UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CENTRO DE AQUICULTURA DA UNESP

Avaliação da qualidade ecológica de ambientes aquáticos da bacia hidrográfica do Rio Itanhaém através da assembleia de macrófitas aquáticas: proposta para a formulação de um índice de integridade biótica

Cristiane Akemi Umetsu

Jaboticabal, São Paulo
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Orientador: Dr. Antonio Fernando Monteiro Camargo

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Aos meus pais, Kenichi e Dolores Umetsu, dedico.

“Viver é a coisa mais rara do mundo. A maioria das pessoas apenas existe.”

Oscar Wilde

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RESUMO

Este trabalho teve como principal objetivo avaliar questões referentes à avaliação da qualidade ecológica de ambientes aquáticos, com ênfase em macrófitas aquáticas. Inicialmente fizemos uma análise cienciométrica a fim de verificar o panorama global dos estudos sobre qualidade ecológica e índices de integridade biótica e tentamos fornecer uma visão geral sobre os ecossistemas, organismos e regiões que mais utilizam esses indicadores, bem como uma análise crítica sobre as tendências, aplicações e limitações dessa abordagem. Nossos resultados indicam que há uma falta de uniformidade na distribuição do número de estudos por continente, principalmente em regiões tropicais. Além disso, poucos estudos consideram mais do que um grupo de organismos para avaliar a qualidade ecológica de ambientes aquáticos. Em seguida, utilizamos dados de macrófitas aquáticas presentes na bacia hidrográfica do Rio Itanhaém, situada no litoral sul do Estado de São Paulo, para desenvolver mais dois estudos. O primeiro deles avaliou a influência das variáveis ambientais sobre a riqueza de espécies e riqueza de formas de vida, com o intuito de verificar quais fatores naturais e antropogênicos são mais importantes na estruturação da assembleia de macrófitas aquáticas. Tanto fatores naturais quanto antrópicos foram responsáveis por explicar a riqueza de espécies e formas de vida nos ambientes aquáticos estudados. Por fim, nós exploramos o uso de macrófitas aquáticas como indicadoras da integridade ecológica dos ambientes aquáticos da bacia do Rio Itanhaém, a fim de desenvolver um índice multimétrico. Nossos resultados mostraram que a estrutura da assembleia de macrófitas é distinta em locais degradados e não impactados, permitindo o desenvolvimento do índice. Embora o processo de validação do índice não tenha apresentado bons resultados, este estudo permitiu um melhor entendimento das condições ecológicas destes ambientes e consiste em uma relevante contribuição para futuros estudos envolvendo avaliação e biomonitoramento em ambientes aquáticos tropicais.

Palavras-chave: ambientes aquáticos tropicais, monitoramento, bacia costeira, avaliação ecológica

ABSTRACT

This study aimed to evaluate issues related to ecological quality of aquatic environments, with emphasis in aquatic macrophytes. Initially we developed a scientometric analysis in order to verify the global view of the studies dealing with ecological quality and index of biotic integrity (IBI), and try to provide an overview of ecosystems, organisms and regions that mostly used these indicators, as well as a critical analysis of the trends, applications and limitations of this approach. Our results indicate a lack of uniformity in the distribution of the number of studies by continent, mainly in tropical regions. Moreover, few studies have focused in more than one biological group to assess the ecological quality of aquatic environments. After, we used macrophyte aquatic data from Itanhaém River Basin, located in the south littoral of São Paulo State, to develop two more studies. First, we evaluated the influence of environmental variables on species richness and life form richness in order to verify which natural and anthropogenic factors are most important in structuring the plant assemblages. Both natural and anthropogenic factors were responsible for explaining the species richness and life forms richness in aquatic environments studied. Finally, we explored the use of macrophytes as indicators of the river ecological conditions in tropical rivers, in order to develop a multimetric IBI. Our results showed that the macrophyte assemblage structure is distinct in degraded and undisturbed sites, allowing the development of an IBI. Although the validation of the IBI has not shown good results, this study allowed a better understanding of ecological conditions in these aquatic environments and consists in a relevant contribution for future studies involving assessment and biomonitoring in tropical aquatic ecosystems.

Keywords: tropical freshwater, monitoring, coastal watershed, ecological assessment

APRESENTAÇÃO

O presente estudo foi dividido em três capítulos, sendo estes organizados em manuscritos e formatados de acordo com as normas específicas de publicação de cada periódico escolhido para posterior submissão. No entanto, somente para fins de melhor compreensão e facilitação da leitura, os capítulos não obedecem à norma de manter as figuras e tabelas no final do manuscrito e são então apresentados ao longo do texto.

Antes de apresentar a tese, peço licença para compartilhar um pouco da história do meu doutoramento e do desenvolvimento deste trabalho. Antes de ingressar no doutorado, eu lecionava na Universidade do Estado do Mato Grosso, Universidade esta onde me formei em Ciências Biológicas no ano de 2006. Após finalizar o mestrado em Ecologia de Ambientes Aquáticos Continentais – PEA/UEM retornei para o Mato Grosso para assumir uma vaga temporária de professor substituto das disciplinas de Botânica e Ecologia. O contrato tinha duração de apenas um semestre e por este motivo já ansiava por retomar os estudos. Neste intervalo encontrei o edital de seleção de novos pós-graduandos do Centro de Aquicultura da UNESP. Apesar de não possuir nenhuma familiaridade com aquicultura propriamente dita, me interessei pela linha de pesquisa do Prof. Antonio Camargo, que inclui Biologia e Manejo de Ecossistemas Aquáticos Naturais. Sendo assim, o procurei, acertamos os ponteiros e prestei a prova no segundo semestre de 2011. Felizmente fui aprovada e em março de 2012 iniciamos os trabalhos.

Para se candidatar ao processo seletivo do Caunesp, não era necessário apresentar um projeto de pesquisa. Sendo assim, dispúnhamos de algum tempo para pensar em uma proposta. A ideia geral desta tese nasceu durante a disciplina de Poluição Aquática e Aquicultura, ministrada pelo Prof. Antonio Camargo. Durante a aula discutíamos sobre a legislação referente à avaliação da qualidade da água no Brasil, como por exemplo, a resolução Conama 357/2005, que dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes. Ao pesquisar um pouco mais sobre o assunto, foi possível perceber que a legislação ambiental brasileira não utiliza indicadores bióticos para a avaliação da qualidade de nossos ambientes aquáticos, diferentemente da União Europeia, que implementou a

Water Framework Directive, que estabelece um conjunto abrangente de objetivos para a qualidade das águas europeias. Esta diretiva estabelece um quadro de ação comunitária em política e gestão da água, e se aplica a todas as águas, incluindo as águas continentais, subterrâneas e costeiras. Os objetivos da WFD podem ser resumidos em uma meta global: alcançar uma boa qualidade de todas as águas da união Europeia até 2015.

Apesar dos inúmeros estudos sobre a utilização de índices de integridade biótica em ambientes aquáticos no mundo todo, uma rápida pesquisa na base de dados *ISI Web of Science* mostrou que no Brasil, apenas 25 trabalhos foram publicados sobre este tema, e nenhum deles utilizou componentes vegetais para avaliar a integridade ecológica dos ambientes estudados. Neste contexto, assumindo a minha experiência e a de meu orientador em macrófitas aquáticas, pensamos em unir o útil ao agradável e propor um projeto para a formulação de um índice de integridade biótica utilizando como ferramenta a assembleia de macrófitas aquáticas. A área de estudo escolhida foi a bacia hidrográfica do Rio Itanhaém. Esta bacia apresenta uma rica diversidade biológica e ambiental, apresentando diferentes corpos aquáticos, com rios de águas claras, brancas e pretas, além de zona estuarina. Além disso, apresenta características topográficas, geomorfológicas e vegetacionais diferenciadas ao longo de um gradiente longitudinal e sofre com diferentes graus de influência antrópica. Esta bacia vem sendo monitorada a mais de vinte anos pelo laboratório de ecologia aquática da Unesp de Rio Claro, sob responsabilidade do Prof. Dr. Antonio Camargo.

Dito isso, nos empenhamos ao máximo em desenvolver o projeto, definir metodologias e formas de analisar os dados. A maior dificuldade em definir os métodos estava no fato de que a maioria dos trabalhos foi desenvolvida em ambientes temperados, e em ambientes aquáticos como riachos, lagos e zonas costeiras. Sem contar com muito embasamento científico para a definição de nosso desenho amostral, optamos por tentar amostrar os ambientes da bacia de modo mais eficiente possível, buscando não perder informação. Para isso, utilizamos o ArcGis como ferramenta para distribuir pontos aleatórios ao longo de toda a bacia, a fim de definir pontos amostrais que representassem satisfatoriamente todos os ambientes aquáticos da bacia. Feito isso, foi possível amostrar um total de 137 pontos, apesar de todas as dificuldades de acesso a certos locais, como por exemplo, o Rio Castro, que só pôde ser amostrado com auxílio de caiaque. Em cada ponto amostral foi possível extrair informações sobre a composição e estrutura da assembleia de macrófitas aquáticas, bem como variáveis da água e do entorno. Assim, dispomos de uma quantidade considerável de informações que foram utilizadas para o desenvolvimento de dois capítulos desta tese.

O capítulo que corresponde ao tema central dessa tese foi desenvolvido graças ao auxílio de pesquisadoras do Centro de Estudos Florestais do Instituto Superior de Agronomia da Universidade de Lisboa, Francisca Aguiar e Teresa Ferreira. Foi possível obter a colaboração das mesmas devido à concessão de uma bolsa doutorado sanduíche no exterior pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, que permitiu que eu ficasse por um período de seis meses em Lisboa, suficientes para aprender as diferentes técnicas utilizadas pela Comunidade Europeia para a formulação de índices de integridade biótica. Além disso, o intercâmbio científico e cultural foi de extrema importância para minha formação pessoal e intelectual. Conviver com investigadores de outro país nos engrandece muito, ao lidar com diferentes formas de pensar e trabalhar. Considero que esse estágio foi de suma importância para o desenvolvimento de parte desta tese, além de um presente pessoal para meu crescimento científico/acadêmico.

INTRODUÇÃO GERAL

A avaliação da qualidade ecológica dos ecossistemas aquáticos tem despertado grande interesse tanto por parte da comunidade científica (Eisele et al. 2003; Penning et al. 2008; Korte et al. 2010) quanto para órgãos gestores responsáveis por sua caracterização e monitoramento ambiental (*por exemplo*, EU WFD – *European Water Framework Directive*). Isso se deve ao fato de os ecossistemas aquáticos estarem entre os mais ameaçados em todo o mundo, apesar da sua reconhecida importância para a diversidade global e dos serviços ecossistêmicos essenciais prestados. O termo ―qualidade ecológica", no âmbito de ambientes aquáticos, pode ser definido como a totalidade das funcionalidades e características da água que incidem sobre sua capacidade de suportar uma flora e fauna naturais adequadas (Pugh 1996). Dessa forma, os protocolos de avaliação da qualidade ecológica de ecossistemas aquáticos levam em consideração não somente as variáveis físicas e químicas da água, mas também seus componentes bióticos, que são influenciados pela qualidade do meio aquático.

Atualmente, a avaliação física e química da água por si só tem sido a principal ferramenta legislativa usada para avaliar a qualidade dos corpos aquáticos (Donohue et al. 2006). Entretanto, relacionar a qualidade ecológica baseada somente na física e química da água parece ser um tanto vago, uma vez que deveriam ser consideradas as interações entre os fatores bióticos e abióticos (Testi et al. 2012). No Brasil, por exemplo, a principal forma de avaliar a qualidade das águas dos corpos aquáticos se dá através do monitoramento das variáveis físicas e químicas. A Agência Nacional de Águas (ANA) possui o Programa Nacional de Avaliação da Qualidade das Águas (PNQA), que visa ampliar o conhecimento sobre a qualidade das águas superficiais no Brasil. Para este fim, são utilizados seis índices de qualidade que sintetizam informações sobre inúmeras variáveis físicas e químicas da água. Estes índices, por sua vez, não levam em consideração informações sobre os componentes bióticos do sistema aquático.

Dada a importância da utilização de componentes bióticos presentes em ecossistemas aquáticos para a avaliação a qualidade ecológica dos mesmos, a presente tese propôs a utilização de um componente biológico específico para ser utilizado em um índice de integridade biótica, com o objetivo de mensurar a qualidade ecológica de ambientes de uma bacia hidrográfica situada no sudeste do Brasil. O grupo biológico escolhido foi o das macrófitas aquáticas, frente à sua importância ecológica, uma vez que, dentre outras coisas, aumentam a complexidade do hábitat, atuam como substrato para refúgio, desova e alimentação de peixes (Padial et al., 2009; Pelicice et al., 2008) e apresentam um imenso potencial para a remediação de diversos contaminantes (Dhir et al., 2009). Além disso, estes organismos são diretamente influenciados pelas características limnológicas da água e reagem a mudanças na concentração de nutrientes, que consequentemente pode refletir na composição taxonômica dessa comunidade (Melzer 1999; Croft & Chow-Fraser 2007).

Além das próprias condições ambientais afetarem os atributos da assembleia de macrófitas aquáticas, a influência antrópica pode ser responsável por modificações mais intensas em suas condições naturais (Aznar et al. 2003; Akasaka et al. 2010; Mackay et al. 2010). Neste contexto, assumindo que a mudança nas condições ambientais dos habitats aquáticos é capaz de influenciar os atributos da assembleia de macrófitas aquáticas, elas podem então representar um interessante indicador das condições desses ambientes.

A seguir apresentaremos os resultados de quatro anos de trabalho, compilados em três distintos capítulos, formatados segundo as normas da publicação científica de cada período escolhido para submissão. O primeiro capítulo constitui-se em um manuscrito realizado através de uma análise cienciométrica que objetivou verificar o panorama global da avaliação da qualidade ecológica de ambientes aquáticos através de índices de integridade biótica. O manuscrito foi realizado em coautoria com Antonio F. M. Camargo e encontra-se formatado nas normas do periódico *Environmental Assessment and Monitoring.* O segundo capítulo relacionou alguns atributos da assembleia de macrófitas aquáticas com as variáveis ambientais com objetivo de verificar quais fatores naturais e antrópicos estruturam essa assembleia. O manuscrito foi desenvolvido em coautoria com Douglas Toledo, Leonardo F. Cancian e Antonio F. M. Camargo, e encontra-se formatado nas normas do periódico *Aquatic Botany*. O terceiro e último capítulo, a pedra angular desta tese, teve como objetivo desenvolver um índice de integridade biótica utilizando como indicador a assembleia de macrófitas aquáticas, a fim de avaliar a qualidade ecológica dos ambientes aquáticos da bacia hidrográfica do rio Itanhaém. O trabalho foi desenvolvido em parceira com as pesquisadoras Francisca C. Aguiar e Teresa Ferreira, do Centro de Estudos Florestais do Instituto Superior de Agronomia da Universidade de Lisboa. O trabalho também teve a colaboração de Leonardo F. Cancian e Antonio F. M. Camargo. O manuscrito encontra-se nos moldes do periódico *Ecological Indicators*.

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MANUSCRITO 1

The current scenario of the assessment of ecological quality of aquatic environments through biotic integrity indices: approaches, issues and prospects

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Abstract

Indices of biotic integrity (IBI) have been widely used to assess the ecological quality of aquatic environments, and are fundamental tools for monitoring and management of the related ecosystems. The present study aims to analyze the worldwide importance of IBI and to provide an overview of ecosystems, organisms and regions that mostly used these indicators, as well as a critical analysis of the trends, applications and limitations of this approach. We performed a systematic review of publications from ISI Web of Knowledge database until 2013. The screening of references allowed a final working pool of 455 scientific published works. Our results showed that publications in IBI increased exponentially in the past decade, especially in Europe. The greatest number of studies was conducted in coastal zones, rivers and/streams, and mostly were macroinvertebrate-based indices, while other biological groups, such as plankton and riparian vegetation were almost overlooked. In addition, several studies pointed out to the advantages of using a joint approach of more than one biological quality element. Thus, studies evaluating more than one biotic component may be useful in assessing the ecological quality of aquatic ecosystems. The recent European legislation in ecological quality and conservation of aquatic habitats could be responsible for the increase in the number of the studies. Our results enabled us to produce an overview of the status of studies related to ecological quality and biotic indicators.

Keywords: indicators; monitoring; environmental assessment; aquatic ecosystems

Introduction

Aquatic environments are among the most threatened in the world, primarily due to land use intensification (Sass et al. 2010; Akasaka et al. 2010), urbanisation (Seilheimer et al. 2007) and agriculture (Tilman 1999), which have resulted in a significant reduction of the ecological quality of these ecosystems. In this context, developed countries have attempted to mitigate anthropogenic effects on their water bodies to ensure the conservation of these habitats, which have high biodiversity and endemism (Balian et al. 2008).

Based on the assumption that biological components respond to changes in local conditions, several indices have been developed based on the predictability of the responses of these organisms to anthropogenic influences. In the early 80‘s, Karr (1981) pioneered in suggesting the use of an Index of Biotic Integrity (IBI) based on ichthyofauna to assess the water quality of rivers in North America. Biotic integrity was defined by Karr and Dudley (1981) as —the capability of supporting and maintaining a balanced integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that natural habitat of the region”. The expression —ecological quality”, in turn, defined as —the totality of the functionalities and characteristics of water that support its capability to maintain an appropriate natural fauna and flora” (see Pugh 1996), is currently used to assess the conditions of aquatic ecosystems. The use of this expression reflects the concern not only about the water quality in relation to their consumption and multiple uses, but also the conditions for the existence and well-being of its aquatic organisms. Thus, the assessment of the ecological quality of aquatic environments has attracted considerable interest from both the scientific community and governmental agencies (*i.e.,* the *EU* WFD – European Water Framework Directive).

Several indices have been developed for a wide variety of aquatic organisms, such as macroinvertebrates (Couceiro et al. 2012; Lund and Rech, 2012), algae (Feio et al. 2012; Wu et al. 2012) and aquatic macrophytes (Mackay et al. 2010; Kuhar et al. 2011; Radomski and Peleberg 2012). Recent studies have demonstrated that these biotic components can be useful indicators of the ecological quality of aquatic environments, based on their interactions with physical, chemical and anthropogenic factors (Croft and Chow-Fraser 2007; Testi et al. 2012). In this sense, because of the growing concern with the integrity of diverse natural ecosystems that face several anthropogenic pressures, many studies have been developed to find new ways to assess the effects of these impacts on biotic communities and suggest mitigation actions. Thus, the aim of the present study is to analyze the worldwide importance of IBI to assess the ecological quality of aquatic environments and to provide an overview of ecosystems, organisms and regions that mostly used these indicators, as well as a critical analysis of the trends, applications and limitations of this approach.

In the current study we intend to answer the following questions: (*i*) What is the temporal trend in publications on biotic indicators related to ecological quality? (*ii*) Which continents are the most advanced in studies of ecological quality? (*iii*) What types of aquatic ecosystems receive the most attention? and (*iv*) Which aquatic assemblages are studied most frequently as biotic indicators?

Methods

In August 2014, a survey was conducted using the Thomson Reuters ISI Web of Science database (apps.isiknowledge.com) to list papers published in specialised journals and addressing the assessment of ecological quality of aquatic ecosystems using biotic indices. The survey covered publications in the database appearing during or before 2013. Before performing the survey, we conducted trials with different possible keyword combinations to identify only the terms that were able to filter papers that matched our goals. Based on these trials, we used the following combination of terms in our search by title, abstract and keywords: (("biotic ind*" OR ind*) AND "ecolog* qual*") to filter words such as biotic index, biotic indices, biotic indicators, and necessarily, ecological quality.

Because of the simple counting of the absolute number of publications might cause erroneous deductions regarding the numerical performance of a given research area, we calculated an Activity Index (*AI*) to normalize the publication profile, since it considers the numerical trends of the overall literature. Thus, this approach has been used in several scientometric evaluations (see Kumari 2006, Caliman et al. 2010, Evangelista et al. 2014). To assess the progress of the scientific publications on the ecological quality using IBI, we compared the publications related to IBI and ecological quality to all publications related to only ecological assessment in the same period. We computed *AI* as follows: $AI = (NY/NT)/(TY/TT)$; where *NY* is the number of the publications related to ―ecological quality and IBI" (herein eqIBI) in a respective year (*y*); *NT* is the total number of eqIBI publications for all years studied; *TY* is the number of overall ecological assessment publications in a year (*y*); and *TT* is the number of overall ecological assessment publications for all years studied. *AI* = 1 indicates that publications on eqIBI were published at the same relative rate as those in the overall literature; *AI* >1 indicates that eqIBI were published at a high relative frequency compared to the overall literature; and *AI* < 1 indicates that eqIBI were published at a low relative frequency compared to the overall literature.

The selected articles were fully evaluated, and the following information was obtained: (*i*) the continents where the studies were conducted, (*ii*) the specialised journals in which these types of studies were published, (*iii*) the types of aquatic ecosystems studied, and (*iv*) the aquatic assemblages used as biotic indicators.

Results

Our survey found 674 articles. All these articles were preliminarily evaluated to verify if the search terms were efficient in filtering only those articles that addressed the assessment of ecological quality of aquatic environments through biological components. After this preliminary procedure, 455 articles were retained for further analysis.

For 1996-1997, we did not find any articles related to the assessment of ecological quality of aquatic environments through biotic indices. The number of publications increased over time, primarily after 2004, reaching a peak from 2010 (Fig. 1A). However, although the *AI* was highly variable, it approached 1.0 between 2003 and 2013 (Fig. 1B), indicating that the frequency of publications on ecological quality and IBI was similar to the frequency of publications focusing on other topics related to ecological assessment. *AI* values were predominantly lower until 2009, indicating that research efforts in assess the ecological quality of aquatic systems through IBI were less than the overall ecological assessment output during this time span.

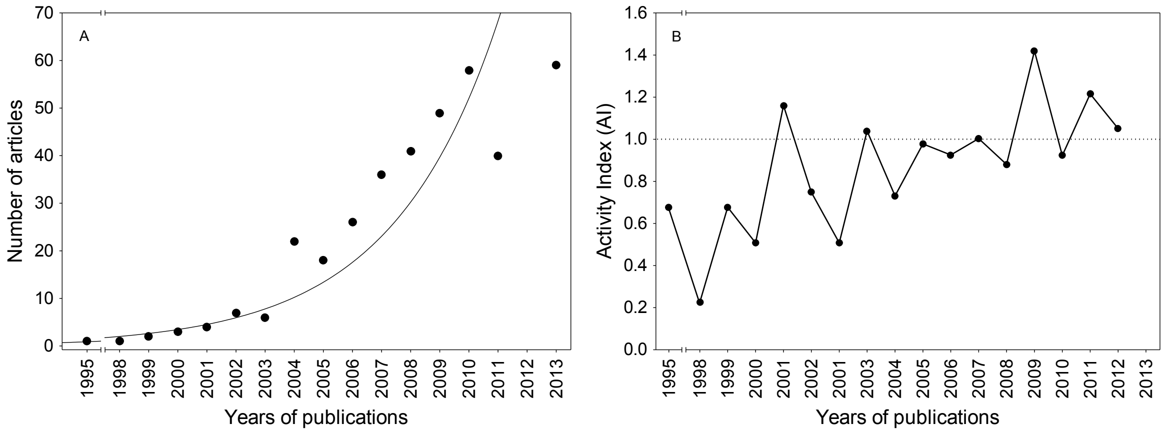

Fig. 1 Temporal trend in the number of papers published per year related to ecological quality and biotic indicators (A). Temporal trend of the activity index (AI) in research output that focuses on ecological quality and IBI relative to all of the studies investigating ecological quality in the same period (B).

Most studies were conducted in Europe (82.25%). Among the European countries, we highlighted Portugal, Spain and Italy as the countries with the highest number of publications (Fig. 2). The smallest number of articles came from the Americas, representing only 4.4% of the total publications, followed by Asia, Africa and Oceania, which together represented 7.47%.

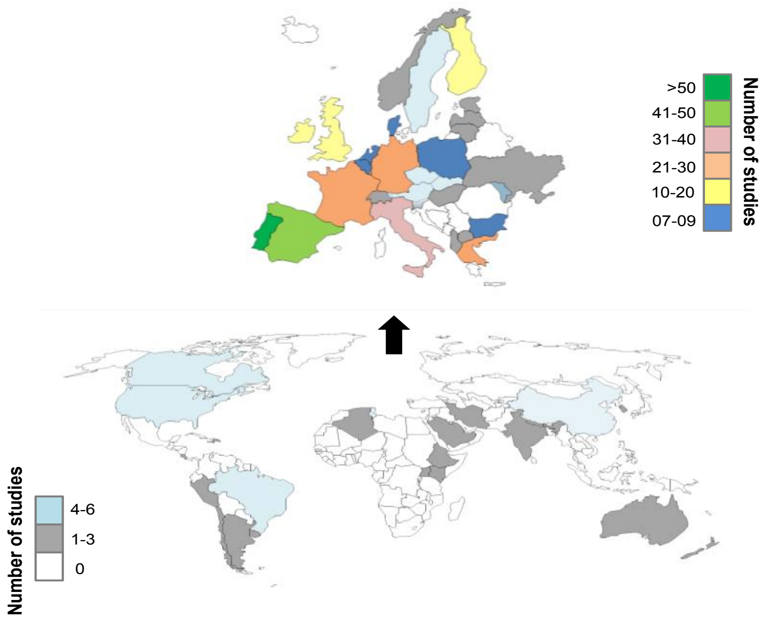

Fig. 2 Geographical distribution of studies related to ecological quality and IBI using colour coding that reflects the number studies per country, with emphasis in the European continent.

The retrieved papers were published in 113 journals, and *Ecological Indicators*, *Hydrobiologia* and *Marine Pollution Bulletin* were the most representative in terms of the number of publications on the subject (Fig. 3). These three journals contributed 18.02%, 15.6% and 14.29%, respectively, of the total number of publications. The journals with fewer than 5 publications per journal contributed 33.41% of the total number of publications.

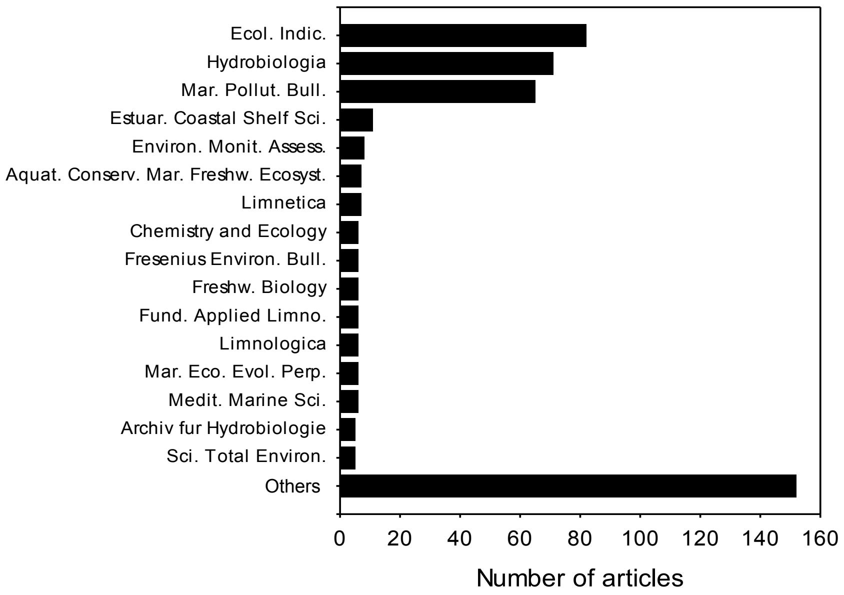

Fig. 3 Number of papers related to ecological quality and biotic indicators published per specialised periodical.

The results of the search showed that coastal zones were the most studied type of environment (43.3%) and that, among freshwater ecosystems, rivers and streams were the most frequently investigated (39.78%), followed by lakes/lagoons (13.63%) (Fig. 4). Only 2.86% of the papers investigated other aquatic environments such as reservoirs, groundwater and ponds.

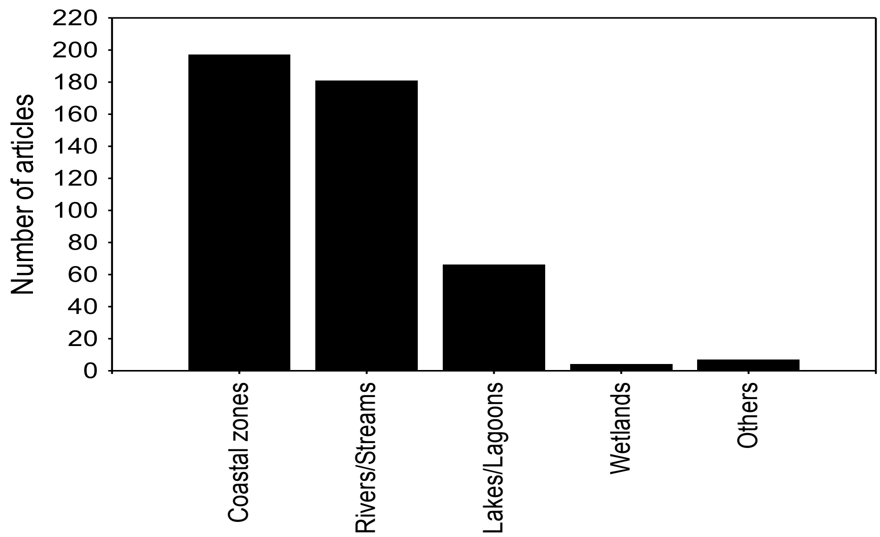

Fig. 4 Number of papers related to ecological quality and biotic indicators published per type of aquatic environment.

Finally, 60.22% of the studies were performed with macroinvertebrates as biotic indicators (Fig. 5). Fish and algae were the subject of an intermediate number of studies, with approximately 10% each.

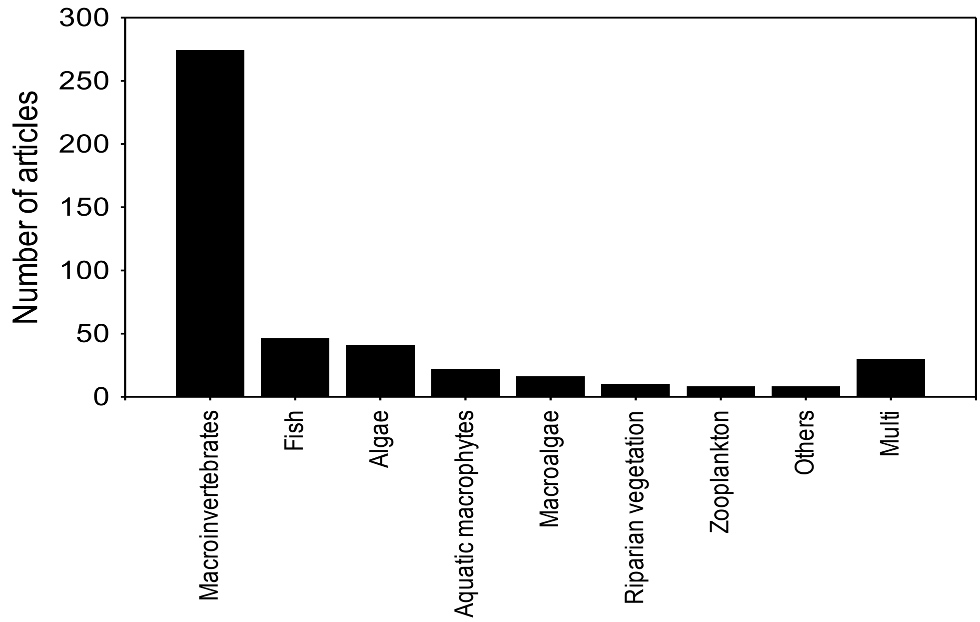

Fig. 5 Number of papers related to ecological quality and biotic indicators published per taxonomic group.

Studies of other groups, such as microorganisms (bacteria), mangroves and coral reefs, received the least attention in the investigations. Studies employing a combination of two or more taxonomic groups represented only 6.6% of the total.

Discussion

Actually, many different terms have been used by the scientific community to assess the quality of the local conditions of aquatic environments. Because of this large number of terms, such as water quality, water health, ecological status, and ecological integrity, among others, in this study we chose to perform the search with only a single term, “ecological quality”, to find an important portion of the studies and then provide a singular overview of this issue. Our results showed that of the papers found by our keyword searches, almost 33% failed to meet the objective of our study. This finding demonstrates that the term “ecological quality” has been used indiscriminately in scientific work without necessarily evaluating the essential meaning of the term. Despite this inconsistency, it was still possible to obtain a considerable number of papers that effectively assessed the ecological quality of aquatic environments through biotic indicators. Thus, it was possible to develop an overview of these studies worldwide.

The number of publications related to the assessment of ecological quality through IBI has increased, especially during the past 10 years. However, the *AI* indicated that research efforts in assess the ecological quality of aquatic systems through IBI was effective from 2009. This increase probably has resulted from the new policy adopted in 2000 by the European Union, the *Water Framework Directive* (WFD). The WFD was implemented to generate a protocol that guarantees the protection of surface waters, groundwater and coastal zones in Europe so that all aquatic ecosystems will be in good condition by 2015. According to the policy, aquatic environments must achieve good ecological and chemical status with the aim of protecting human health, water supply, and biodiversity (for more information, see http://ec.europe.eu/environment/water/water-framework/index_en.html).

The finding that the greatest number of studies was conducted in Europe agrees with the efforts of European countries to meet the objectives of the WFD and restore the good condition of their water bodies. Regarding North America, we expected a larger number of publications, mainly because they were the pioneers in developing multimetric index. However, this result can be explained by the different approach they use, such as predictive models, and not necessarily biotic indices. The low percentage of publications from the other continents, especially Africa, Asia and South America, can indicate that the assessment of ecological quality of aquatic ecosystems in the tropics is still in the early stages. The same conclusion was reached by Ruaro and Gubiani (2013), who found that studies citing Karr (1981), focusing on indices of biotic integrity, have been conducted primarily in North America and Europe. The present water policy of many developing countries still consists of assessing the conditions of aquatic ecosystems only through indices of water quality without considering the biotic components of their environments. In South America, for example, the legislation for monitoring and assessment of surface and groundwater requires the use of indices based on purely physical and chemical variables regardless of the biotic compartments of the aquatic systems. This clear geographical bias leads us to believe that there is currently a limited amount of information that can be useful as a basis for measures to mitigate human impacts on tropical aquatic environments. Thus, planning and implementation of conservation strategies for development in tropical countries are hampered by the lack of basic biological and ecological expertise (Pringle 2000).

Three journals were typically the sources of most publications identified in our survey. *Ecological Indicators* had the greatest number of papers. This predominance may be due to the scope of the journal, which includes ―monitoring and evaluation of ecological and environmental indicators, combined with management practices”. If studies on ecological quality assess and monitor the environments studied, this periodical provides an excellent option, primarily because it offers authors the opportunity to present direct applications of their findings to management. *Marine Pollution Bulletin* also contributed a considerable number of publications. This finding is logical because most studies of interest in this context have been conducted in marine environments. The substantial number of studies conducted on freshwater ecosystems can explain the high ranking of *Hydrobiologia*. Moreover, this journal publishes papers ―investigating the biology of all aquatic environments, including the impacts of human activities”.

In terms of the type of aquatic ecosystems studied, most studies were developed in coastal zones, rivers and streams. Freshwater environments are vulnerable to human stressors that result in, among other things, loss of water quality (Dudgeon et al. 2006). Marine ecosystems, in turn, are among the most threatened by human activities (Halpern et al. 2007), primarily because coastal waters are directly influenced by river inputs or sewage discharges. Thus, the pollution of coastal areas has caused many problems with the structure and functioning of other aquatic communities, also including public health (Islam and Tanaka 2004). For this reason, in recent years, several sets of legislation worldwide (e.g. Oceans Act in USA, WFD in Europe, National Water Act in South Africa, etc.) have been developed in order to address ecological quality, within estuarine and coastal systems. In this sense, the major concern for coastal zones, rivers and streams is totally plausible because an improvement in one produces improvements in the other. Furthermore, the most studied coastal zones were distributed throughout Mediterranean coastal ecosystems, where the WFD policy has been broadly applied. Wetlands, however, received less attention despite their high biodiversity (Junk et al. 2006) and important ecosystem services.

The high percentage of research involving macroinvertebrates that was found in our study indicates that these organisms are the preferred taxa for assessing the ecological quality of aquatic environments. Most likely, the explanation for this result is that this group has a range of characteristics that allow the evaluation of local modifications of habitats, such as sensitivity to pollutants, rapid response to a numbers of stressors, and a sufficiently long life cycle to provide a record of environmental quality. In addition, these taxa have a broad distribution, are abundant and are easily identified (Metcalfe 1989). The number of studies on other groups of organisms was considerably less than the number of studies on macroinvertebrates. Of these other groups, fish and algae were the most frequently represented. Although ichthyofauna were the object of study for the development of the first IBI for assessing the quality of aquatic ecosystems (Karr 1981), our results showed that fish have not generally been investigated for this purpose, whereas macroinvertebrates have clearly been preferred.

Studies investigating other aquatic assemblages, such as aquatic macrophytes, riparian vegetation and plankton, are poorly represented. Aquatic macrophytes, for example, are important components of several aquatic habitats (Lancoul and Freedman 2006) and have an important role in ecosystem structure and functioning (Bornette and Puijalon 2011). Previous studies have demonstrated that this group can respond rapidly to anthropogenic stressors that may be responsible for more marked changes in the composition of macrophyte communities (Aznar et al. 2003; Akasaka et al. 2010; Mackay et al. 2010). Nevertheless, this group has received little attention. In addition, our results showed that the simultaneous use of two or more taxonomic groups for the assessment of ecological quality has apparently been overlooked. This gap indicates that the interest of researchers is to focus only on a single group, although it seems that the simultaneous use of multiple groups of organisms may provide a broader perspective for assessment (Cheimonopoulou et al. 2011). In this regard, since most studies use a single group as indicator of ecological quality, we can only extrapolate the results to other groups if we find high rates of agreement between them. However, studies indicate that the levels of agreement between groups are low (see Heino 2010), greatly restricting our ability to extrapolate. Thus, we suggest that is necessary assess most often other groups, to provide more information to be possible have a less biased analysis.

In summary, our results were able to produce an overview of the studies related to ecological quality and biotic indicators. It was possible to note a lack of uniformity in the distribution of the number of studies by continent. Europe was the focus of almost all the studies. This finding indicates that the European countries are more advanced in terms of environmental legislation. Few studies have focused on assessing ecological quality in tropical aquatic ecosystems. In this context, we highlight the need for future studies in these ecosystems because this type of study can be useful especially for conservation issues and the improvement of legislative tools. Finally, we found a few number of publications that have focused on assessing the ecological quality of aquatic ecosystems based on the examination of more than one group of organisms. These are important and necessary aspects for conservation issues, which should receive greater emphasis in future studies.

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MANUSCRITO 2

Richness and life form richness of aquatic macrophytes in relation to environmental factors in a southeast watershed in Brazil

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Abstract

The species richness of aquatic macrophytes was studied using count data in 137 sampling sites distributed throughout the Itanhaém river basin. Total species richness and richness of emergent, floating and submerged macrophytes was investigated separately. Generalized linear models (GLM) were used to study the role of environmental variables in explaining species richness in aquatic habitats. The GLM identified some variables that positively influence the richness of all taxa and richness of life forms. Overall, water temperature and radiation were the most representative variables. Agriculture, conductivity and nutrients were the variables that most affected macrophyte composition and the species richness decreased with the increasing of these variables.

Keywords: life form; tropical rivers; aquatic plants; GLM; negative binomial

1. Introduction

The modeling of aquatic macrophytes attributes has been widely performed in order to verify their relationship with, mainly, abiotic factors (Chappuis et al., 2012; Hrivnák et al., 2014). Understanding the interactions between macrophyte species and their biotic environments can be very important for predicting ecosystems change and has a broad ecological implication, including assessment, restoration and management. Considering the importance of the aquatic macrophytes to ecosystem functioning, many studies in freshwater ecosystems have concentrated their efforts to explain the response of aquatic macrophytes for both natural covariates and human modifications (Aznar et al., 2003; Akasaka et al., 2010; Alahuhta et al., 2013; Alahuhta et al., 2014). Thus, find out which factors govern the richness and distribution of aquatic macrophytes is one important issue in aquatic ecology.

Species richness is an integrative descriptors of the community, often associated with the health and ecological quality of ecosystems. It is known that macrophyte richness responds differently to ecological gradients. Studies have shown that macrophytes richness can be explained by water quality and climate (Alahuta, 2015), hydro-morphology (Alahuta et al., 2014), morphometric variables and canopy openness (Bando et al., 2015), or even show no relationship with physical characteristics (Balanson et al., 2005). Therefore, in general, it is difficult to draw consistent conclusions about the role of the environmental conditions in explaining aquatic macrophytes due to inconsistencies among results. Many of the relationships between macrophytes and environmental characteristics also depend upon macrophyte life forms. Overall, emergent macrophytes are more influenced by the surroundings conditions and sediment, while floating and submerged species are more influenced by water conditions, such as nutrient availability and light. Some reviews provide a broad discussion about how environmental characteristics can influence aquatic macrophytes (see Barendregt and Bio, 2003; Bornette and Puijalon, 2011).

Once the macrophyte assemblage is directly affected by environmental conditions, previous studies have tried to show the increasing relevance of anthropogenic impacts leading to changes in community composition (Hrivnák et al., 2007; Mackay et al., 2010; Pozo et al., 2011). In this sense, aquatic macrophytes have been widely used as biotic indicator due to their response to changes in local conditions. This assemblage was recognized as good indicator of water quality changes (Søndergaard et al., 2010; Mackay et al., 2010; Aguiar et al., 2011) and integrate many measures of environmental quality (Kuhar et al., 2011; Feio et al., 2012).

Most studies evaluating the influence of environmental conditions on the biological attributes of communities have been developed using multivariate analysis as statistical tool (*e.g.* Rolon and Maltchik, 2006; Capers et al., 2010; Alahuhta et al., 2013). In this work we analyze the determinants of life form occurrence using generalized linear models (GLM), which extend the standard linear models and can accommodated various non-normal errors distributions (Nelder and Wedderburn, 1972). Over the past four decades, advances in statistical analysis provided by GLM have improved the investigation of ecological processes (see Guisan et al., 2002). Currently, many ecological studies have used this statistical approach, because of its ability to handle a larger class of error distributions.

Since many of the data collected in biological studies are poorly represented by normal distributions, GLM provide suitable means for analyzing ecological relationships. In addition, GLM is wide used to deal with typical situations in nature, such as data with excess zeros (Minami et al., 2007; Chelgren and Dunhan, 2015; Hoogenboom et al., 2015), and it was also our case. Thus, the purpose of this study was relate the aquatic macrophytes to the environmental variables in order to identify which natural and anthropogenic factors are most important in structuring the plant assemblages in an entire coastal watershed, using GLM.

2. Material and methods

2.1. Study Area

This study was conducted in the Itanhaém river basin that has an area of approximately 950 km^2 in the southeast littoral of São Paulo state, Brazil (23° 50‘- 24° 15‘S/ 46° 35‘- 47° 5‘W; Fig. 1). The climate of the region is classified as Cfa according to the Köppen system, and is characterized by hot and humid summers and mild winters with no dry months. There are no well-defined seasons and the rainfall is homogeneous throughout the year and there are no seasonal changes in water level.

Most of the basin is located in a coastal plain that extends approximately 16 km from the coast to the escarpment of the sea mountain range, wherein the gap in relation to sea level is about 20 m.

This basin has some particular features such as: *i*) in the upper part of the basin, the headwaters are located in well-preserved areas within the conservation area of the *Parque Estadual da Serra do Mar* (Serra do Mar Park State). However, along the river path is possible to find areas extensively used for banana farming. In these areas the rivers show greater transparency and margin slope and less depth; *ii*) in middle part of the basin the region is predominantly flat and the rivers present few agricultural areas and have a wide variety of natural waters, such as white, black and clear water; *iii*) the lower part of the basin has an estuarine area predominantly occupied by urban area. Thus, this basin was chosen because of its diverse aquatic habitats, including streams, small and large rivers. Moreover, as described above, this area presents different characteristics along natural and anthropogenic gradients. It is therefore an excellent area to assess patterns of species richness and richness of life forms at a water body level.

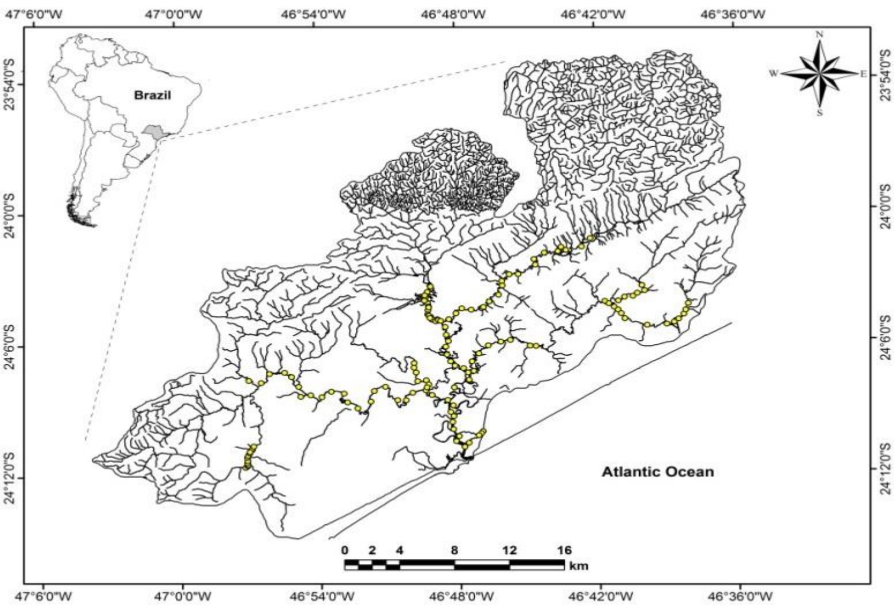

Fig. 1 Sampling points in the Itanhaém river basin environments. Yellow circles represent the 137 sampling sites.

2.2. Sampling

We used aquatic macrophyte data from 137 sampling sites distributed throughout the basin (Fig. 1), sampled between April 2013 and August 2014. The aquatic plants were verified by boat moving at a slow and constant velocity, covering ~ 100 m of the river bank in each sampling site. Submerged species were recorded using a grapple attached to a line. Only macrophytes growing in the water were recorded and the survey included a list of species. Macrophytes were identified to the lowest taxonomic level possible. Then, the species were grouped according to their life form, into emergent, rooted floating, free floating, rooted submerged, free submerged and epiphyte. After inventory, we decided to use three main life form groups: emergent, floating and submerged. Although we had recorded species belonging to rooted floating (*Eichhornia azurea* and *Nymphaea* sp.), free submerged (*Utricularia foliosa*) and epiphyte (*Oxycarium cubense* and *Vigna lasiocarpa*), we think we could include these few species into floating, submerged and emergent life form groups, respectively, and we believe that this decision is unlikely to affect the results. Macrophyte richness, estimated as the number of taxa, was calculated separately for all taxa, emergent, floating and submerged macrophytes (Table 1).

We collected a total of 17 exploratory variables to determine their influence on macrophyte composition (Table 1). Water and physical characteristics, including conductivity, pH, turbidity, dissolved oxygen and temperature were measured *in situ* at the time of macrophyte survey, using multiparameters sensors (Table 1). To determine the concentration of nutrients in water and sediment, samples were collected and conditioned in plastic bottles (3 replicates) for further analysis, according to standard methods (Table 1). In the center of channel we recorded the current velocity of water, depth and photosynthetically active radiation (PAR), and in the margins we determined riparian vegetation cover (Table 1). The digital elevation model of the terrain was obtained through interpolation of topographic maps from the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística—IBGE) on a 1:50,000 scale and vertical topographic lines of 20 m (available at http://www.ibge.gov.br) of the study area with ArcGIS 9.3 (Environmental Systems Research Institute—ESRI). Data on the land use of watershed comprise proportion of agricultural and urbanization, determined using buffer zones with distances of 250 m from the river shoreline, and data were also derived from the GIS database.

Table 1 Descriptive statistics of explanatory variables for 137 sampling sites distributed throughout the Itanhaém river basin. Mean ± standard deviation, range (minimum and maximum) and their respective methods employed.

	Methods	Mean ± SD	Range
Explanatory variables			
Water Chemistry			
Conductivity (µS.cm^{-1})	HORIBA U10	0.93±2.41	0.02 - 10.48
pH		5.97±0.70	4.67 - 7.16
Turbidity (NTU)		16.88±13.57	3.59 - 86.21
Dissolved Oxygen (mg.L^{-1})	Oxímetro digital YSI - 55	5.98±1.38	2.44 - 8.68
Total nitrogen (mg.L^{-1})	Mackereth et al. (1978)	0.29±0.8	0.1 - 4.9
Total phosphorus (µg.L^{-1})	Golterman et al. (1978)	25.75±19.82	10.63 - 141.36
Physical			
Depth (m)	Laylin SM5 Depth Sounder	0.65±0.11	0.29 - 0.98
Declivity (°)	Suunto Clinometer	19.49±12.6	0.78 - 83.58
Temperature (°C)	HORIBA U10 multiparameters	20.52±0.42	19.47 - 21.31
Photosynthetically active radiation (µmol.s^{-1}.m^{-2})	LI-COR LI-250A Light Meter	198.05±72.69	59.19 - 394.79
Current velocity m.s^{-1}	Global Water Flow Probe	0.52±0.18	0.18 - 1.13
Sediment total nitrogen (%)	Allen et al (1974)	17.88±8.24	4.41 - 64.53
Sediment total phosphorus (%)		15.82±16.47	1.45 - 92.27
Landscape			
Vegetal cover (%)	Wildco canopy densitometer	53.57±5.85	0 - 83.58
Elevation (m)	Digital elevation model	16.04±5.98	0 - 20
Agriculture (km^2)	GIS database	0.031±0.059	0 - 0.196
Urban área (km^2)		0.004±0.022	0 - 0.139

2.3. Data Analysis

Prior to performing the analysis, we tested for correlation among environmental variables, by constructing a correlation matrix using Pearson‘s correlation coefficients. We considered variables with significant correlation coefficients when -0.75 > *r* > 0.75. Thus, only one of the variables was retained for further analysis. In this study, the following variables were excluded: salinity and elevation, which were highly correlated with conductivity, and urban area that was highly correlated with all nutrients from both water and sediment.

Total species richness (all taxa) and richness of each life form (emergent, floating and submerged taxa) was modeled separately by GLM as responses to the explanatory variables. The models were initially fit using the Poisson distribution, appropriate for count data. The Poisson distribution assumes that the variance is equal to the mean. However, in our case, the excessive number of zeros led to overdispersed data, term used for any data set or model where the variance exceeds the mean (McCullagh and Nelder, 1989). Overdispersion count data have been modeled using several approaches, typically based on quasi-Poisson or the negative binomial models (Hinde and Demétrio, 1998; Ver Hoel and Boveng, 2007; Okamura et al., 2012). We used this last approach to accommodate the overdispersion, using negative binomial model with canonical link function. A backward selection process using Akaike information criterion (AIC; Akaike, 1974) was used to select the most relevant environmental variables. Models were validated using the examination of model residuals (Half-normal plot; Atkinson, 1985). The statistical analyses were performed using the *glm.bn* model from the package *MASS* (Venables and Ripley, 2002) and *hpn* from the package *HPN* (Moral et al., 2015) in *R* software, version 3.0.2 (R Core Team, 2013).

3. Results

3.1. Total richness of macrophyte assemblage

A total of 44 taxa of aquatic macrophytes was recorded (Appendix 1). The most frequent species found in the basin were *Egeria densa* (55 occurrences), *Eichhornia azurea* (41 occurrences), *Salvinia molesta* (35 occurrences), *Cabomba furcata* (28 occurrences) and *Urochloa arrecta* (25 occurrences). Macrophytes species richness ranged from zero to nineteen species per sampling site, and in terms of proportion of taxa per life form, emergent is the most representative life form, presented 61.36% of all macrophytes; the rest was shared between floating (25%) and submerged (13.64%). The Figure 2 illustrates the descriptive results of all plant richness and life forms richness registered in the sampling sites.

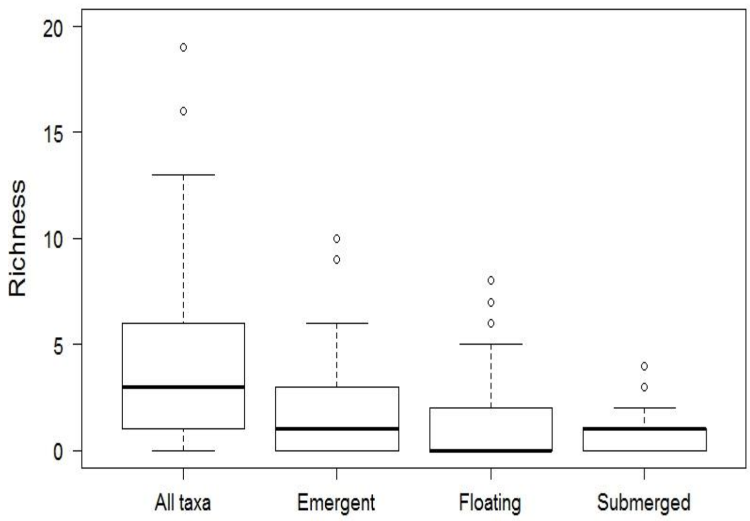

Fig. 2 The box plots of macrophyte richness in the Itanhaém river basin. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, and whiskers are non-outlier ranges beyond the boxes. Empty circles represent outliers values.

For all response variables, the models were validated examining the residuals through half-normal plot with a simulation envelope (Fig. 3). For all taxa, the best model selected five significant variables, from which PAR and water temperature were positively related to macrophyte richness, while agriculture, conductivity and pH presented a negative relationship (Table 2). The half-normal plot in Fig. 3a shows no evidence of an inadequate model, since most of observed residuals lying out of simulated envelope.

3.2. Life form richness

A total of 27 emergent macrophyte taxa were recorded in this study. Emergent taxa ranged between 0 and 10 per site. The most frequent emergent species registered were the Poaceae *Urochloa arrecta* (18.24%) and *Hymenachne amplexicaulis* (17.51%). The variable selection retained six significant variables and the emergent plants were positively related with PAR, total phosphorous in sediment and water temperature, and negatively related with total nitrogen in sediment and turbidity. For emergent taxa, the Fig. 3b shows that most of the deviance residuals are within the simulate envelope, indicating a good fit.

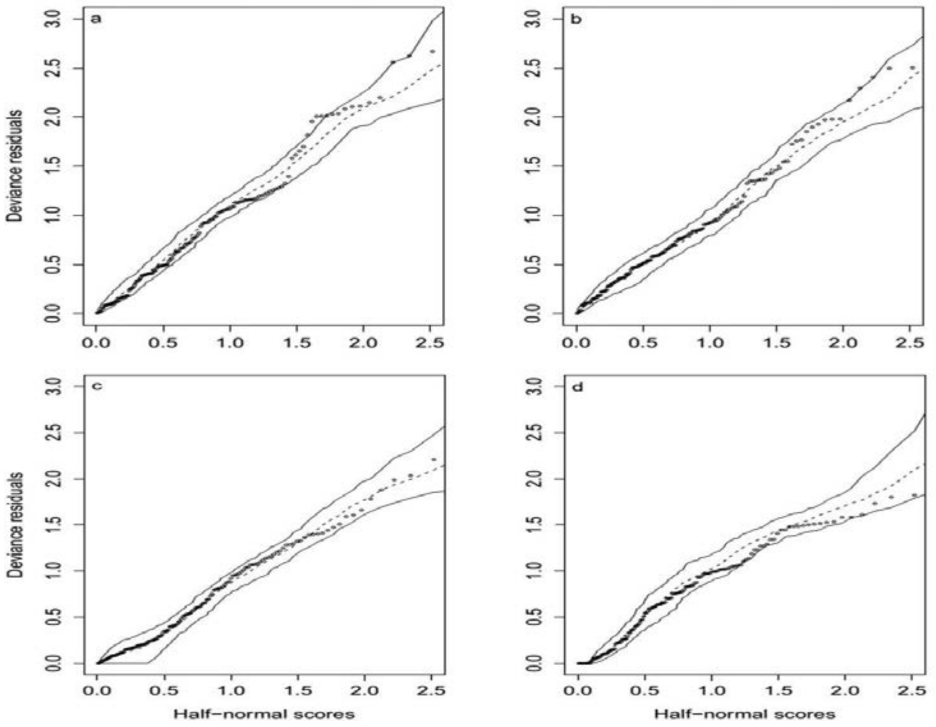

Fig. 3 Half-normal plots of deviance residuals for negative binomial models for (a) total richness, (b) emergent taxa, (c) floating taxa and (d) submerged taxa.

We recorded 11 floating taxa and ranged from 0 to 8 taxa per site. The most frequent floating species were *Salvinia molesta* (25.54%) and *Eichhornia crassipes* (14.59%). For floating macrophytes, GLM selected six significant variables. Water temperature was positively related with floating plants, along with total nitrogen, while pH and sediment total nitrogen and total phosphorous were negatively related.

Table 2 The environmental variables significance based on the results of negative binomial models for all dependent variables

	Estimate	SE	z-value	p-value
All taxa				
Agriculture	-0.43	0.11	-3.78	***
Conductivity	-0.19	0.09	-2.15	*
pH	-0.33	0.08	-3.79	***
PAR	0.27	0.06	4.31	***
Temperature	0.18	0.07	2.54	*
Emergent				
Agriculture	-1.11	0.21	-5.27	***
PAR	0.32	0.06	4.72	***
Sediment total nitrogen	-0.73	0.15	-4.68	***
Sediment total phosphorous	0.42	0.09	4.31	***
Temperature	0.24	0.06	3.52	***
Turbidity	-0.43	0.10	-4.13	***
Floating				
Agriculture	-1.75	0.55	-3.15	**
pH	-0.80	0.16	-5.00	***
Sediment total nitrogen	-0.99	0.32	-3.07	**
Temperature	0.63	0.17	3.62	***
Total nitrogen	1.38	0.34	4.05	***
Total phosphorous	-2.24	0.49	-4.54	***
Submerged				
Conductivity	-4.00	1.75	-2.28	*
Dissolved oxygen	-0.34	0.13	-2.54	*
PAR	0.21	0.10	2.11	*
Sediment total nitrogen	0.24	0.07	3.30	***

Significance codes: 0 ‗*** ' ; 0.001 ‗** ' ; 0.01 ‗* ' .

A total of 6 submerged macrophytes were registered, ranging from 0 to 4 per site. The most frequent species were *Egeria densa* (40.14%) and *Cabomba furcata* (20.43%). The GLM identified five significant variables and submerged plants were positively related with PAR and sediment total nitrogen, but negatively with conductivity, dissolved oxygen and sediment total phosphorus.

Considering only life forms, overall, it was possible to verify the negative relationship with nutrients. Besides, with exception of submerged macrophytes, agriculture was the most relevant variable that influenced the macrophyte composition. Regarding the adjust of the models for floating and submerged taxa, both models presented a satisfactory fit, since Fig. 3c,d shows that all the observed residuals are lying within the simulated envelope. Overall, we consider that binomial negative model was adequate to analyze this data set.

4. Discussion

Considering only regional (landscape) variables, macrophyte assemblage richness is negatively related, overall, to land use. Agriculture was negatively related to richness of all taxa, emergent and floating species. It is expected that land use had a stronger effect on macrophytes (Akasaka et al., 2010; Alahuta et al., 2014), since it is often used as a proxy from anthropogenic-derived nutrients increase in water (Alahuta et al., 2011). However, many other explanations can possible help to understand the role of this land use in our result. Based in our data and prior knowledge of basin, we considered the negative relationship between richness (for both all taxa and emergent and floating taxa) and agriculture a spurious correlation.

The agriculture *per se* is not responsible for the lower richness of all taxa and life forms. The sites with high agriculture pressure are located in upper part of the basin, with influence of elevation, declivity of margin and current velocity of water. These natural characteristics do not promote the colonization of emergent and floating species. In the other hand, it seems to favor the occurrence of submerged species, reason why submerged was the only life form that was not negatively related to agriculture. This type of situation is quite common in ecology and the imprecise interpretation of the data can suggest erroneous causal relationships (see more in Garsd, 1984).

The urbanization, in turn, is a category of land use we believe exert more influence on macrophyte richness, due to the nutrient input, as confirmed by several studies (Edvardsen and Okland, 2006; Alahuta et al., 2013, 2014; Baláži et al., 2014). The urbanization was removed from our analysis since it was strongly related to all nutrients. Thus, the concentration of nutrients may be used as a surrogate of urban development. Different levels of external nutrient input promote a succession of aquatic macrophytes. In the pioneer stadium of enrichment, the vegetation mainly consists in submerged species, being gradually replaced by floating and emergent species (Portielje and Roijackers, 1995). In upper part of the basin, the flora is dominated by submerged species, where we found lower values of nutrients. In the middle part of the basin it was possible to find higher diversity of species and life forms, since we found intermediate degree of trophy. In the lower part of the basin, where the area is predominantly urban, the flora consists only of emergent species. The higher concentration of nitrogen and phosphorous in sediment were negatively related to floating and submerged life forms. Despite floating species are generally dominants in eutrophic environments, we believe that other factors are involved to the decline of species richness in this zone. In the lower part of the basin, the highest conductivity gradient registered is related to the salinity since this area is near to mouth of the Itanhaém river that flows into the Atlantic Ocean. Thus, despite the availability of nutrients, salinity is a limiting factor for the development of the species, being restricted colonization by tolerant species (Barendregt and Bio, 2003; Thouvenot et al., 2012), such as *Spartina alterniflora* and *Scirpus californicus*, both emergent species. This result corroborate with the negative relationship of conductivity with richness of all taxa.

Considering local factors, GLM identified some variables that positively influence the richness of all taxa and richness of life forms. Overall, temperature and PAR were the most representative variables. This result is in accordance with the findings by others studies (Madsen et al., 2006; Lacoul and Freedman, 2006). Water temperature of water seems to be one of the most important variables determining the distribution, productivity (Dar et al., 2014) and composition of macrophyte assemblages (Baláži et al., 2014).The higher values of these variables were found in the middle part of the basin and is congruent with the higher diversity of species and life forms in this area. In our case, we believe that water temperature and PAR may be associated with reduced riparian vegetation cover, which usually provides shade over water bodies. The increase in incident solar radiation due to the lack of vegetation cover may favor the establishment of emergent species, which, in turn, may act as anchors for floating species, leading to an increase in the number of species. In fact, emergent species can promote additional structural complexity to the littoral regions and provide habitats for other assemblages (Agostinho et al., 2007; Amorim et al., 2015).

Human modifications, such as alteration of riparian vegetation integrity, may strongly influence macrophytes composition, especially as regards the replacement of specialized species by generalist or even exotic species (Aznar et al., 2003). In this sense, it is important to remember that the most frequent emergent species registered in the sampling sites is the exotic highly invasive Poaceae *Urochloa arrecta.* This species is known to invade tropical and subtropical zone worldwide (Reinert et al., 2007; Pott et al., 2011) and recent studies have shown its negative impacts on native biota (Michelan et al., 2010; Amorim et al., 2015). Studies have suggested that the presence of riparian vegetation can prevent the invasion by grasses in aquatic ecosystems owing to the reduction in light availability at the water surface (Loo et al., 2009; Thomaz et al., 2012). Based in this kind of knowledge we can speculate that our results are important to support management strategies.

The aquatic macrophyte community may be influenced by environmental variables at different scales in the landscape (Barendregt and Bio, 2003). The diversity in local assemblages can be regulated by regional and local factors, and the relative importance of these factors on biotic composition is still uncertain since they act in different ways depend on the ecosystem studied. Most studies relating the relative roles of environmental variables in driving variation in aquatic macrophytes are developed in lakes, ponds and reservoirs. In this study we tried to provide basic information about the environmental factors which support the species richness and richness of life forms in an entire watershed.

According to the results presented in this paper, we verified that some specific variables are related to the structure of macrophyte assemblage along the basin. In Itanhaém river basin, it is possible that the interaction between water variables and external factors may be responsible to determine the characteristics of the upper, middle and low part of the basin and, this reflects in the response of the aquatic macrophyte. In the upper part of the basin, the diversity and richness of life form were more influenced by natural conditions. In the middle-low part, natural variables along with variables indicative of human activities strongly affected the assemblage. It is interesting to highlight that some variables can often be related to variables that have not been selected by the model, but also help to explain the patterns found. However, this interaction occurs in different ways in nature, making it difficult to ascertain which are the main determinants of the macrophyte composition. Therefore, we believe that future researches could focus in study the individual relative importance of natural covariates, human disturbance and spatial variables in explaining the variation of aquatic macrophyte assemblages, in order to help to elucidate what govern their distribution in aquatic ecosystems.

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Appendix 1

Aquatic macrophyte taxa recorded in Itanhaém river basin. Life form code: EM - emergent, FF - free floating, RF - rooted floating, RS - rooted submerged, FS - floating submerged and EP - epiphyte.

Family	Taxa	LF
Acanthaceae	*Hygrophila costata* Nees & T. Nees	EM
Alismataceae	*Echinodorus cordifolius* L. Griseb.	EM
Amaryllidaceae	*Crinum procerum* Herb. & Carrey	EM
Araceae	*Lemna valdiviana* Phil	FF
	Pistia stratiotes L.	FF
Araliaceae	*Hydrocotyle ranunculoides* L. f.	EM
Cabombaceae	*Cabomba furcata* Schult. & Schult. f.	RS
Characeae	*Nitella furcata* (Roxb. ex Bruz.) Ag. emend. R.D. Wood	RS
Commelinaceae	*Commelina schomburgkiana* var. *brasiliensis* Seub.	EM
Cyperaceae	*Cyperus giganteus* Vahl	EM
	Eleocharis interstincta (Vahl) Roem. & Schul.	EM
	Eleocharis mínima Kunth	EM
	Fiurena umbellata Rottb.	EM
	Oxycaryum cubense (Poepp. & Kunth) Lye	EP
	Rhynchospora corymbosa (L.) Britton	EM
	Scirpus californicus Steud.	EM
Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc.	EM
Hydrocharitaceae	*Egeria densa* Planch	RS
	Limnobium laevigatum (Humb. & Blonpl. ex Willd.) Heine	FF
Leguminosae	*Vigna lasiocarpa* (Mart. ex Benth.) Verdc.	EP
Lentibulariaceae	*Utricularia foliosa* L.	FS
Mayacaceae	*Mayaca fluviatilis* Aubl.	EM
Menyanthaceae	*Nymphoides indica* (L.) Kuntze	RF
Nymphaeaceae	*Nymphaea sp*	RF
Orchidaceae	*Habenaria repens* Nutt.	EM
Poaceae	*Echinochloa polystachya* (Kunth) Hitchc.	EM
	Hymenachne amplexicaulis (Rudge) Nees	EM
	Leersia hexandra Sw.	EM
	Panicum pernambucense (Spreng.) Mez ex Pilg.	EM
	Paspalum repens Berg.	EM
	Spartina alterniflora Loisel	EM
	Urochloa arrecta (Hack. ex. T. Durand & Schinz) Morrone & Zuloaga	EM
Potamogetonaceae	*Potamogeton pusillus* L. subsp. *pusillus*	RS
	Potamogeton polygonus Cham. & Schltdl.	RS
Polygonaceae	*Polygonum acuminatum* Kunth	EM
	Polygonum ferrugineum Wedd.	EM
Pontederiaceae	*Eichhornia azurea* (Sw.) Kunth	RF
	Eichhornia crassipes (Mart.) Solms	FF
	Heteranthera multiflora (Griseb.) C. N. Horn	EM
Pteridaceae	*Ceratopteris thalictroides* (L.) Brongn	FF
Ricciaceae	*Ricciocarpos natans* L. (Corda)	FF
Salviniaceae	*Azolla filiculoides* Lam	FF
	Salvinia molesta D.S. Mitch	FF
Typhaceae	*Typha domingensis* Pers.	EM

MANUSCRITO 3

Can we monitor the ecological quality of rivers in Brazil using macrophytes? A case study in the Itanhaém River Basin, São Paulo.

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Abstract

This study sets out to explore the use of macrophytes as indicators of the river ecological conditions in tropical rivers, in order to develop a multimetric index of biotic integrity (IBI). We used environmental, floristic and anthropogenic disturbance data from a 950 km^2 river basin at the south littoral of the São Paulo State, Brazil (Itanhaém river basin). Vegetation surveys were carried out in 2013 in 137 sites representing a range of limnological and watershed characteristics. Using the environmental data set, we first performed a hierarchical clustering with Ward's method to define the existing river types. Then, we identified undisturbed and degraded sites of each river type using the gradient of stressor variables. A set of candidate metrics was compiled based on cover, diversity and functional attributes of aquatic vegetation (e.g. origin, life forms), and compared in each river condition. We found that degraded sites had significantly less species richness and lower diversity of life forms than undisturbed ones for the floodplain river type, allowing the development of an IBI with a good discriminatory efficiency. The present results allow a better understanding of ecological conditions in tropical rivers and consists a relevant contribution for the implementation of legislation to monitor and assess the ecological status of superficial waters in the tropics.

Keywords: tropical basin; macrophyte IBI; clustering; river monitoring

1. Introduction

Aquatic environments are among the most threatened in the world. Among them, freshwater ecosystems are threatened by intensive agriculture (Tilman, 1999), urbanization (Seilheimer et al., 2007), overfishing (Kang et al., 2009). Consequently, freshwater diversity is endangered all over the world, so what, in the recent years there has been an attempt to develop new politics to assess and monitor the ecological quality of these environments.

The importance of the assessment of biological conditions in aquatic ecosystems was recognized since Karr (1981) provided the ―Index of Biotic Integrity", as a tool to assess the effect of multiple stressors in aquatic environments. Actually, the basis of water policy and management has been made through direct measurements of the biotic condition of aquatic ecosystems, in function of recent legislative determinations (*e.g.* Clean Water Act – USA and Water Framework Directive - EU). These kind of legislative tools aim to improve the water quality through the holistic environmental view, putting aquatic ecology at the base of management decisions. For that, the development of reliable methods to ensure the physical, chemical and biological integrity has been the central point of the efforts for the aquatic monitoring.

In several countries, the legislation for monitoring and assessment of surface and groundwater still remains in the use of indices based on purely physical and chemical variables regardless of the biotic compartments of the aquatic systems. Thus, currently there is a limited amount of biological information that can be useful as a basis for measures to mitigate human impacts on aquatic environments. This kind of information is very valuable especially in development countries such as Brazil, where critical habitats have been affected, polluted or even destroyed by agricultural intensification (Barretto et al., 2013), deforestation (Fernside, 2005), and constructions of dams (Fernside, 2006; Sanches et al., 2006).

Based on the premise that biological members respond to changes in local conditions, several indices were developed supported in the predictability of responses of these organisms to human influence. We highlight, for example, macroinvertebrates (Couceiro et al., 2012; Lund and Resh, 2012), algae (Feio et al., 2012; Wu et al., 2012) and macrophytes (Mackay et al., 2010; Dodkins et al., 2012).

Aquatic macrophytes, which are the focus of this study, are important components of many aquatic environments and display an important role in the functioning of the ecosystems. This group may be useful for the monitoring of ecological status of aquatic ecosystems and has been recognized as a fundamental biological indicator for river assessment by the Water Framework Directive (European Commission, 2000).

Many studies have tried to use macrophytes as ecological indicators of quality of the different aquatic environments, such as lakes (Nichols et al., 2000; Beck et al., 2010; Radomski and Peleberg, 2012), rivers (Kuhar et al., 2011) and streams (Feio et al., 2012). This community has become increasingly important for their use as ecological indicators because the plant community can respond to anthropogenic influence that may be responsible for more intense changes in their natural conditions (Aznar et al., 2003; Akasaka et al., 2010; Mackay et al., 2010). Thus, our main goal was to develop a macrophyte-based IBI to test the suitability of aquatic macrophytes to discriminate between rivers sites of different environmental conditions in a Brazilian littoral river basin. This river basin is influenced by different kinds of anthropogenic pressures, such as agriculture and urbanization. So, we believe that the development of an index that can be used to indicate the degree of human impact and the resultant influence on aquatic macrophyte assemblage can be a significant contribution for conservation and management. Besides, despite long research traditions, bioassessment through aquatic macrophytes in the tropics is still limited.

In this study we first tried to establish a typology of rivers based on environmental characteristics in order to identify similar group sites. After that, we distinguish the disturbed and undisturbed sites of the selected groups based on stressor variables. Lastly, we qualified the macrophyte response to different types of river conditions. Thus, in this work, we: (i) tested the suitability of aquatic macrophytes to assess the ecological quality of tropical rivers through a biotic index of integrity, and (ii) analyzed of performance of the aforementioned approach.

2. Material and methods

2.1. Study area

Itanhaém River basin is located in the south littoral of the São Paulo State, Brazil (23° 50‘- 24° 15‘S/ 46° 35‘- 47° 5‘W) (Fig.1). The catchment area is approximately 950 km^2 and most of the basin is located in a coastal plain that extends about 16 km from the coast to the escarpment of the sea mountain range. The basin is predominantly flat from the coast to the regions adjacent to the sea mountain range, wherein the gap in relation to sea level is about 20 m. The climate of the region is classified as humid subtropical climate (Cfa), according to the Köppen Climate Classification System, and is characterized by hot and humid summers and mild winters with no dry months. The long-term average annual rainfall in the study area is 2175 mm and is homogeneous throughout the year and there are no seasonal changes in water level. There are no defined seasons and annual mean of temperature is 22.74 °C.

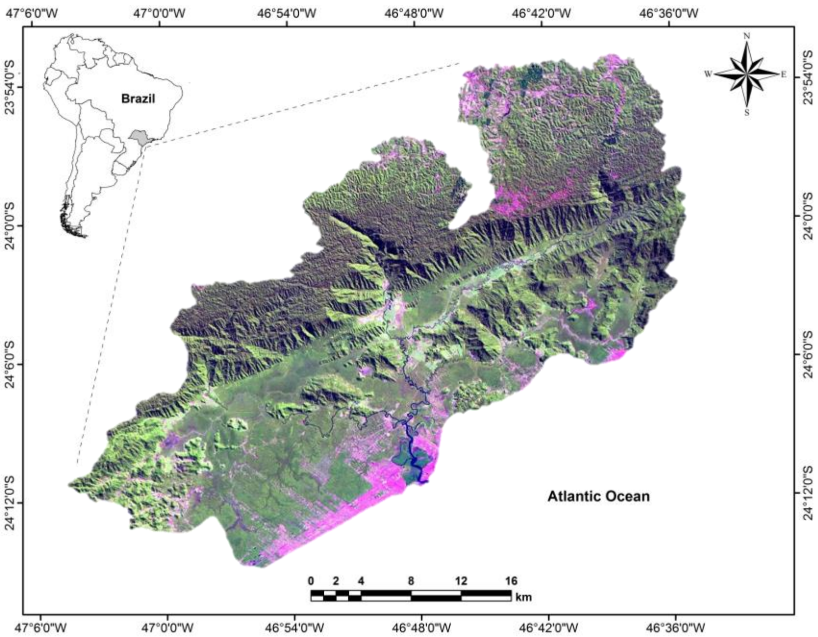

Fig. 1 Satellite image of Itanhaém river basin.

137 sampling sites were surveyed throughout nine rivers geographically located in study area (Fig. 2a). All sites were surveyed once during 2013-2014, as part of the official monitoring approach for Itanhaém river basin (Long Term Ecological Research, PELD-ITA).

2.2. Environmental surveys

Water and sediment samples were collected for determination of nutrients analyzed according to standard methods (Allen et al., 1974; Mackereth et al., 1978;

Golterman et al., 1978). Conductivity, dissolved oxygen, pH, turbidity and water temperature were measured *in situ* at the time of macrophyte survey, using Horiba U10 multiparameters. Water velocity and underwater radiation in each site were recorded with a Global Water Flow Probe and with LI-COR LI-250A light meter, respectively. The depth was measured with Laylin SM5 Depth Sounder. The digital elevation model was obtained through interpolation of topographic maps from the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística—IBGE) on a 1:50,000 scale and vertical topographic lines of 20 m (available at http://www.ibge.gov.br) of the study area with ArcGIS 9.3 (Environmental Systems Research Institute—ESRI). In addition, we gathered data on land-use at a 250 m buffer around the site and classified land-uses into 2 classes: urban areas and agricultural systems. Data on the land use of watershed were also derived from the GIS database.

2.3. Macrophyte surveys

Macrophytes surveys were conducted over a 100 m length at each of the 137 sampling sites, including both inner banks and the main channel. The aquatic macrophytes were verified by boat moving at a slow and constant velocity, covering ~ 100 m of the shoreline in each sampling point. Only river macrophytes growing in the water were recorded and the survey included a list of species and estimated vegetation cover. The cover of each macrophyte species was sampled using quadrats (1m x 1m) that were placed along transects perpendicular to the shore line. Within the transects, the distance between successive quadrats was constant, and the number of quadrats per transect varied according to the length of macrophyte stand. Inside each quadrat ($1m^2$), macrophytes were estimated visually and converted to a categorical value using classic Braun-Blanquet scale (Braun-Blanquet, 1928) to cover percentages (0, 5, 15, 37.5, 62.5, 87.5% cover), and submerged species were verified using a grapple attached to a line. Macrophytes were identified to the lowest taxonomic level possible. Aquatic macrophytes have been first grouped according to their life form, into emergent, rooted floating, free floating, rooted submerged, free submerged and epiphyte. However, as we had few and rare species in epiphyte (2), rooted floating (2), and free submerged (1), we decided to include them into wider groups as emergent, floating and submerged, respectively. Total macrophyte cover per site was determined as the average of the quadrats recorded at each sampling site. Species richness was calculated as the total number of taxa recorded per site, and the percentage of each growth form was calculated as a proportion of the total number of species present at each site. For the purpose of macrophyte index development, several metrics were proposed. Table 1 summarizes these candidate metrics.

Table 1 Potential macrophyte assemblage metrics tested to the construction of the macrophyte index of biotic integrity for Itanhaém river basin (MIBI-ITA) and their descriptions.

Metrics of macrophyte assemblage	Unit
Number of taxa	
Total	Total richness per site
S emergent S floating S submerged S native S exotic	Number of taxa per site
Macrophyte cover	
Emergent taxa cover Floating taxa cover Submerged taxa cover Native taxa cover Exotic taxa cover	Percentage cover expressed by Braun-Blanquet scale
Proportion of taxa	
Emergent Floating Submerged Native Exotic	Percentage of taxa per site

2.4. Development of Macrophyte index of biotic integrity in Itanhaém (MIBI-ITA)

The index was developed in the following steps:

Step 1: Determination of a river typology - we carried out a classification analysis using the environmental data based on local, landscape and climate variables, in order to create a typology based only on natural characteristics of the basin (Table 2). We used only environmental variables we considered important to describe the natural conditions of the sampling sites, and which not represent pressure gradients, that is, all stressors variables were excluded from this step. This procedure is necessary since river assessment must be based on river type specific conditions to be liable to comparison. For this, we performed a cluster analysis using thirteen variables, with a matrix of similarity built using the Euclidean distance coefficient, available in *vegan* package (Oksanen et al., 2011) in the software R (R Development Core Team, 2013). We chose Euclidean distance because our input data describe only environmental variables. The cluster was ordered by the “ward” method. After verify the pattern exhibits in the cluster analysis, we explore the influence of variables in sampling sites through a principal component analysis (PCA);

Step 2: Classification of the undisturbed and degraded sites - after verifying the discrimination of groups based on their natural conditions, we tried to identify undisturbed and degraded sites within each typology group using the gradient of stressor variables, also through a cluster analysis. For this purpose, we used eight variables to describe potential anthropogenic impacts as nutrients, dissolved oxygen, turbidity and land use (Table 2). After that, we used box plots to confirm the distribution values of undisturbed and degraded sites;

Step 3: Selection of candidate metrics - to determine if the biological descriptors could be discriminated by undisturbed and degraded sites we used macrophyte composition data to calculate univariate assemblage metrics. Each potential metric was transformed to dimensionless number for aggregation into a quality index, following Aguiar et al., (2009). After that, each metric was tested for each group type responding to environmental stress. Box plots were used to correlate metrics against undisturbed and degraded sites. A metric was selected as candidate if the interquartiles (25th/75th percentile) of undisturbed and degraded sampling sites were not overlapping;

Step 4: Index development - we used three quality scores for macrophyte metrics selected, following Karr (1986): 1 – Bad, 3 – Moderate, 5 – Good quality. The good-fair boundary was limited by mean of undisturbed sites and the poor-fair boundary by the mean of degraded sites. The MIBI-ITA for each sampling site was obtained by the sum of the quality scores of all core metrics, subtracted by the total number of core metrics, following Aguiar et al., (2009). The WFD requires that bioassessment must be presented in the form of Ecological Quality Ratio (EQR). Thus, we followed the standard procedure, and all metrics results of MIBI-ITA were transformed into EQR values. The EQR is represented by the following equation:

$$\frac{\mathrm{EQR}\ \ (\mathrm{MIBI\ ITA})\ \ n}{md}$$

where *n* is the number of metrics and *md* is the median value from the distribution of the reference sites of MIBI-ITA. The EQR is expressed as a numerical value between 0 and 1. High ecological status is represented by values close to 1, whereas bad ecological status is represented by values close to 0. We used five ecological quality classes: 1 – Excellent, 2 – Good, 3 – Moderate, 4 – Poor and 5 – Bad. The reference value of excellent/good boundary was determined as the median value of the MIBI-ITA at the reference sites. The boundary value of the four remaining classes of ecological quality is obtained by dividing equally the interval limited by the Excellent/Good boundary and the lower extreme of the gradient (Aguiar et al. 2009);

Step 5: Assessment of index performance - after the development of MIBI-ITA, we applied it, in turn, in a similar study area to evaluate the ecological conditions of this aquatic environment based on our index. For this, we sampled a total of 27 sites in Bertioga coastal plain, geographically close to Itanhaém river basin (approximately 80 km), and with similar environmental characteristics. We applied the MIBI-ITA using the same criteria in order to calculate the EQR for each sampling site.

3. Results

3.1. River sites typology

The cluster analysis with environmental data carried out in order to create a typology based on natural variables revealed two groups. The result of the clustering is shown in an illustration form, where it is possible to verify the formation of two distinct groups: one in the upper part of the basin and one in the middle-low part (Fig. 2a, Table 2).

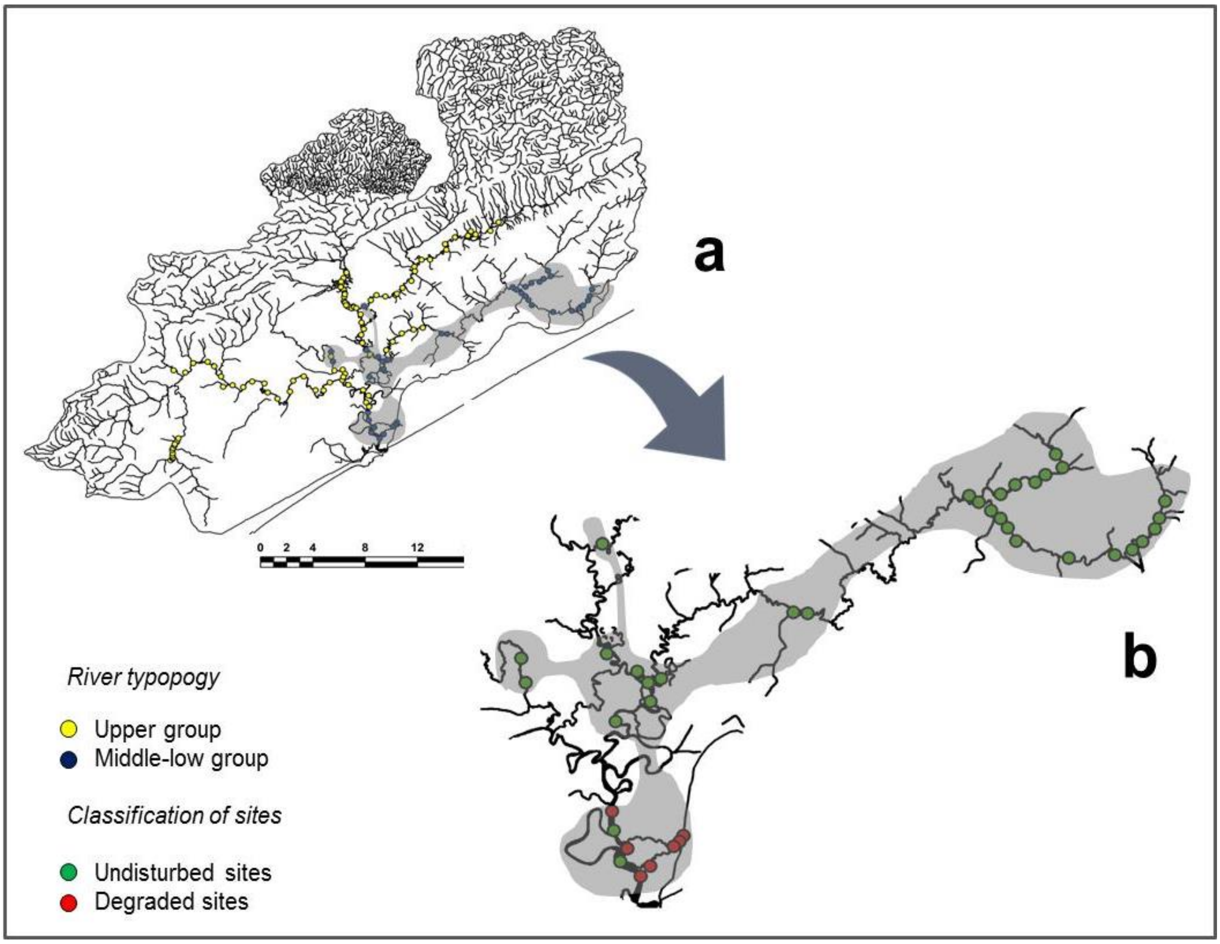

Fig. 2 Sites sampled in the Itanhaém river basin: (a) two main groups distinguished by typology; (b) middle-low group used to IBI development indicating degraded and undisturbed sites.

The upper part of the basin comprises most of sampling sites, representing almost 71% of all sites sampled. The middle-low part, in turn, represented only 29%. Sites varied widely in natural and anthropogenic variables.

Table 2 Range, mean and standard deviation of variables measured in this study. Natural category represents the environmental variables and anthropogenic category represents the pressure variables.

Variable	Middle-low part (n=40)		Upper part (n=97)	
NATURAL	Mean	Range	Mean	Range
Water Chemistry				
Conductivity (µS.cm^{-1})	2.36±3.92	0.03-10.48	0.34±0.84	0.02-4.92
pH	5.74±0.59	5.18-6.81	6.07±0.72	4.67-7.16
Physical				
Depth (m)	0.63±0.13	0.40-6.92	0.66±0.10	0.41-0.90
Temperature (°C)	20.48±0.49	19.47-21.29	20.54±0.39	19.91-21.31
Radiation (µmol.s^{-1}.m^{-2})	297.6±46.31	190.2-394.8	157.01±28.42	59.19-221.85
Current speed	0.64±0.17	0.34-0.65	0.48±0.16	0.18-1.13
Annual rainfall (mm)	2292±163	2111-2292	2128±87.49	1992-2370
Annual temperature (°C)	22.75±0.10	22.5-22.9	22.75±0.11	21.9-22.9
Landscape				
Declivity (°)	9.24±8.24	0.78-32.16	23.72±11.1	3.29-36.99
Elevation (m)	12.62±7.63	0.01-20	17.46±4.44	3.32-20
Substrate				
Clay (%)	33.75±23.41	0-50	2.06±9.94	0-50
Sand (%)	46.25±13.16	0-50	59.79±19.84	0-100
Gravel (%)	12.5±21.65	0-50	38.14±21.26	0-50
ANTHROPOGENIC				
Water Chemistry				
Total nitrogen (mg.L^{-1})	0.66±1.41	0.09-4.86	0.14±0.02	0.05-0.19
Total phosphorus (µg.L^{-1})	33.25±32.83	12.49-141.36	22.66±8.81	10.63-42.14
Turbidity (NTU)	20.35±17.85	3.59-86.22	15.45±11.03	4.42-62.51
OD (mg.L^{-1})	5.44±1.22	1.22-8.33	6.19±1.39	2.88-8.68
Physical				
Sediment nitrogen (%)	18.65±5.2	12.01-36.31	17.57±9.19	4.42-64.54
Sediment phosphorus (%)	25.69±25.82	2.69-92.27	11.75±7.18	1.45-23.8
Landscape				
Agriculture (m)	1563±9758	0-62504	43769±66104	0-19624
Anthropic (m)	16389±39720	0-139794	277.3±2252	0-21755

The PCA employed to verify the contribution of the environmental variables for each group revealed that the sites located in the upper part of the basin in an area closest to mountain range, belong to a region characterized by environments with steep margins, and the substrate is predominantly gravel and sandy. So, these sites are positively related, as expected, with substrate type, declivity and elevation (Fig. 3).

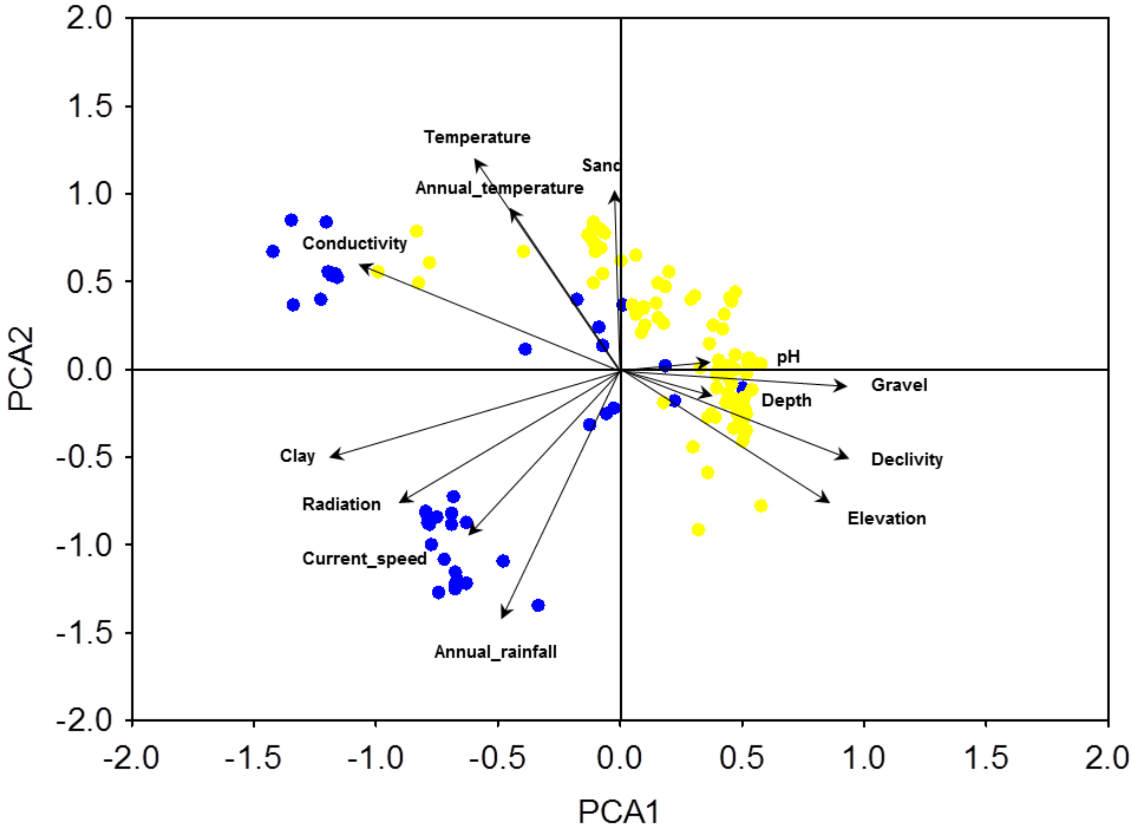

Fig. 3 Biplot from principal component analysis (PCA) of 137 sampling sites distributed in Itanhaém river basin, based on only natural variables. Blue and yellow circles represent the middle-low part and upper part of the basin, respectively.

Sites in the middle-low part are positively influenced by conductivity, radiation and current speed. Since these sites are located predominantly in a coastal plain of the basin, naturally they have higher conductivity by marine influence, lower current speed and less vegetal cover which reflects a greater radiation.

3.2. Classification of the undisturbed and degraded sites

After distinguish the sampling sites by their purely natural characteristics, as second step in the development of the index, we classified the undisturbed and degraded sites in each typology group using the gradient of stressor variables, as described in *Step 2*. This step is important to confirm if each typology exhibits a consistent pressure gradient that can exercise some influence on macrophyte assemblage. Using the stressors variables aimed to classify the undisturbed and degraded sites in upper group, it was not possible to observe differences between them, showing the same variation (Fig. 4).

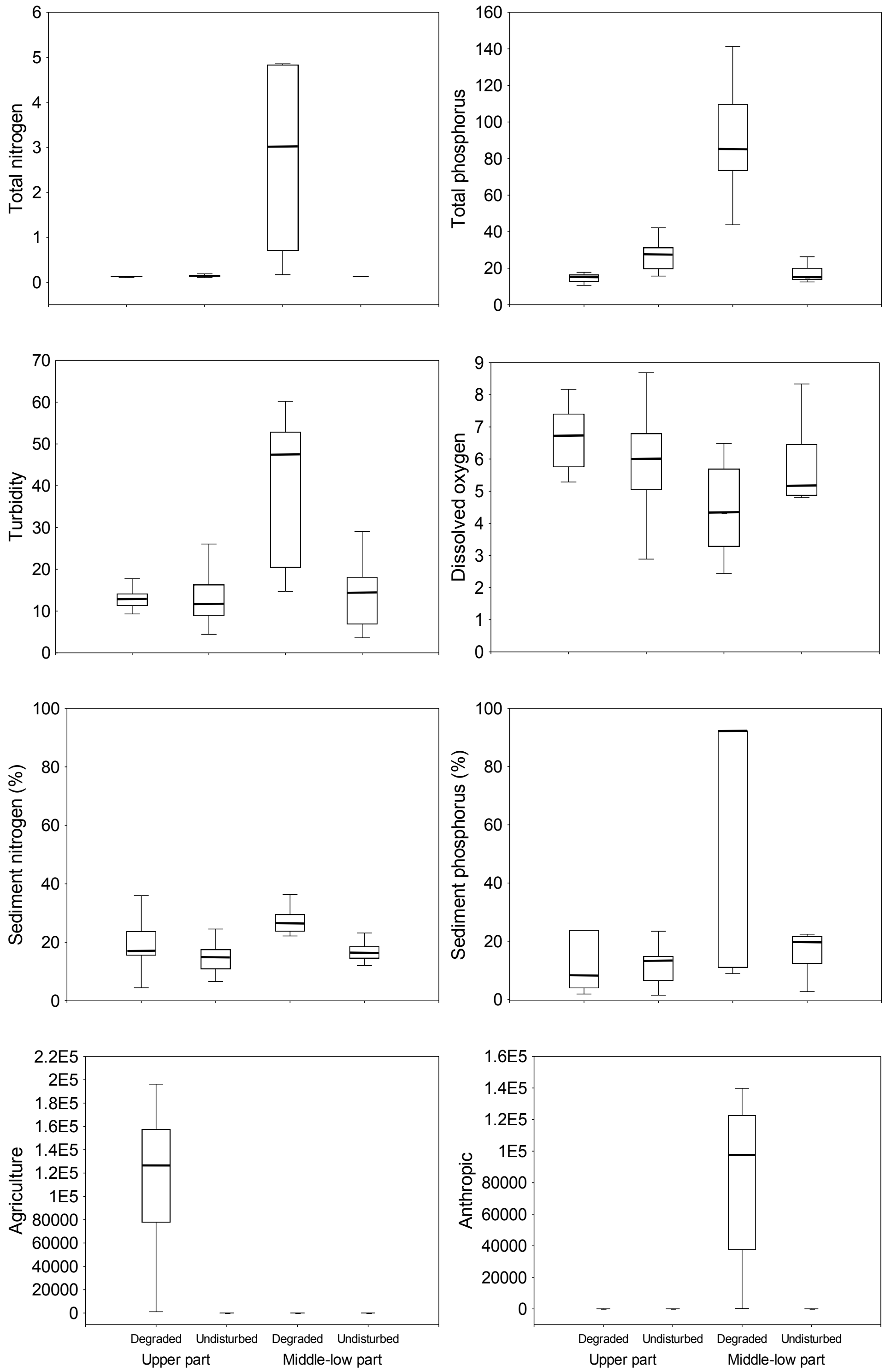

Fig. 4 Box plots of pressure variables of degraded and undisturbed sites in upper and middle-low part of Itanhaém basin. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, and whiskers are non-outlier ranges beyond the boxes.

The only variable stressor really significant was land use, represented by agriculture, because this part of the basin is extensively used for banana farming.

However, this anthropogenic pressure seems does not affect the water quality and the macrophyte assemblage. The macrophyte metrics tested in the upper group did not have a good discriminatory efficiency. On the other hand, the cluster analysis applied in the middle-low group distinguished 8 degraded sites from 40 sampling sites (Fig. 2b). It was possible to observe the differences, overall, in all stressor variables between degraded and undisturbed sites presenting a good discriminatory efficiency. The nutrient enrichment of degraded sites is clearly visible, since nitrogen and phosphorus, both in water and sediment, showed the highest values. For this reason we decided move forward with the analysis only of middle-low group.

3.3. Selection of candidate metrics

Forty-four macrophyte taxa were recorded from the study area (Appendix 1). Emergent taxa represented about 62% of total taxa while floating and submerged taxa represented 25% and 13%, respectively. About one-third of the macrophyte taxa registered in the middle-low group were Poaceae or Cyperaceae. However, the most representative species in terms of frequency of occurrence were *Eichhornia azurea* and *Egeria densa.* Emergent taxa were predominant in both degraded sites and undisturbed sites.

The relative performance of the potential 16 metrics was visually evaluated considering the differences between undisturbed and degraded sites. The substantial overlap between the adjacent classes of almost all metrics constituted the major problem in defining and separating ecological classes. So, only four metrics responded markedly to the stress gradient: richness and the proportion of life forms taxa (Fig. 5).

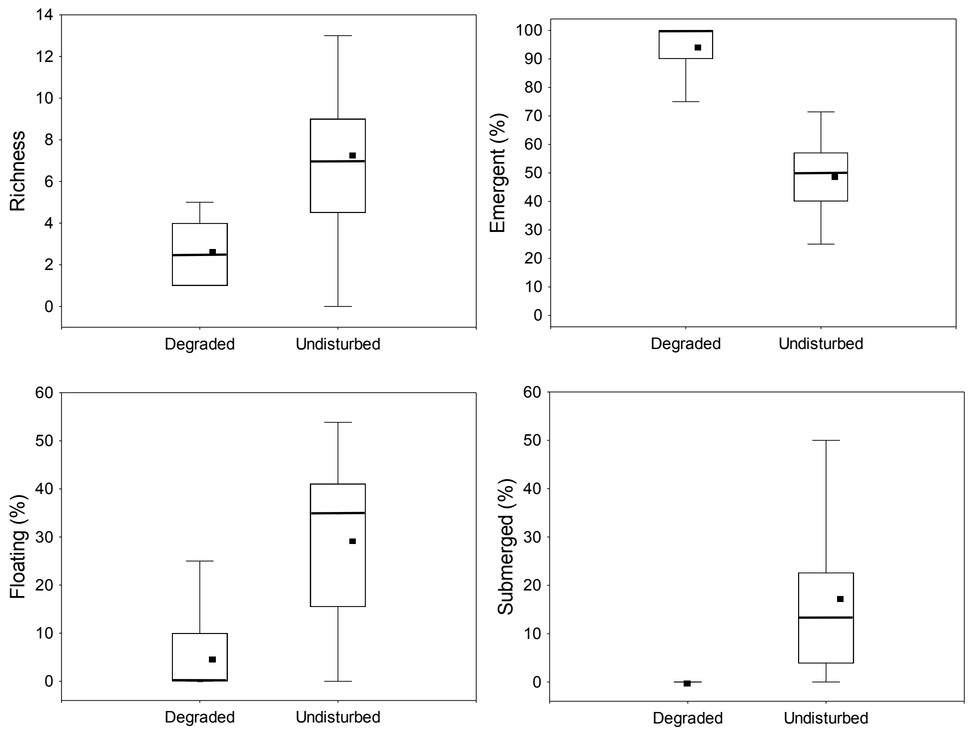

Fig. 5 Box plot of macrophyte metrics used to the development of MIBI – ITA. Boxes show interquartile ranges (25^{th} and 75^{th} percentiles), middle lines are medians, and whiskers are non-outlier ranges beyond the boxes.

Degraded sites had significantly less species and lower proportion of life forms, with exception of emergent life form, which presented the highest value, when compared with undisturbed sites. So, the classification for these individual metrics showed a good discriminatory efficiency, allowing the development of the MIBI-ITA.

3.4. Index development

After choosing the best performing macrophyte metrics, the next step is establishing three quality classes, as described in *Step 4*. Table 3 shows the suggested indicator boundaries used in MIBI-ITA. Although most of indices classify the boundaries of selected metrics based on the median, we chose to use the mean as reference, since the metrics —floating” and —submerged” presented zero as median in degraded sites. Overall, the good quality class is considered when the sites had greater values of richness and proportion of life forms, with exception of emergent life form, which has been classified as bad quality class associated with higher values.

Table 3 Suggested boundary values of the MIBI-ITA for the three classes of ecological quality

Core metrics	1- Bad	3 - Moderate	5 - Good
Richness	≤ 2.6	> 2.6 - 7.1	≥ 7.1
Emergent (%)	≥ 94.4	50.3 - 94.4	≤ 50.3
Floating (%)	≤ 5.6	5.6 - 29.3	≥ 29.3
Submerged (%)	0	< 17.2	≥ 17.2

We calculated the EQR for all sampling sites of the middle-low group (Fig. 6). The reference value of excellent/good boundary based on median value of the reference sites was 0.75. The boundary value of the four remaining classes of ecological quality obtained by dividing equally the interval limited by the Excellent/Good boundary and the lower extreme of the gradient was 0.18.

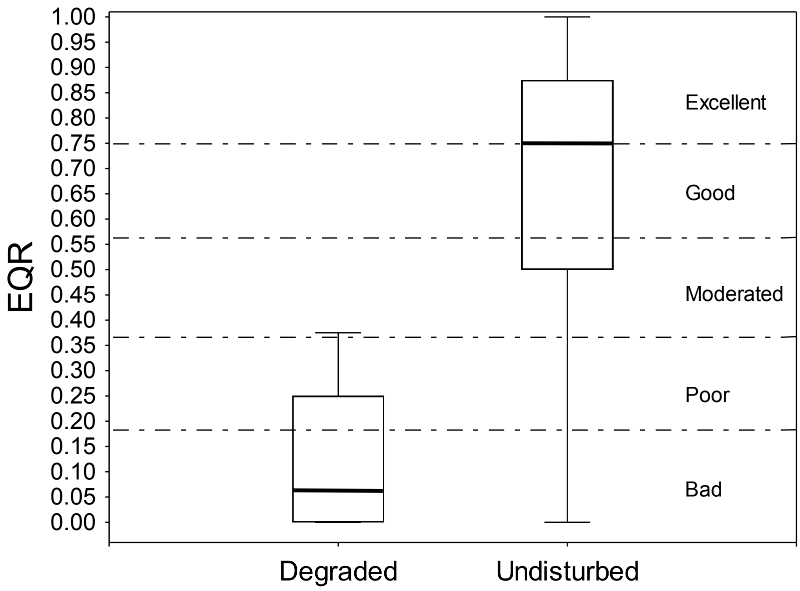

Fig. 6 Boundary values of Ecological Quality ratio (EQR) of the MIBI-ITA for the five classes of ecological quality. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, squares are means, and whiskers are non-outlier ranges beyond the boxes.

Box plot of EQR calculated showed good separation between degraded and undisturbed sites. The scores were classified in Excellent (value ≥ 0.75), Good (value ≥ 0.56 < 0.75), Moderate (value ≥ 0.37 < 0.56), Poor (value ≥ 0.18 < 0.37) and Bad (value < 0.18). Once developed the index and set the EQR scores, the next step is test the performance of the index.

3.5. Assessment of index performance

The MIBI-ITA created was tested with the independent validation dataset (n=27). This process indicates how well the MIBI-ITA would be expected to work with novel sample sites. For this, to determine whether MIBI-ITA was efficient in determine the ecological quality of the sites based on macrophyte composition, we plotted the EQR against a pressure gradient constructed from the 1st PCA axis employed using the stressors variables sampled in the new dataset. These stressor variables were the same used in development of the index (Fig. 7).

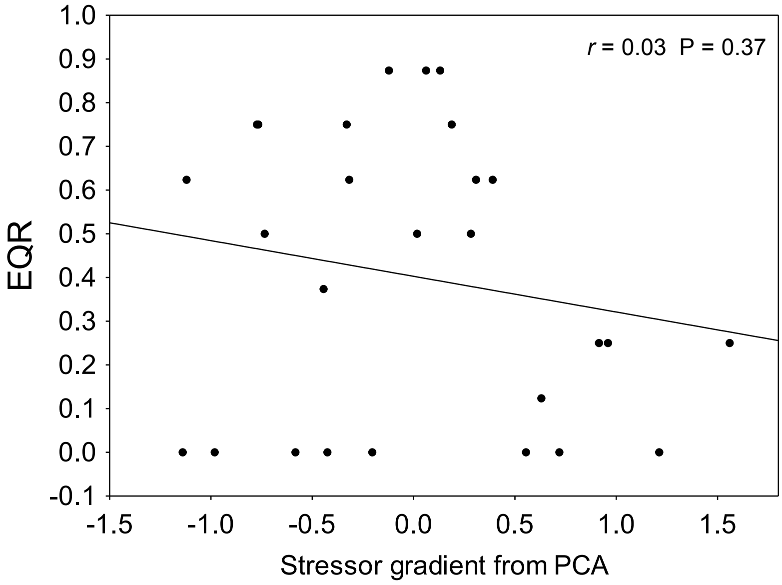

Fig. 7 Relation between EQR values calculated for 27 sampling sites and its pressure gradient calculated from stressor variables.

We expected a negative relationship between EQR and pressure gradient, indicating that the water quality can reflect in macrophyte metrics. However, it was possible to observe no significant correlation between them. Despite the relationship showed a slight negative trend, our results suggest that our index performed poorly in this validation set.

4. Discussion

This study assesses the use of aquatic macrophyte assemblage to develop a desirable integrity biotic index to evaluate the ecological quality of aquatic habitats in tropical environments. In developing the MIBI-ITA, our principal goal was verify if macrophyte community can reflect ecological quality conditions, because it usually respond to human-induced impacts on aquatic environments. In the development of any biotic index, is necessary to assume that the effects of environmental alterations in ecosystems will result in compensatory responses from biota. Although we recognize that some changes in the species composition of the plant community are not reflective of altered water quality, the anthropogenic changes are known to cause impacts in this assemblage (Kolada, 2010; Pozo et al., 2011).

Our results allowed the development of one single index, in spite of the fact that we found two different types of environmental conditions across the watershed. In the upper part of the basin was not possible to develop an index because we did not find any evidence of a pressure gradient, unlike the middle-low part, which has a clear one. In the middle-low part, the urbanization was the stressor most significant associated with changes in proportion of taxa. The results showed that emergent taxa seem to be related with pressure gradients, occurring in a highest proportion in degraded sites. This result is plausible because emergent macrophytes predominantly colonize zones nearer the shore and therefore are exposed to nonpoint source nutrients input from human wastes. The proportion of floating and submerged taxa, in turn, would increase with habitat quality. Submerged taxa, for example, provided a useful metric of water quality since degraded sites completely lacked submerged plants. This life form is strongly dependent on water quality and had proved to be vulnerable to eutrophication (Søndergaard et al., 2010).

In this study, a large number of metrics tested were not appropriated to index development. From sixteen metrics proposed, only four have good performance characteristics. Although the metrics selected showed a good discriminatory efficiency in the development dataset, they may be insufficient as predictors of ecological quality. Testing MIBI-ITA in the validation set, we did not notice a clear relationship with pressure gradient. We expected a negative and significant relationship between them, indicating the effect of human activity on aquatic macrophytes, mainly through the deterioration of water quality. It is possible that the small number of metrics does not provide the robustness necessary for the effective functioning of the index. Some studies employed more than thirty potential metrics to index development (Birk et al., 2006; Aguiar et al., 2009). Species traits such as dispersal, reproduction and survival together with ecological preference have been used to identify signs of habitat degradation (Baattrup-Pedersen et al., 2016). For now, we were not able to get much information about our species to include in the index development. So, we highlight the need to use of several macrophyte metrics to improve the precision of the index.

Another explanation for the poor performance of the index may be the great heterogeneity found in neotropical habitats, both in biological and environmental aspects. In Europe, for example, the similarity of macrophyte flora and environmental conditions are factors that make their indices applicable and comparable to several other parts of the continent (Jusik et al., 2015). The same is not feasible for our case, since our results showed that within a watershed is possible to find many different environments with its particular characteristics. Although the validation dataset had similar environmental conditions when compared with index development dataset, it suffers with different anthropogenic pressures and based on our results, the aquatic macrophytes seem to respond differently.

Based on our results, we assumed that our macrophyte index of biotic integrity has limited applicability as direct indicators of water quality disturbance in our study area and clearly demonstrates a need for alternative methods to establish the assessment and monitoring of aquatic environments in Brazil and tropical regions. We believe further testing is needed, and how we noticed there are few studies on the limitations and the practical use of macrophyte-based index, we decided to list some recommendations in order to help future studies in tropical environments:

1. Given the environmental diversity found in tropics, is necessary to identify the vegetation patterns in different environments and relate the composition of macrophytes to environmental variables in a broader geographical area;
2. To test other metrics based on ecological concepts and patterns displayed by biological data to help to improve the effectiveness of the developed index;
3. Use a large dataset integrating information from other coastal watersheds;
4. More sampling is required in order to balance the number of degraded and undisturbed sites;
5. Use other analytical approaches to data analysis;

Despite of the limitation of this study, we highlight our efforts represent a first attempt to develop a biotic integrity index through aquatic macrophyte and assess its ability to evaluate the ecological quality of aquatic environments in a tropical watershed of Brazil. We believe our results may become relevant in further studies involving assessment and biomonitoring of tropical aquatic ecosystems.

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Appendix 1

Aquatic macrophyte taxa recorded in Itanhaém river basin. Life form code: EM - emergent, FF - free floating, RF - rooted floating, RS - rooted submerged, FS - floating submerged and EP - epiphyte.

Family	Taxa	LF
Acanthaceae	*Hygrophila costata* Nees & T. Nees	EM
Alismataceae	*Echinodorus cordifolius* L. Griseb.	EM
Amaryllidaceae	*Crinum procerum* Herb. & Carrey	EM
Araceae	*Lemna valdiviana* Phil	FF
	Pistia stratiotes L.	FF
Araliaceae	*Hydrocotyle ranunculoides* L. f.	EM
Cabombaceae	*Cabomba furcata* Schult. & Schult. f.	RS
Characeae	*Nitella furcata* (Roxb. ex Bruz.) Ag. emend. R.D. Wood	RS
Commelinaceae	*Commelina schomburgkiana* var. *brasiliensis* Seub.	EM
Cyperaceae	*Cyperus giganteus*Vahl	EM
	Eleocharis interstincta (Vahl) Roem. & Schul.	EM
	Eleocharis mínima Kunth	EM
	Fiurena umbellata Rottb.	EM
	Oxycaryum cubense (Poepp. & Kunth) Lye	EP
	Rhynchospora corymbosa (L.) Britton	EM
	*Scirpus californicus*Steud.	EM
Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc.	EM
Hydrocharitaceae	*Egeria densa* Planch	RS
	Limnobium laevigatum (Humb. & Blonpl. ex Willd.) Heine	FF
Leguminosae	*Vigna lasiocarpa* (Mart. ex Benth.) Verdc.	EP
Lentibulariaceae	*Utricularia foliosa* L.	FS
Mayacaceae	*Mayaca fluviatilis* Aubl.	EM
Menyanthaceae	*Nymphoides indica* (L.) Kuntze	RF
Nymphaeaceae	*Nymphaea sp*	RF
Orchidaceae	*Habenaria repens* Nutt.	EM
Poaceae	*Echinochloa polystachya* (Kunth) Hitchc.	EM
	Hymenachne amplexicaulis (Rudge) Nees	EM
	Leersia hexandra Sw.	EM
	Panicum pernambucense (Spreng.) Mez ex Pilg.	EM
	Paspalum repens Berg.	EM
	Spartina alterniflora Loisel	EM
	Urochloa arrecta (Hack. ex. T. Durand & Schinz) Morrone & Zuloaga	EM
Potamogetonaceae	*Potamogeton pusillus* L. subsp. *pusillus*	RS
	Potamogeton polygonus Cham. & Schltdl.	RS
Polygonaceae	*Polygonum acuminatum* Kunth	EM
	Polygonum ferrugineum Wedd.	EM
Pontederiaceae	*Eichhornia azurea* (Sw.) Kunth	RF
	Eichhornia crassipes (Mart.) Solms	FF
	Heteranthera multiflora (Griseb.) C. N. Horn	EM
Pteridaceae	*Ceratopteris thalictroides* (L.) Brongn	FF
Ricciaceae	*Ricciocarpos natans* L. (Corda)	FF
Salviniaceae	*Azolla filiculoides* Lam	FF
	Salvinia molesta D.S. Mitch	FF
Typhaceae	*Typha domingensis* Pers.	EM

CONSIDERAÇÕES FINAIS

A presente tese teve como principal objetivo, o desenvolvimento de um índice de integridade biótica que fosse capaz de avaliar a qualidade ecológica dos ambientes aquáticos continentais brasileiros. Neste sentido, a proposta, apesar de ambiciosa, poderia gerar informações relevantes e potencialmente inovadoras no que diz respeito ao uso de indicadores bióticos como ferramenta para a avaliação da qualidade das águas superficiais do Brasil. As evidências observadas no terceiro capítulo demonstram que ainda há muito trabalho a ser desenvolvido para gerar um índice que efetivamente represente a condição atual dos habitats aquáticos estudados. No entanto, cremos que um importante passo foi dado, visto que em ambientes aquáticos tropicais ainda dispomos de pouquíssimos trabalhos que tem usado macrófitas aquáticas para fins de bioavaliação. Esperamos que nossos resultados funcionem como potencial instrumento de suporte a novos estudos, pois, acreditamos que ao desenvolver um índice capaz de indicar o grau de influência antrópica que resulta em impactos na biota, pode ser uma importante contribuição para o manejo e conservação dos nossos ambientes aquáticos.

O primeiro e segundo capítulos apresentados nos auxiliam a reforçar o argumento supracitado, uma vez que foi possível observar que poucos estudos sobre qualidade ecológica são realizados nos trópicos e, além disso, a assembleia de macrófitas é ferramenta de estudos majoritariamente em ambientes temperados. O segundo capítulo nos revela que esse grupo biológico é capaz de responder às mudanças nas condições ambientais onde estão inseridas, sendo fortemente influenciadas por interferências antrópicas. Dessa forma, destacamos e incentivamos a continuidade de estudos neste sentido, no âmbito da geração de conhecimentos que aprimorem as técnicas aqui empregadas.