	0.036
Pleidae	1	0	1	0.590	0.008
Velidae	1	0	1	0.751	0.017
Ancilidae	0	1	2	0.369	0.033
<i>Melanoides</i>	0	1	2	0.463	0.004
<i>Perilestes</i>	0	1	2	0.416	0.001
<i>Gripopteryx</i>	1	0	1	0.809	0.002
<i>Guaranyperla</i>	1	0	1	0.623	0.007
<i>Paragripopteyx</i>	1	0	1	0.674	0.015
<i>Anacroneuria</i>	1	0	1	0.841	0.001
<i>Helicopsyche</i>	1	0	1	0.671	0.047
<i>Atopsyche</i>	1	0	1	0.667	0.007
<i>Leptonema</i>	1	0	1	0.827	0.001
<i>Nectopsyche</i>	1	0	1	0.791	0.001
<i>Triplectides</i>	1	0	1	0.737	0.009
<i>Barypenthus</i>	1	0	1	0.579	0.011
<i>Marilia</i>	1	0	1	0.602	0.005
<i>Grumicha</i>	1	0	1	0.585	0.015

The indicator analysis (indVal) showed Geomorphology for richness found similar taxa with abundance in taxonomic resolution and data type (Table 3). Few taxa were found in the genera and the family levels (36 and 24, respectively). Furthermore, the order Coleoptera (*Hexanchorus sp.*, *Macrelmis sp.*, *Phanocerus sp.*, *Promoresia sp.*) was a significant indicator exclusively associated with genera.

Table 2. Indicator value (IndVal) richness of significant macroinvertebrate taxa (mostly genus) obtained by Geomorphology.

Taxa	Rocky	Sedimentary	index	IndVal	p.value
Blattaria	0	1	2	0.598	0.014
<i>Hexanchorus</i>	1	0	1	0.768	0.001
<i>Macrelmis</i>	1	0	1	0.670	0.042
<i>Phanocerus</i>	1	0	1	0.692	0.037
<i>Promoresia</i>	1	0	1	0.592	0.012
<i>Gyrinus</i>	1	0	1	0.447	0.029
<i>Hydroblus</i>	1	0	1	0.492	0.038
<i>Macrobrachium</i>	0	1	2	0.423	0.006
<i>T.fluviatillis</i>	0	1	2	0.540	0.001
<i>Baetodes</i>	1	0	1	0.792	0.001
<i>Leptohyphes</i>	1	0	1	0.744	0.001
<i>Leptohyphodes</i>	1	0	1	0.566	0.007
<i>Traverhyphes</i>	0	1	2	0.694	0.001
<i>Farrodes</i>	1	0	1	0.755	0.001
<i>Hagenulopsis</i>	0	1	2	0.681	0.001
<i>Thraulodes</i>	1	0	1	0.400	0.048
Belastomatidae	1	0	1	0.490	0.01
Helotrephidae	1	0	1	0.615	0.003
Naucoridae	1	0	1	0.657	0.033
Pleidae	1	0	1	0.548	0.013
<i>Melanoides</i>	0	1	2	0.463	0.004
<i>Perilestes</i>	0	1	2	0.401	0.017
<i>Gripopteryx</i>	1	0	1	0.756	0.001
<i>Guaranyperla</i>	1	0	1	0.599	0.008
<i>Paragripopteyx</i>	1	0	1	0.706	0.002
<i>Anacroneuria</i>	1	0	1	0.736	0.012
<i>Kempnyia</i>	1	0	1	0.576	0.028
<i>Phylloicus</i>	1	0	1	0.737	0.004
<i>Helicopsyche</i>	1	0	1	0.692	0.001
<i>Atopsyche</i>	1	0	1	0.700	0.001
<i>Leptonema</i>	1	0	1	0.779	0.001
<i>Nectopsyche</i>	1	0	1	0.758	0.001
<i>Triplectides</i>	1	0	1	0.706	0.004
<i>Barypenthus</i>	1	0	1	0.566	0.01
<i>Marilia</i>	1	0	1	0.560	0.024
<i>Grumicha</i>	1	0	1	0.566	0.011

## Discussion

Stream classifications are used for a wide range of purposes. Several methods for the establishment of stream classifications have been developed, and although there has been considerable discussion of their merits, it is acknowledged that more systematic methods are needed to both validate and improve classifications (Snelder et al. 2008).

In this study, we tested the performance of stream classifications by using the top-down and the bottom-up approaches. In general, both showed significant results, but the bottom-up classifications have the highest values. These results corroborate with Snelder et al. (2012), who found that bottom-up approaches have been using more complex modeling methods that can produce classifications with a significantly better performance. The bottom-up classifications were influenced by the choice of biological and environmental dissimilarity measures, and by the choice of clustering methods. Cluster 4 had the best performance in spite of their taxonomic resolutions and the data type. However, presented a scarcity of sites in the two classes that limits the interpretation of results. Therefore, our evaluation indicated that the Geomorphology is some of the most capable of discriminating variation in biologic patterns.

Our results also showed that the taxonomic resolution was different. The genera level obtained higher values than those of the family when we observed the different classification schemes (for both the top-down and the bottom-up approaches). This result is in agreement with Vasconcelos et al. (2013), who found variation in macroinvertebrate composition. Furthermore, the data type (abundance and richness) showed different results among classifications. In general, the abundance values were higher than the richness values.

Overall, the ANOSIM R values were higher for the bottom-up than for the top-down approaches. These results suggest that the bottom-up classification systems were the most successful at increasing within-class similarity and decreasing between-class similarity. Although there has always been significant differences in ecological characteristics of the classes of the stream classifications ( $P > 0.05$ ), the R value was low (less than 0.5), indicating that the within-class variation was high and mean differences between classes were weak. These results corroborate with Snelder et al. (2008). Our results indicate that the bottom-up approaches to classify stream sites obtained the best performance with the abiotic variables (Cluster 4) that have the best ability to discriminate the variation of the macroinvertebrate in a range of ecological

characteristics. Therefore, our evaluation of the classification performance selected that a geomorphology was the most capable of discriminating variation in biological patterns. Stream classifications that are based on geomorphic processes are useful tools for providing a means of communication for stream studies involving scientists and managers with different backgrounds.

Our analysis was that all stream classifications (the top-down and even the bottom-up approaches) had weak classification strengths, regardless of their taxonomic resolution and data type used. This produced low within-group similarity and between-group heterogeneity. These patterns suggest that stream macroinvertebrate assemblages are dominated by taxa that are widely distributed across the stream classifications. Besides, the widespread species occurring across the different landscape which decreases between-group heterogeneity, while rare species increase noise and decrease within-group similarity, both contributing to low classification strengths (Heino & Mykra 2006). Several studies use landscape classifications, i.e., ecoregions, watershed, and drainage systems, for classifying streams. According to these studies, these classifications may represent the variability of stream assemblages to some extent. We found weak CS value in all classification streams which corroborates with other studies (Hawkins et al. 2000, Van Sickle & Hughes 2000, Dallas 2004, Heino & Mykra 2006).

Slightly similar taxa numbers were found in the indicator analysis (IndVal) in taxonomic resolution (genera and family) and data type (abundance and richness). These taxa have high values and, thus, most are representatives of the reference sites, and the frequency of occurrence would facilitate their use as bioindicators (Caro-Borrero et al. 2016). In contrast, Simuliidae is a tolerant organism that maintained a frequency of occurrence in many sites. The importance of tolerant organisms is that they provide an initial indication of changes in the conditions of ecological quality. The stream sites in this study were characterized by taxa that inhabit the upper reaches of rivers with colder and oxygen-rich waters, in areas of cobbles and small boulders. These sites supported the greatest presence of Trichoptera, Ephemeroptera, and Plecoptera. In general, these taxa are considered to have high oxygen requirements, and their presence is associated with good water quality (Jacobsen et al. 2008).

For the purpose of conservation planning, physical classification is probably more heuristic than environmental variables, thereby justifying their limitations in capturing the variability of assemblage structure, species distributions, and richness. Stream classification is essential for designing sampling programs and communicating

information. Thus, it also has the potential for the management of aquatic resources by providing a framework within which the bioassessment is undertaken.

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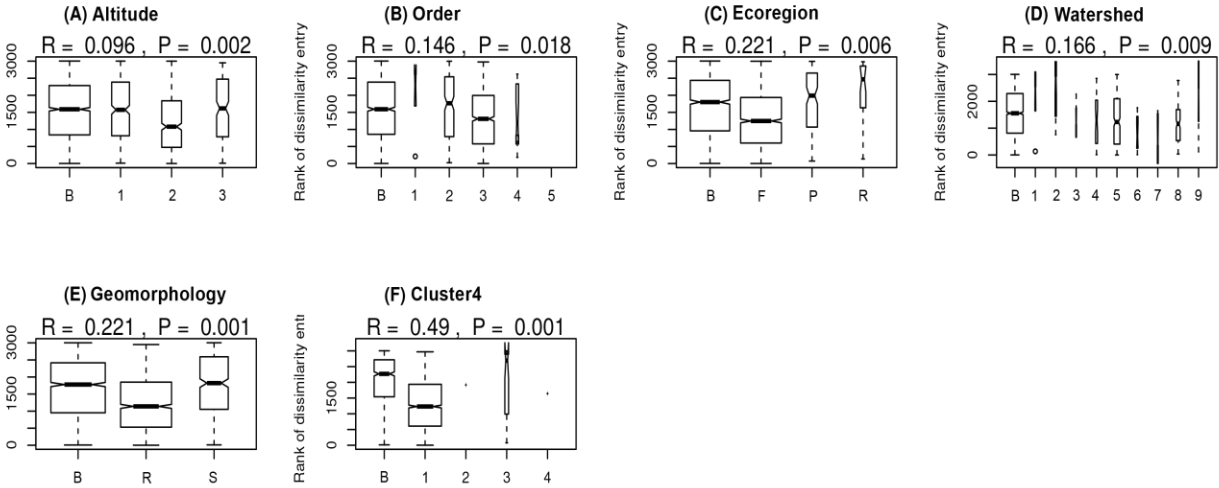
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**Appendix 1.** Summary of the abiotic variables used in the study.

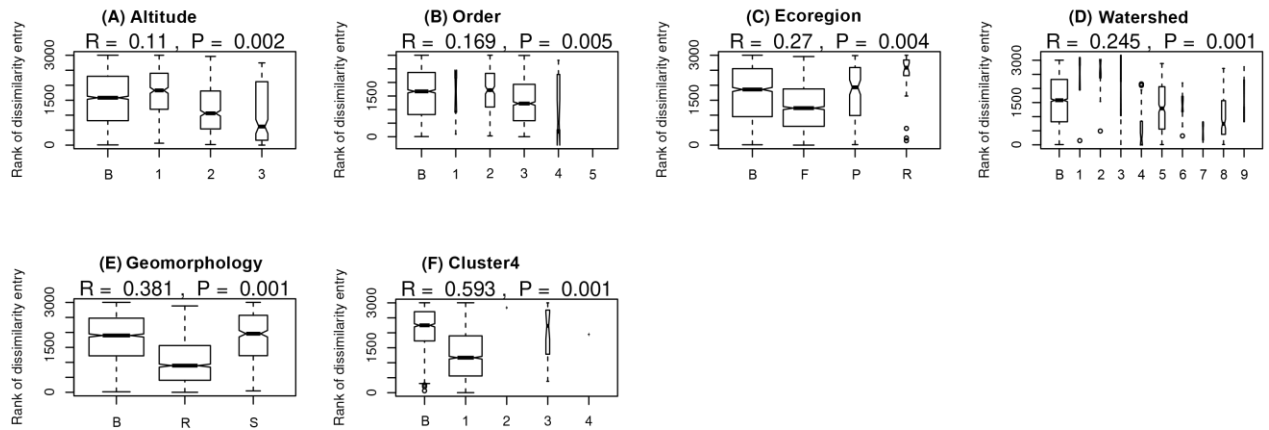
Variables	Reference			
	Mean	SD	Min.	Max.
Mean width (m)	9.05	10.01	1	76.10
Altitude	486.84	394.53	10	1402.07
pH	6.50	1.25	5,4	8.20
Log10_Conductivity (S/cm)	0.48	0.20	0.15	1.01
Log10_Chloride (mg/L)	0.33	0.16	0.05	0.98
Log10_Total alkalinity (mg/L)	0.46	0.25	0.00	1.17
Log10_Total Hardness (mg/L)	0.35	0.30	0.00	1.67
Dissolved oxygen (mg/l)	8.03	1.39	5.12	13.80
Log10_Calcium (mg/l)	0.10	0.09	0.00	0.38
Log10_NH <sub>3</sub> (mg/l)	0.01	0.05	0.00	0.31
HAP	16.48	2.36	10.50	20.00



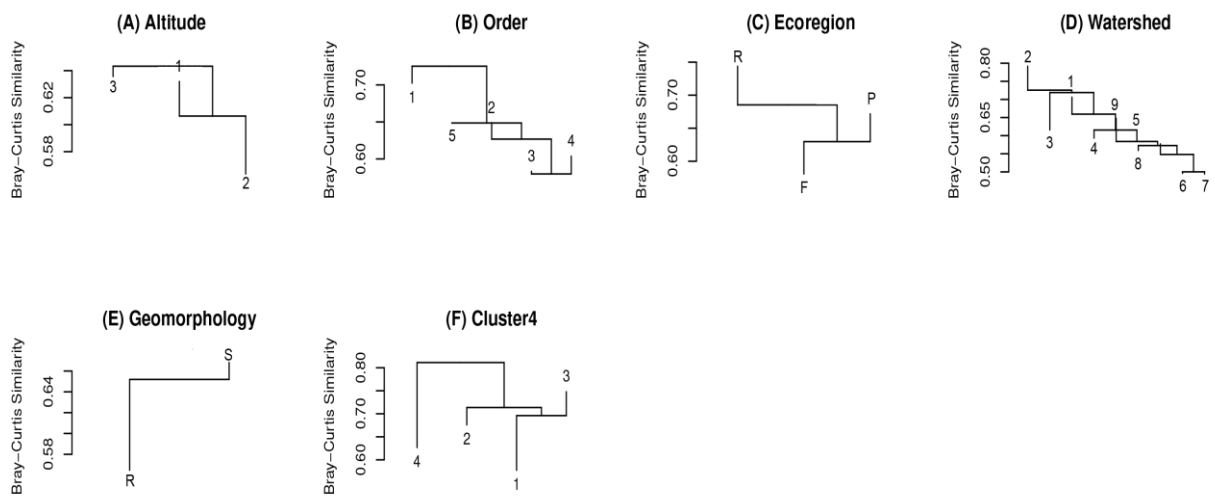
**Appendix 2.** Box-and-whisker plots for macroinvertebrate abundance to test whether there is a significant difference between groups in the family taxonomic resolution of sampling units using ANOSIM with dissimilarity matrix produced by Bray-Curtis distance (B – Between; Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).



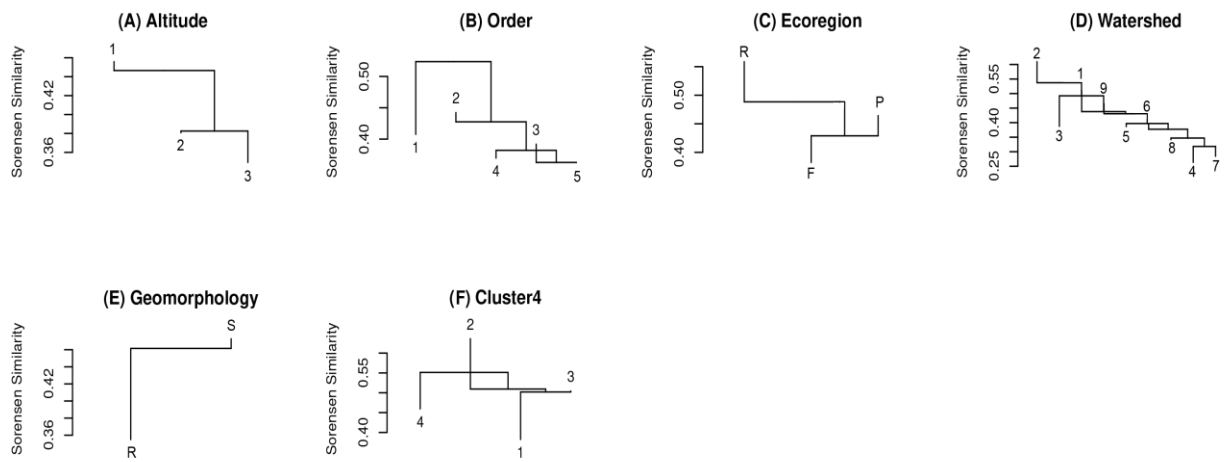
**Appendix 3.** Box-and-whisker plots for macroinvertebrate richness to test whether there is a significant difference between groups in the family taxonomic resolution of sampling units using ANOSIM with dissimilarity matrix produced by Sorensen distance (B – Between; Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).



**Appendix 4.** Mean similarity dendrograms to the family level for the abundance of six stream classifications for macroinvertebrate assemblages in the Atlantic Forest. The vertical lines represent the mean between-class similarity (B), and the horizontal lines terminate at the mean within-class similarity (W). (Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).



**Appendix 5.** Mean similarity dendrograms to the family level for the richness of six classifications streams for macroinvertebrate assemblages in the Atlantic Forest. The vertical lines represent the mean between-class similarity (B), and the horizontal lines terminate at the mean within-class similarity (W). (Altitude 1 - <200 m, 2 - 200–800 m, 3 - >800 m; Ecoregion F – Fluminense, P – Paraíba do Sul, R – Ribeira de Iguape; Geomorphology R – Rocky, S – Sedimentary).



## CONCLUSÕES

Este estudo avaliou uma área de intensa atividade e desenvolvimento e que a cada momento vem sendo modificado por ação antrópica. No entanto, poucos estudos ambientais têm sido realizados na região, o que dificulta uma avaliação temporal dessas intervenções. Os capítulos desta tese contribuem para a implantação de programas de biomonitoramento e gestão no Estado do Rio de Janeiro. O Capítulo I, com a utilização de uma ferramenta integrada baseada em mecanismos morfológicos e comportamentais de aquisição de alimentos (razões de FFG) e o Capítulo II, fornecendo informações que contribuem para a padronização de um sistema de classificação de rios que melhor explica a variação da comunidade de macroinvertebrados.

No primeiro capítulo, foram avaliados os efeitos de variáveis de riachos na abundância e riqueza de FFG e os atributos do ecossistema (razão de FFG) como uma ferramenta para caracterizar a condição ambiental dos rios da Mata Atlântica. Os modelos de efeito misto mostraram que os FFG diferiam em suas respostas às variáveis abióticas quanto à abundância e riqueza. Além disso, eles diferem significativamente nas áreas impactadas quando comparados às áreas intermediária e de referência. As razões de FFG encontraram diferenças significativas ao longo do gradiente de impacto. A análise da razão de FFG evidenciou-se como uma ferramenta rápida e barata, com potencial para ser utilizada no monitoramento de ecossistemas aquáticos no bioma Mata Atlântica. No entanto, mais estudos serão necessários para calibrar o método especificamente na região da Mata Atlântica do Estado do Rio de Janeiro.

No segundo capítulo foram avaliados o desempenho de diferentes sistemas de classificações de rios e calcular o valor do indicador ecológico (IndVal). Os resultados indicam que os sistemas de classificações do tipo bottom-up obtiveram um melhor desempenho. apesar da resolução taxonômica (gênero e família) e tipo de dados (abundância e riqueza). Devido variáveis abióticas resultantes de agrupamentos hierárquicos aglomerativos de quatro grupos (Cluster 4) apresentar uma escassez de áreas classificadas em duas escalas, o que limita a interpretação dos resultados, foi selecionada a Geomorfologia como o melhor capaz de discriminar a variação nos macroinvertebrados. Táxons semelhantes foram encontrados na análise do indicador

(IndVal) na resolução taxonômica e tipo de dados. Esses táxons têm valores elevados e, alguns são representativos de áreas de referência. A classificação de rios é essencial para projetar programas de amostragem e comunicar informações. Também tem potencial para o manejo dos recursos aquáticos, fornecendo uma estrutura dentro da qual a bioavaliação é realizada.

Considerando a nova mudança de paradigma de interpretação do conceito de qualidade da água, a deterioração e melhora da integridade ecológica é definida pela resposta da biota, em conjunto as mudanças nas variáveis físico-químicas. O Biomonitoramento deve ser considerado hoje ação primordial para a conservação dos recursos hídricos, uma vez que a biota é o melhor indicador da saúde dos ecossistemas como um todo. A comunicação das condições dos sistemas biológicos para os diversos setores da sociedade pode transformar o biomonitoramento de um exercício acadêmico em uma metodologia efetiva para o manejo e a conservação dos corpos hídricos.