UNIVERSIDADE FEDERAL DO PARANÁ
FABRÍCIO DE ANDRADE FREHSE

ARTIFICIAL AQUATIC HABITATS: A GLOBAL REVIEW, COLONIZATION EXPERIMENTS BY FISH AND COMPARISON OF SAMPLING METHODS IN RESERVOIRS

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FABRÍCIO DE ANDRADE FREHSE

ARTIFICIAL AQUATIC HABITATS: A GLOBAL REVIEW, COLONIZATION EXPERIMENTS BY FISH AND COMPARISON OF SAMPLING METHODS IN RESERVOIRS

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, da Universidade Federal do Paraná, como requisito parcial para obtenção do título de Doutor em Ciências Biológicas, área de concentração em Ecologia e Conservação.

Orientador: Prof. Dr. Jean Ricardo Simões Vitule

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Aos meus pais, Fátima e Nelson Luiz

Aos meus avós, Ilona e Andrade, e Marlene e Nelson

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Se vencer uma batalha
É matar o perdedor
Na guerra que vivo em vida
Não me vejo ganhador
Pois sem matar nem ferir, ai meu Deus
Eu me sinto vencedor
A luz da experiência
A luz da experiência
O caminhar nas estradas
O cruzar encruzilhadas
Me ensinou a jogar
Jogar o jogo da vida
Na vida vencer o jogar
O jogar pela vitória
Entristece o jogador
Quando pensa que venceu, colega velho
Vê que é o perdedor
Camarada

Vencer - Mestre Moraes - Ladainha de Angola

RESUMO

Devido ao aumento da população humana e ao consequente incremento na exploração e transformação de ambientes naturais, uma infinidade de tipos de estruturas tem sido instalada em ambientes aquáticos para os mais variados propósitos. Desde meados do século 20, diversos estudos começaram a avaliar o efeito de estruturas submersas como habitat para organismos em diferentes ecossistemas aquáticos, considerando distintas perspectivas. Neste sentido, dado a urgente necessidade de conservação dos ecossistemas aquáticos em todo o mundo, é importante sintetizar o conhecimento acerca deste tópico de pesquisa, a fim de encontrar generalizações e identificar lacunas, a fim de orientar estudos futuros. Desta forma, uma revisão global sistematizada da literatura sobre habitat artificiais aquáticos foi realizada com o objetivo de detectar padrões temporais, geográficos e metodológicos em publicações científicas sobre este assunto (capítulo 1). A maioria dos estudos foi realizado em ambientes marinhos temperados. Habitat experimentais, feitos principalmente de concreto, foram os mais estudados. O censo visual foi o método de amostragem mais utilizado e houve predomínio de estudos de curto prazo (< 12 meses de duração) e em águas rasas (< 5 m de profundidade). Peixes foi o grupo mais estudado, no entanto, poucos estudos relataram a ocorrência de espécies não-nativas associadas a habitat artificiais. Posteriormente, devido à falta de estudos em ecossistemas de água doce, especialmente na região Neotropical, experimentos em campo foram realizados em dois reservatórios neotropicais, a fim de identificar padrões gerais no uso de habitat artificiais por peixes em reservatórios. Para isso, o uso de habitat artificiais (tubos de cerâmica e pequenas árvores) e seminaturais (rochas e habitat não estruturados) por peixes foi comparado em relação à origem das espécies (nativas ou não-nativas) (capítulo 2). Pequenos indivíduos de Cichlidae e Centrarchidae (< 80 mm de comprimento total) foram dominantes, no entanto, a abundância e composição de espécies variaram de acordo com o tipo de habitat. Espécies nativas e não-nativas de Cichlidae parecem não co-ocorrer com Centrarchidae. Tubos de cerâmica foram usados principalmente por Cichlidae nativos, enquanto as árvores parecem ser preferidas por Centrarchidae. Finalmente, dois métodos observacionais de amostragem, censo visual (VC) e vídeo subaquático (UWV), foram comparados em relação ao número de espécies, composição e classe de tamanho de peixes registrados (capítulo 3). O número total de espécies registradas por ambos os métodos de amostragem foi similar, no entanto, UWV registrou maior número médio de espécies do que VC. No geral, VC foi eficiente no registro de espécies crípticas e residentes, enquanto UWV, espécies com maior movimentação. Além disso, VC foi mais eficiente que UWV na detecção de indivíduos de menor tamanho (31-79 mm de comprimento total). A aplicabilidade de habitat artificiais para o manejo e conservação de reservatórios neotropicais, e o potencial e limitações de amostragens de peixes através de VC e UWV em tais ecossistemas são discutidos.

Palavras-chave: Ecologia de peixes água doce. Ecossistemas lênticos. Espécies exóticas. Introdução de peixes. Métodos não-destrutivos. Recifes artificiais

ABSTRACT

Due to the increase of the human population and the growing exploitation and transformation of natural environments, a wide range of structures has been deployed in aquatic ecosystems for different purposes. Since the mid-20th century, several studies began to evaluate the effects of submerged structures as habitats for organisms in different aquatic ecosystems, from different perspectives. In this sense, given the urgent need for aquatic ecosystems conservation worldwide, it is important to synthesize what has been done in this research topic, in order to find generalizations, identify knowledge gaps, and guide future studies. Thus, a systematic global literature review concerning artificial aquatic habitats was conducted aimed at detecting temporal, geographic and methodological patterns in scientific publications on this subject (chapter 1). The majority of studies were carried out in marine temperate environments. Experimental habitats, made mainly of concrete, were the most studied. Visual census, was the most common sampling method, and the majority of studies were short-term (< 12 months in duration) and shallow waters (< 5 m depth). Fish was the most studied group, however, few studies reported the occurrence of non-native species associated with artificial habitats. Posteriorly, due to the lack of studies in freshwater ecosystems, especially in the Neotropical region, manipulation experiments were performed in two Neotropical reservoirs in order to identify general patterns in the use of artificial habitats by fish in reservoirs. For this, the use of artificial (ceramic pipes and trees) and semi-natural (rocks and non-structured) habitats by fish was compared regarding the origin of species (i.e. native or non-native) (chapter 2). Small individuals (< 80 mm in total length) of Cichlidae and Centrarchidae were dominant, however the abundance and composition of species varied according to the habitat. Native and non-native Cichlidae appear not to co-occur with Centrarchidae species. Ceramic pipes were mostly used by native Cichlidae, while trees seem to be preferred by Centrarchidae. Finally, two observational sampling methods, visual census (VC) and underwater video (UWV), were compared regarding the fish species number, composition, and size class recorded (chapter 3). The total number of species recorded by both sampling methods was similar, however, UWV recorded higher mean number of species than VC. In general, VC was efficient in recording cryptic and resident species, while UWV, highly mobile species. Moreover, VC was more efficient than UWV in detecting smaller fish individuals (i.e. 31-79 mm in total length). The applicability of artificial habitats for management and conservation of Neotropical reservoirs and the potential and limitations of fish surveys through VC and UWV in such ecosystems are discussed.

Keywords**:** Artificial reef. Exotic species. Freshwater fish ecology. Fish introductions. Lentic ecosystems. Non-destructive surveys.

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THESIS PRESENTATION

This thesis is composed of three scientific papers elaborated over four years of PhD graduation in the post-graduation program "Ecologia e Conservação" of "Universidade Federal do Paraná" – Brazil. The three chapters containing scientific content were developed and are formatted according to the scientific journal chosen for publication. Chapter one is formatted as a review for submission to the *Global Ecology and Conservation* journal (classification according to the Brazilian agency CAPES: Qualis B2), and has as a main objective to review the scientific literature on artificial aquatic habitats in order to detect geographic, temporal and methodological patterns of publications in this theme. The second chapter is formatted as an original article for submission to the *Ecological Applications* journal (classification according to the Brazilian agency CAPES: Qualis A1), and has as a main objective investigate the use of artificial habitats by fish in Neotropical reservoirs in view of the growing global process of non-native fish introductions. The third chapter is formatted as an original article for submission to the *Journal of Applied Ichthyology* (classification according to the Brazilian agency CAPES: Qualis B1), and has as the main objective to compare the number of fish species, abundance and size class recorded by two observational sampling methods (visual census and subaquatic video) in reservoirs. Beyond these three chapters, this thesis is also composed by an introductory article (in Portuguese and in English) for scientific divulgation, in a non-formal scientific language. This article will be disclosed in a local online newspaper promoted by the post-graduate program in "Ecologia e Conservação", in order to spread scientific novelty to non-academic community.

SCIENTIFIC DIVULGATION ARTICLE

ARTIFICIAL AQUATIC HABITATS: WHAT IS IT AND WHY IS IT IMPORTANT?

Habitat artificiais aquáticos: o que é isso e por que são importantes?

Artificial aquatic habitats: what is it and why is it important? Habitat artificiais aquáticos: o que é isso e por que são importantes?

Fabrício de Andrade Frehse
Jean Ricardo Simões Vitule

Água é um recurso essencial à vida. Os ecossistemas aquáticos fornecem diversos serviços à humanidade, como por exemplo o fornecimento de água e alimentos, geração de energia, irrigação de plantações, ciclagem de nutrientes, e atividades recreativas. Porém, devido ao aumento da população humana e à crescente exploração e transformação de ambientes naturais, uma infinidade de tipos de estruturas tem sido instalada em ambientes aquáticos pelo homem para os mais variados propósitos. Uma vez embaixo d'água, tais estruturas tornam o ambiente físico mais complexo, adicionando novos relevos e substratos, e criando novos habitat para diferentes organismos. Alguns exemplos de habitat artificiais aquáticos (termo usado neste

Water is an essential resource for life. Aquatic ecosystems provide a great diversity of services to mankind, such as water supply, food, power generation, irrigation, nutrient cycling, and recreation. However, due to the continuous human growth and the never ending increasing exploitation and transformation of natural environments, myriad of types of structures have been deployed by man in aquatic environments for a variety of purposes. Once underwater, such structures make the physical environment more complex, adding new reliefs and substrates, and creating novel habitats for different organisms. Some examples of artificial aquatic habitats (term used in this study to refer to any submerged structure, through

estudo para se referir a qualquer estrutura submersa, através da ação humana) são: recifes artificiais, trapiches, píer, molhes, plataformas de petróleo, estruturas para aquicultura, embarcações naufragadas.

Habitat artificiais influenciam o ambiente aquático onde são inseridos, e podem potencialmente alterar a distribuição e abundância de organismos. Neste sentido, diversos estudos têm abordado o papel de estruturas submersas como habitat para organismos aquáticos. Uma revisão da literatura avaliou tendências e lacunas temporais, geográficas e metodológicas, em publicações sobre habitat artificiais aquáticos. Nessa constatou-se que o número de estudos começou a crescer a partir do final dos anos 90, levado pela realização de conferências internacionais sobre o tema. A maioria das publicações avaliou o efeito de habitat artificiais em peixes e em ecossistemas marinhos. Curiosamente, poucos estudos foram realizados em ambientes aquáticos

human action) are: artificial reef, pier, pontoon, jetties, breakwater, seawall, oil platforms, aquaculture structures, wrecks and others.

Artificial aquatic habitats influence the environment where they are deployed and can potentially change the distribution and abundance of organisms. In this sense, several studies have evaluated the role of submerged structures as habitats for aquatic organisms. A literature review evaluated temporal, geographical, and methodological trends and gaps, in publications concerning artificial aquatic habitats. It was found that the number of studies began to grow from the end of the 90s, driven by international conferences on the subject. Most publications evaluated the effect of artificial habitats on fish marine ecosystems. Interestingly, few studies have been conducted in tropical and/or freshwater aquatic environments. The most frequent

tropicais e/ou de água doce. O método de amostragem mais utilizado nos estudos foi o censo visual. Além disso, uma minoria de estudos registrou a ocorrência de espécies não-nativas (isto é, espécies que foram translocadas, pela ação humana, do seu local de origem para um novo local) associadas à habitat artificias aquáticos.

Apesar do crescente número de barragens construídas em rios para a criação de reservatórios, especialmente em países em desenvolvimento, poucos estudos avaliaram o uso de habitat artificiais por peixes nestes ambientes. Com base nisso, experimentos com habitat artificiais foram realizados em dois reservatórios de abastecimento público de água na região de Curitiba. Neste trabalho científico, objetivou-se comparar o uso de habitat artificiais frente ao crescente processo global de introdução de espécies não-nativas de peixes. Durante um ano e meio de monitoramento, constatou-se que os

sampling method adopted in the studies was visual census. Additionally, a minority of studies have reported the occurrence of non-native species (i.e. species that have been translocated by human action from their origin place to a new location) associated with aquatic artificial habitats.

Despite the increasing number of dams in rivers for reservoirs creation, especially in developing countries, few studies have evaluated the use of artificial habitats by fish in these environments. Based on this, manipulation experiments with aquatic habitats were carried out in two water supply reservoirs in Curitiba, Brazil. In these experiments, the aim was to compare the use of artificial habitats in the face of the growing global process of introduction of non-native species of fish. During a year and a half of surveying, it was found that artificial habitats in reservoirs were mainly used for juvenile individuals of fish,

habitat artificiais nos reservatórios foram usados principalmente por indivíduos juvenis de peixes, entretanto, a composição e abundância de espécies variaram de acordo com tipo de habitat. Por exemplo, tubos de cerâmica foram mais usados pelo Cará (*Geophagus brasiliensis*), uma espécie nativa de peixe, enquanto que pequenas árvores submersas foram mais usadas pela espécie não-nativa "*Black Bass*" (*Micropterus salmoides*), um peixe piscívoro, conhecido mundialmente por predar e competir com outras espécies de peixes. Neste sentido, fica evidente a importância de se considerar a origem das espécies (isto é, nativa ou não-nativa) associadas à habitat artificiais aquáticos, de modo a evitar que espécies não-nativas invasoras se beneficiem de estruturas artificias submersas em ambientes de água doce.

Cada vez mais, com o desenvolvimento de novas tecnologias, câmeras e sistemas de vídeos tem sido

however, the composition and abundance of species varied according to the type of habitat. For example, ceramic pipes were most used by the Cará (*Geophagus brasiliensis*), a native fish species, while trees were preferred by the non-native largemouth bass (*Micropterus salmoides*), a piscivore, known worldwide for predating and competing with other fish species. In this sense, it is important to consider the origin of species (i.e. native or non-native) associated with artificial aquatic habitats, in order to avoid that invasive non-native species benefiting from artificial structures submerged in freshwater environments.

Increasingly, with the development of novel technologies, cameras and video systems has been used to monitor fish and other aquatic organisms. In another study, two methods for surveying fishes was compared: visual census, where a diver records the fish from direct

usado para o monitorar peixes e outros organismos aquáticos. Em outro trabalho científico, dois métodos para o monitoramento de peixes foram comparados: censo visual, onde um mergulhador registra os peixes dentro do seu campo de visão; e vídeo subaquático, onde uma câmera é submersa e registra o que acontece em baixo d'água. Apesar do censo visual ser mais eficiente para monitorar peixes pequenos e espécies que costumam se esconder dentro dos habitat, o vídeo subaquático parece ser mais adequado para monitorar espécies de peixes maiores e com maior movimentação.

Habitat artificiais são uma realidade crescente em diversos ecossistemas aquáticos. No entanto, é necessário compreender o efeito que tais estruturas exercem na biota aquática circundante, de forma a prevenir que espécies não-nativas se beneficiem do seu uso. Neste sentido, o vídeo subaquático é uma ferramenta alternativa

observation; and underwater video, where a camera is submerged and records what happens underwater. Although visual census is more efficient to survey small fish and species that occupy interstices, underwater video seems to be more suitable for surveying larger and mobile fish species.

In conclusion, artificial habitats are a growing reality in many aquatic ecosystems. However, it is necessary to understand the effect that artificial habitats exert on the aquatic biota, in order to prevent non-native species benefiting from its use. In this sense, underwater video is an alternative and complementary tool to monitor habitat use by fish in reservoirs.

e complementar para monitorar o uso de habitat por peixes em reservatórios.

CHAPTER 1

ARTIFICIAL HABITATS IN AQUATIC ENVIRONMENTS: A GLOBAL SYSTEMATIC REVIEW

Artificial habitats in aquatic environments: a global systematic review

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Abstract

For thousands of years different structures have been deployed in aquatic environments by humans, aiming to attract and facilitate the catch of fish and other aquatic organisms. Since the mid-20th century, several studies began to evaluate the effect of submerged structures as habitats for aquatic organisms. Due to the increasing number of artificial aquatic habitats worldwide, it is important to synthesize what has been done in regard to this research topic, aiming to determine possible gaps in our knowledge, and identify emergent patterns. In this sense, a global systematic review was conducted to detect temporal, geographic and methodological patterns in scientific publications on artificial aquatic habitats. The majority of relevant peer-reviewed publications were field studies carried out in marine temperate environments, there was a lack of studies in tropical and freshwater ecosystems. Habitats made mainly of concrete were the most studied artificial structures. Visual census was the most used sampling method, and there was a dominance of short-term (< 12 months in duration) and shallow waters (< 5 m depth) studies. Fish was the most studied group, however few studies reported the occurrence of non-native species associated with artificial habitats. This review highlights and discusses gaps and trends on artificial aquatic habitats publications, in order to guide future research and assist conservation measures related with this topic.

Keywords: Artificial reef. Artificial structure. Ecological succession. Exotic species. Fish introduction. Freshwater ecosystem. Human-made structure.

Introduction

The human population continues to grow around the world, along with exploration for natural resources and the transformation of natural environments (Vitousek et al., 1997; Martin et al., 2016). Due to the expanding human footprint on Earth, a novel geological era has been proposed, the Anthropocene (Steffen et al., 2011). Water is one of the most fundamental resources for humankind, additionally aquatic ecosystems also provide unique services to humanity (e.g. water supply, food, nutrient cycling, power generation, recreation among others) (Jackson et al., 2001). However, the exploitation of such services has caused profound changes in these ecosystems, such as, landscape alteration (Airoldi and Beck, 2007; Winemiller et al., 2016), pollution (Ivar do Sul and Costa, 2014), resources overexploitation (Christensen et al., 2014; Golden et al., 2016) non-native species introduction (Gallardo et al., 2016) and the creation of novel artificial habitats in aquatic environments (Bulleri and Chapman, 2010; Firth et al., 2016). Artificial habitats are defined in the present study as *any structure (human-made or natural) deployed, intentionally or not, in the aquatic environment by humans*.

The first evidences of artificial habitats in aquatic environments dates to the Neolithic period (9000 - 3000 BC), when coastal vegetation and rocks were submerged to create new fishing grounds along the African coast (Stone et al., 1991; Seixas et al., 2013). The earliest records were in Japan in the mid-17th century, where the "*artificial reef*" concept was created, and then spread to the USA and later to Europe (Fabi et al., 2011). Nevertheless, it was from the mid-20th century that the installation of artificial structures spread throughout the world, with different materials and purposes (Stone et al., 1991; Bolding et al., 2004).

Due to growing interest in the subject, in 1974 the first *International Conference on Artificial Reefs* was organized in Houston (USA). The primary focus was on using, evaluating and managing artificial reefs for fisheries purposes (Bortone, 2015). However, since the beginning of the 90's there has been a perception change regarding the use of artificial structures

in aquatic environments (Seaman, 2002). Artificial reefs began to be seen not only as a tool for fishing resources exploitation, but also as a way to enhance the ecological value of aquatic habitats (Grove and Wilson, 1994). Then, since the seventh edition of the international conference, held in San Remo (Italy), in 1999, the title has changed to CARAH - *International Conference on Artificial Reefs and Related Aquatic Habitats* (for a historical perspective see Bortone, 2015).

Since then, an increasing number of structures has been installed in aquatic ecosystems for different purposes. Consequently, novel materials: coal ash (Frease and Windsor, 1991), decommissioned gas and oil platforms (Quigel and Thornton, 1989); applicability: fish aggregating devices (Matsumoto, 1981), recreational scuba diving (Brock, 1994), anti-trawling (Guillén et al., 1994); and research fields: freshwater (Wilbur, 1978), benthic community (Carter et al., 1985), restoration of corals (Abelson and Shlesinger, 2002), non-native species (Page et al., 2006), protected areas (Wilson et al., 2002), have emerged related to artificial aquatic habitats. Additionally, with the increase of coastal occupation by humans and their aquatic environments use, novel habitats, even if not intentionally, have been created for coastal defence (Wolff, 1999), marina (Holloway and Connell, 2002) and aquaculture (Oakes and Pondella, 2009) purposes.

Due to the increasing number of artificial aquatic habitats worldwide and the growing number of studies on different themes, using different approaches and in distinct ecosystems, a global systematic review is essential in order to detect gaps and research trends in publications. Systematic reviews have been increasingly used in ecology because they aim to answer well-defined questions through a transparent, replicable and updatable method (Gurevitch et al., 2018). In this sense, a scientometric analysis through a systematic review can be useful to update what have been done and guide future researches on this subject. Therefore, the present study seek to answer the following questions: (1) What is the temporal pattern of publications

on artificial habitats in aquatic environments? (2) Which journals publish more on this subject? (3) Which countries and continents publish more on this theme? (4) Which are the most applied research methods? (5) What are the ecosystems, ecoregions and environments more studied? (6) Which are the most studied artificial habitats? (7) What are the most used sampling methods? (8) What is the duration of published studies? (9) What is the depth of the artificial habitats studied? (10) Which taxonomic group has been more studied? (11) How many studies with artificial habitats in aquatic environments have been conducted within protected areas and where? (12) How many studies have recorded the occurrence of non-native species associated with artificial habitats and which is the taxonomic group with the greatest number of exotic species?

Material and methods

A literature survey was conducted using the Web of Science database (ISI – Institute of Scientific Information; access: http://apps.webofknowledge.com) in order to assess a sample of studies on artificial habitats in aquatic environments. Key-words used were "*artificial reef**" OR "*artificial structure**" OR *"artificial habitat*"* AND *aquatic* OR *freshwater* OR *marine* OR *estuar** in the "Topic" search field. The search was completed in August of 2016 and the timespan was for all years. Furthermore, the studies were filtered by "Document Types" (filtered for "*article* and *proceedings paper"*), "Language" (filtered for "*English*") and "Research Areas" (filtered for "*marine freshwater biology, environmental sciences ecology, oceanography, fisheries, biodiversity conservation, water resources, zoology*"). Other search options were left in accordance with the ISI default. Non-related publications were excluded by title, abstract or a careful reading of all text if necessary. To include a study in the survey, it had to focus on artificial habitats in aquatic environments. Some papers were excluded because the research purpose was not directly linked to the different aspects of artificial habitats; they only

cited the subject tangentially, such as a place of a species occurrence, or a possible tool for conservation. Papers using the term *artificial habitat* as a kind of environment, for example artificial channels, tanks or ditches, were also excluded. Papers filtering were planed and performed according to the PRISMA protocol (Moher et al., 2015; see Supplementary Material I for details).

The results are based on the total sample of studies provided by the Web of Science database that matched the proposed inclusion criteria (above described). However, the presented data is not equivalent to the total number of papers, but to the information in the studies. Therefore, the numbers included in the analysis are not always the same. For instance, multiple counts per paper were conducted when necessary (e.g. some studies have been conducted in more than one country, environment and/or depth, or with more than one kind of habitat, sampling method and/or taxonomic group).

A second search was conducted in the same database, to obtain the total number of publications per year within the same research areas in order to compare and discuss temporal pattern of publications on artificial aquatic habitats with general publications. Different journals publish different numbers of papers per year, so the more they publish the more likely they are to have published a paper on any given subject. To correct for this bias, a formula for relative weights (adapted from Braga et al., 2012) was applied to the data. The weight value (w) is given as:

$$w = \left(\frac{n}{p * e * y}\right) * 1000$$

where p is the average number of papers published in the first edition of each year (calculated for a subset of 9 years, 1975, 1980, 1985, 1990, 1995, 2000, 2005, 2010 and 2015), e is the average number of editions per year, y is the number of years that the journal has published within our survey period (maximum value of 43 years as the first paper recorded was published

in 1973 and the last in 2016), and *n* is the number of resulting papers from our survey for the journal.

Research methods were categorized into: field observational, field experimental, laboratory experimental, enclosure experimental (micro and mesocosm), literature review, interview, modelling, and theoretical (discussion on the subject). Ecosystems were categorized into: freshwater, estuary, and marine. Coastal ecosystems studies (marine and estuary), were categorized in marine ecoregions according to Spalding et al. (2007), while continental freshwater studies, were categorized according to the type of environment (e.g. river, lake, reservoir, or others). The artificial habitats were categorized in: aquaculture (human-made structures with the intention of creating organisms with commercial purpose); coastal defence (human-made structures with the intention of protecting and/or maintaining the coast line or beach); experimental habitat (human-made or natural structures with the intention of creating and/or imitating habitats for organisms); marina (human-made structures with the intention to serve as wharf for boats); offshore platforms (human-made structures with the intention of fossil fuels exploitation or power generation); and wreck (shipwrecks). Sampling method refers to the method used for data collection. For study duration and depth, were created categories in months (i.e. ≤1, 2-6, 7-12, 13-24, >24) and meters (i.e. 0-5, 6-15, 16-30, >30), respectively. Non-native species associated with artificial habitats and studies that were carried out in protected areas or conservation units were also recorded.

Results

The search resulted in 635 papers, of which 460 matched the proposed criteria (see methods and Supplementary Material I). Publications on this subject began in the early 70s (1973), but until the late 80s few publications were found in our search. There was a first peak of publications in 1994, but it was only in late 90s that the number of studies began to

progressively increase (Fig. 1A). The year with the most papers was 2013 (*n*=46) followed by 2014 and 2015 (*n*=40 each). Studies were published in 124 different journals. The journals with highest absolute number of studies were *Marine Ecology Progress Series* and *Bulletin of Marine Science*, while *Brazilian Journal of Oceanography* and *Marine Environmental Research* showed the highest weight in publications (i.e. highest number of publications on the topic relative to the total number of publications) (Fig. 1B).

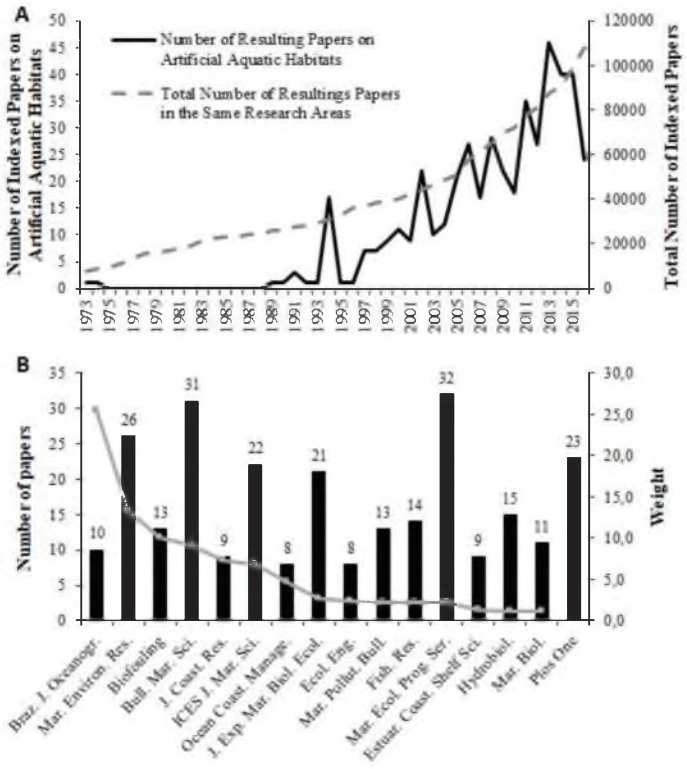


Fig. 1. (A) Temporal variation of the number of publications with artificial aquatic habitats compared to the total number of publications in the same research areas. Black solid line represents the number of resulting indexed papers on artificial aquatic habitats. Gray dashed line represents the total number of resulting papers in the same research areas. (B) General pattern of journals publishing artificial aquatic habitats studies. Black bars represent the number of resulting papers for each journal. The gray line represents the relative weight for each joural (see methods).

Studies were conducted in 59 countries. While most countries had only three or fewer publications, the United States (*n*=87), Australia (*n*=68), Italy (*n*=34) and Brazil (*n*=24) were responsible for the largest number of studies (Fig. 2; Supplementary Material II). Overall, studies were concentrated in Europe (*n*=146) and North America (*n*=103), followed by Oceania

(*n*=79), Asia (*n*=74), South America (*n*=31), Africa (*n*=9), Eurasia (*n*=8) and Central America (*n*=2).

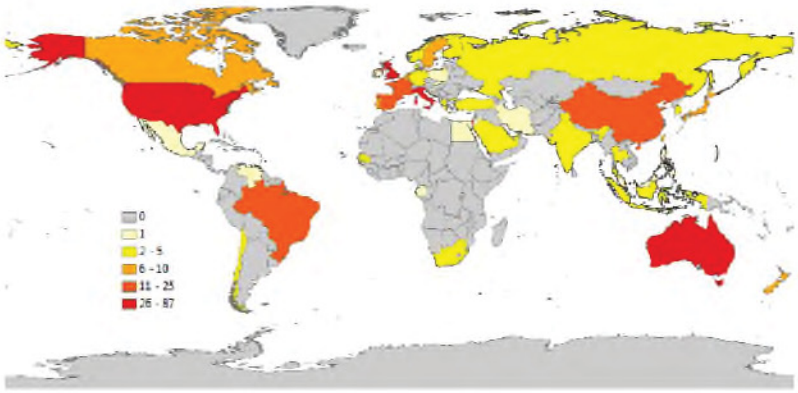


Fig. 2. World map showing the number of artificial aquatic habitats studies by countries. The colors correspond to the number of studies published in each country.

Field based approaches were the most employed research method, primarily experimental studies (43%, *n*=206), followed by observational ones (37%, *n*=177) (Fig. 3). With respect to ecosystems, there is a clear dominance of marine studies (74%, *n*=323), followed by estuary (21%, *n*=89) and freshwater (5%, *n*=21).

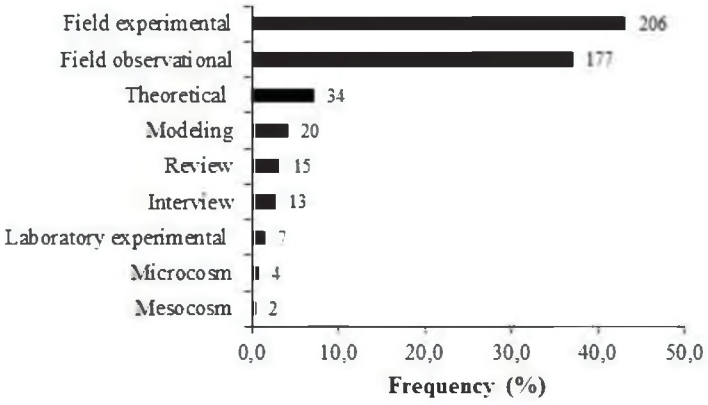


Fig. 3. Number of studies per research method.

In coastal ecosystems, the majority of marine ecoregions were represented, with the exception of the Southern Ocean, with a dominance of studies in the Temperate Northern Atlantic (47%, *n*=187) (Fig. 4). For continental ecosystems, most of the studies were conducted

in lakes (33%, *n*=7), followed by reservoirs and rivers (23%, *n*=5 each) and other kinds of environments (19%, *n*=4).

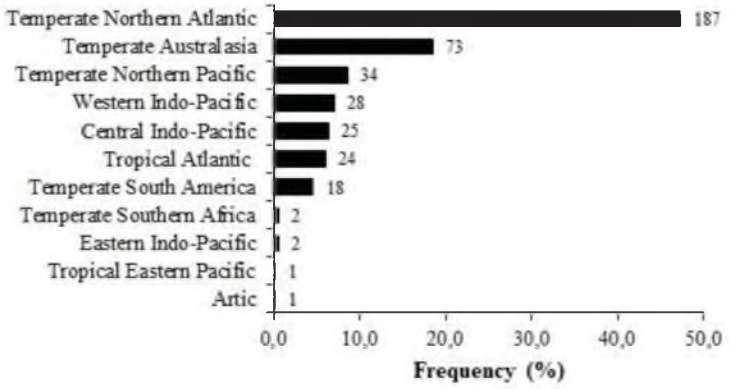


Fig. 4. Number of studies by marine biogeographical realms.

Considering the kinds of artificial habitats, experimental habitat (54%, *n*=248) and coastal defence (13%, *n*=61) were the most studied (Fig. 5), while concrete was the most common material installed in aquatic systems (supplementary material III).

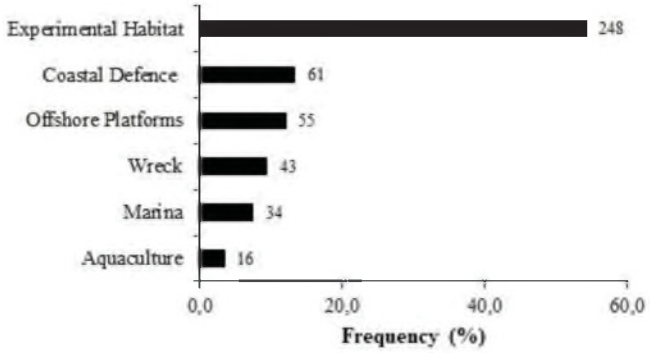


Fig. 5. Number of studies by kind of artificial aquatic habitats.

The most used sampling method was visual census through scuba diving (Supplementary Material IV). Artificial habitats between 0 and 5 meters deep were the most studied, and the number of studies decreased as depth increased (Fig. 6A). Study duration frequently ranged from 2 to 12 months, accounting for almost half of all publications (52%, *n*=174). Only 19% of studies (*n*=65) lasted for more than two years (Fig. 6B).

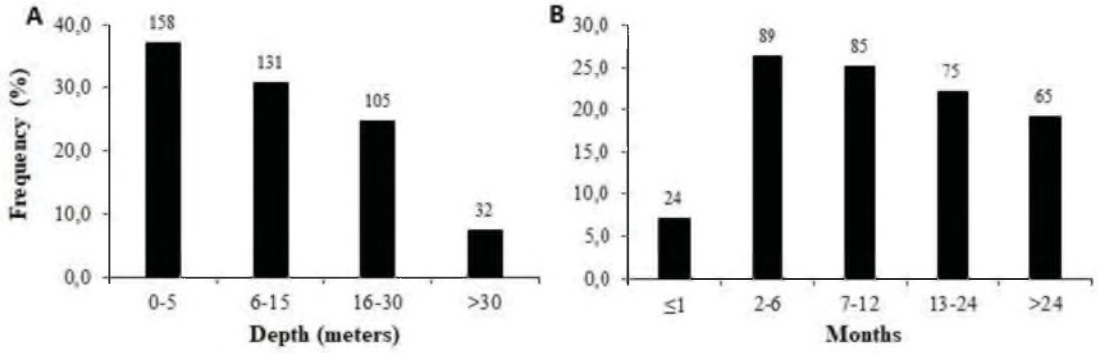


Fig. 6. Number of studies according to sampling depth (A) and study duration (B).

For taxonomic groups, Fish (18%, *n*=184), Crustacea and Mollusca (13% each, *n*=131 and 129, respectively) were the most representative in all studies. Non-native species were recorded in 11% of the studies (*n*=52), totalling 121 species. The taxonomic group with the greatest number of non-native species was Tunicate (*n*=25), followed by Crustacea (*n*=20) (Fig. 7). The non-native species with the greatest number of records were *Diplosoma listerianum* (Tunicate) (*n*=7), followed by *Bugula neritina* (Bryozoa), *Codium fragile* ssp. *tomentosoides* (Algae), *Megabalanus coccopoma* (Crustacea), *Styela plicata* (Tunicate) (*n*=6 each). A small number of artificial habitats studies were carried out in aquatic protected areas (5%, *n*=22), of these, most were in China and Spain (Supplementary Material V).

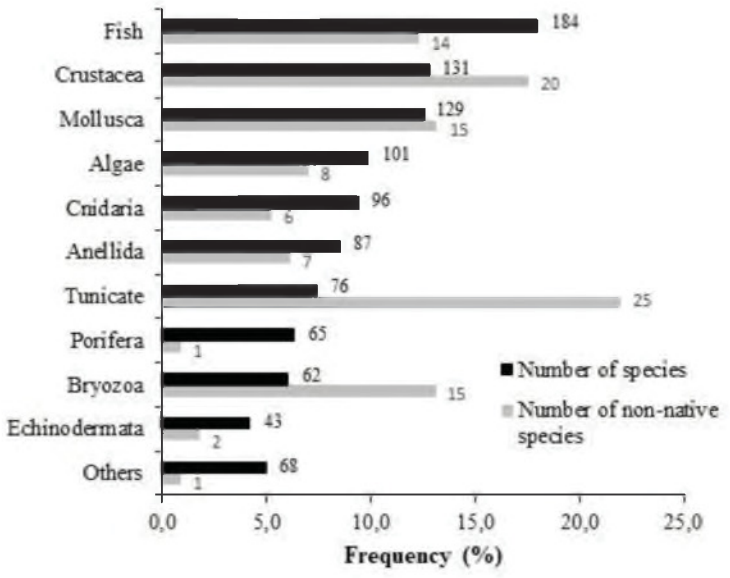


Fig. 7. Proportion of total number of species (black bars) and non-native species (grey bars) for taxonomic groups. The numbers of species are showed beside the bars.

Discussion

The present review aimed to highlight temporal, geographic and methodological patterns and/or trends in artificial aquatic habitats studies. More importantly, taking into account a variety of kinds of artificial habitats, regardless of the deployment purpose. That is because submerged structures will be colonized and, consequently, serve as habitat for different organisms, regardless of human intentions. This approach expands our understanding about the role that artificial habitats play in aquatic ecosystems. In this sense, it is important to consider and seek to understand the effects that different kinds of structures have on the life cycle of aquatic organisms.

The first publication detected in the search was in 1973, after this, surprisingly, few studies were found until the early 90s. Possibly, that was because until the mid-80s most publications on this theme were non peer-review technical reports (Bohnsack and Sutherland, 1985). However, it might also be a bias from the Web of Science database, where old publications may not be indexed as much as recent ones. From the 1990s, the number of publications began to grow progressively, with some peaks followed by retractions, but tending to follow the same pattern of the total number of publication on the same research areas. Most of these peaks coinciding with years in which special issues of CARAH proceedings were published (e.g. 1994, 2002, 2006, 2011, 2015) (Bortone, 2015), demonstrating the importance of these conferences in this research field.

Most of the studies on artificial aquatic habitats were published in journals exclusively marine focused, indicating a trend of publications in this kind of ecosystem. However, it is worth highlighting some possible biases regarding the number and weight of publications. For example, *Bulletin of Marine Science* (the second in number of publications) published four special issues of CARAH proceedings (1985, 1989, 1994 and 2006) (see Bortone, 2015). In the same way, *Brazilian Journal of Oceanography* (the first in weight of publications) also

published a special issue of CARAH proceedings (2011), moreover, this is a recent journal (the first edition was published only in 2004), which probably also contributed to increasing its weight. These numbers indicate that a considerable portion of publications on this subject come from these international conferences.

Publications from 59 countries were registered in the search. This is a relatively modest number given that, currently, there are officially 193 countries in the world (UN, 2018), indicating that in most countries there is no scientific research related to any kind of artificial aquatic habitats. Japan and USA were pioneers in the development of artificial aquatic habitats (Bohnsack and Sutherland, 1985). However, nowadays, USA leads the global number of publications in the area, while the relevant Japan is only the fourth placed on the Asian continent. This may be due to the fact that most Japanese publications are not available in English, and also that the national government of Japan funded most of the large-scale projects in this country (Grove et al., 1989), generating a large amount of information in non-peer reviewed publications (e.g. technical reports), not available on the Web of Science. However, it is important to notice that the dominance of North America and Europe in number of publications is not an exclusive pattern in this research field (e.g. Pysek et al., 2008; Braga et al., 2012), and this seems to be related to greater economic and political incentives to scientific research in countries of such continents (King, 2004).

Bohnsack and Sutherland (1985), reviewing artificial reefs publications, reported that most studies were purely descriptive and less than 20% employed an experimental approach, however, this trend seems to have changed. Most publications were experimental field studies, where artificial habitats were constructed or manipulated intentionally, aiming to test specific hypotheses. Bortone (2006) had already indicated this change in the publications pattern, from a broad and non-specific approach to hypothesis-based designs. However, there are still few studies focusing on data synthesis, modelling, effect predictions or performed in micro or

mesocosms. Such kinds of studies are essential to assess and promote the applicability of artificial habitats and/or to test the effects of such artificial structures in aquatic systems.

Marine ecosystems are by far the most studied. Probably because it was in the sea that the first artificial structures were intentionally submerged (Fabi et al., 2011). Additionally, some kinds of artificial habitats considered in this review (e.g. wrecks, offshore platforms, and coastal defence) are typically marine. On the other hand, publications in estuaries are most related with marina structures (e.g. floating pontoons and piers), because these ecosystems usually provide adequate shelter for boats and ships. Considering coastal ecosystems, most studies are in temperate biogeographic realms (Northern Atlantic, Australasia, Northern Pacific), indicating a lack of studies in tropical oceans and estuaries. Freshwater ecosystems were the least studied, even though there are in the literature studies dating from the late 70s in USA (e.g. Wilbur, 1978). Most of the freshwater studies were carried out in temperate lakes. Despite the increasing number of reservoirs around the world (Zarf et al., 2015) and the potential applicability of artificial habitats in management and conservation of reservoirs (Santos et al., 2008; 2011), few studies have been carried out in such environments.

The fact that experimental habitats were the most frequent kinds of artificial habitats in our review is not a surprise, since the study of artificial habitats began with structures intentionally submerged to mimic some characteristics of natural environment in order to improve fisheries (Fabi et al., 2011). Concrete was the most used material in aquatic structures (mostly in blocks, of different shapes and sizes) probably due, to its low cost, high durability (Bohnsack and Sutherland, 1985), and the effectiveness of this material as substrate for fouling organisms (Fitzhardinge and Bailey-Brock, 1989). However, since the 90s other kinds of structures, submerged for different purposes, began to be studied as novel habitats in aquatic ecosystems. Then the artificial habitat concept began to be applied to a variety of structures, regardless of the deployment purpose. In the future, the number of artificial habitats in aquatic

ecosystems will continue to grow along with human population growth (Dafforn et al., 2015). In this sense, the number of coastal defence structures (mainly breakwaters and seawalls) will keep increasing worldwide, due to the growing need to mitigate effects of the rising ocean levels, caused by global warming (Nicholls and Cazenave, 2010). Likewise, the number of artificial habitats created by aquaculture structures will keep increasing, mainly due to the expansion of such activity in tropical freshwater systems (Lima et al., 2016). It is important to highlight the lack of studies evaluating the effects of artificial habitats created by offshore platforms for oil exploration in developing countries.

The dominance of visual census in artificial aquatic habitats samplings indicates the preference for non-lethal methods. In this sense, new technologies such as underwater cameras and filming systems are increasingly being incorporated into research and monitoring, in addition to enabling sampling at deepest sites (Ebner et al., 2014; Whitmarsh et al., 2016). The inverse relationship between number of publications and sampling depth can be simply explained by the ease of sampling in shallow as opposed to deep water. By way of example, there are many papers analysing shallow artificial habitats, including between-tides structures. Another reason may be the physiological limitation and increased risk of decompression sickness imposed by scuba diving, the most common sampling method (NOAA, 2001).

Overall, short- and medium-term field studies were more published. One reason for this may be that most of peer-reviewed publications are related to postgraduate research (i.e. Master, PhD, and Postdoc) where the study duration is limited by a short deadline, delimited according to the degree. On the other hand, long-term projects have high costs and are usually, funded by governments or non-governmental organizations, having, in general, their results published as technical reports (e.g. Grove et al., 1989). One way to minimize this bias is to make efforts in the sense that governments and non-governmental organizations publish and make available

the results of their researches in peer-reviewed journals, a more accessible and robust publication form.

Regarding taxonomic groups, fish were the most studied, probably because historically, artificial habitats were created to enhance fisheries opportunities and/or production (Bortone, 2006). Most of the other taxonomic groups were represented by fouling organisms' adhered to artificial structures. Interestingly, for non-native species, the most frequent taxonomic groups were fouling animals, indicating that artificial habitats can serve as stepping stones (Mineur et al., 2012; Adams et al., 2014), facilitating the dispersal and establishment of non-native species (Marques et al., 2013). This is a problem especially in offshore platforms and marina structures (e.g. Messel et al., 2015; Schwan et al., 2015) that are in direct contact with vessels, one of the main vectors of species introduction (Frehse et al., 2016), and in aquaculture structures, by the fact that, in some regions, most of the cultivated species are non-natives (Padial et al., 2017). Therefore, it is important to record non-native species occurrence and evaluate, specifically, the role that each kind of artificial habitats exert in the different phases of the invasion process (Blackburn et al., 2011), in order to propose control and eradication measures (e.g. Coutts and Forrest, 2007). In contrast to the possible negative impacts related to non-native species, artificial experimental habitats can serve as a tool for endangered species conservation (Giglio et al., 2014; Bueno et al., 2016) and maintenance of natural habitats (Polak and Shashar, 2012). In this sense, the experimental habitats installation should be preceded by well-defined objectives, previous local assessment (physical and biological), and subsequent monitoring, in order to evaluate if the results are in agreement with the initial purpose (e.g. Brandini, 2014)

In conclusion, this review demonstrated that the number of artificial aquatic habitats publications began to increase from 1990, mostly in Western Northern Hemisphere countries. We highlighted the importance of the CARAHs in the number of publications, the lack of studies in tropical and freshwater ecosystems and trends in duration and kind of study, kind of

artificial habitat, depth, sampling methods, and taxonomic groups. In the last decades, we have observed changes in the pattern of publications on the subject, towards experimental studies, including a wider range of habitats and taxonomic groups. Now it is necessary to take the next step, directing future artificial aquatic habitat research towards data synthesis and effects prediction, especially considering the combined effect with other human actions such as species introduction and climate change.

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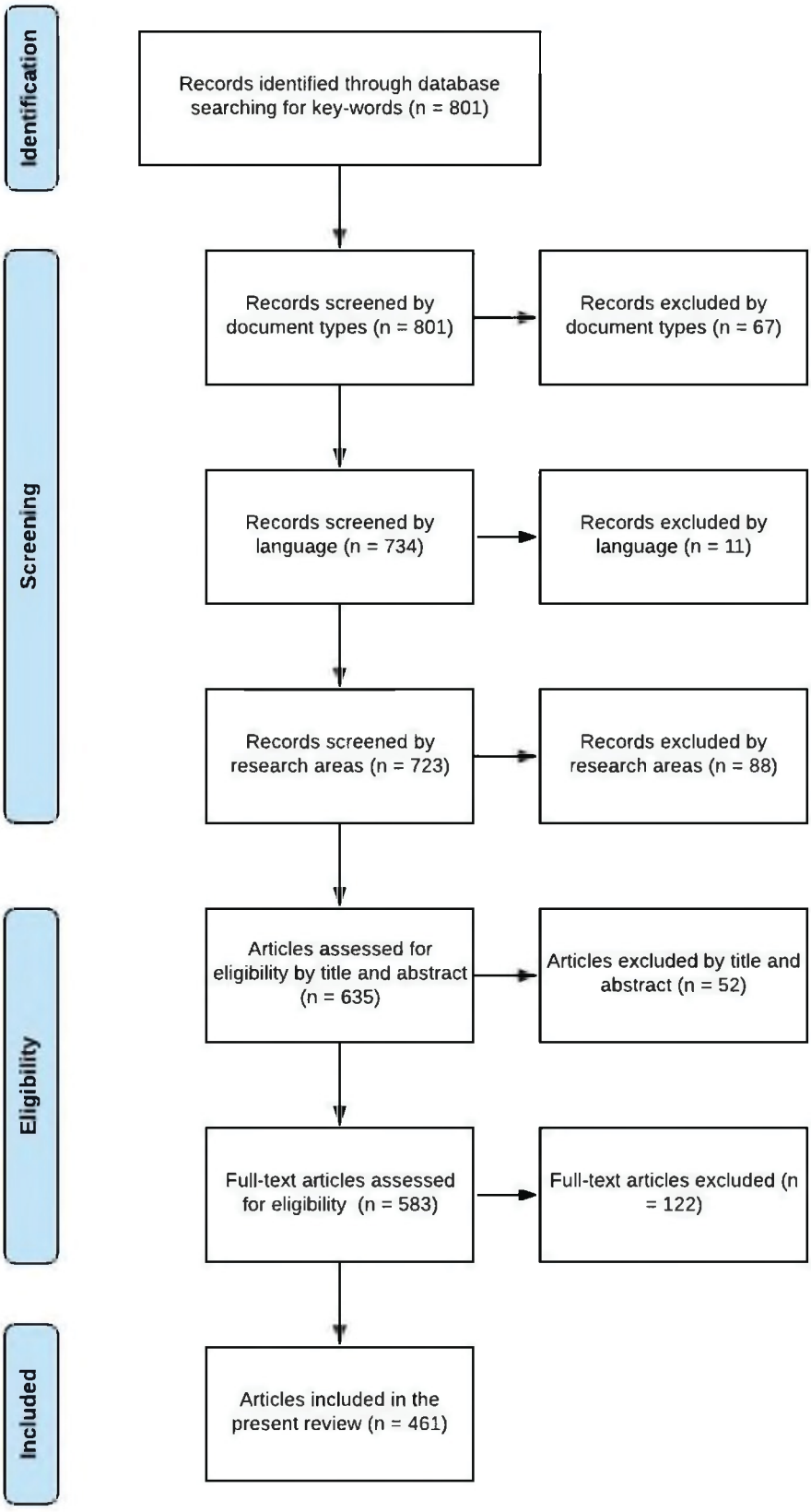
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Supplementary Material I. PRISMA flow diagram, showing the number of records identified, included and excluded in the different phases of the systematic review process.



Supplementary Material II. Number of artificial aquatic habitats studies by countries.

Africa		**Europe**		**Northh America**	
Cabo Verde	3	Italy	34	USA	87
Senegal	2	Spain	17	Canada	8
South Africa	2	England	16	Bahamas	4
Egypt	1	Scotland	16	Barbados	2
Gabon	1	France	11	Alaska	1
		Portugal	9	Mexico	1
Asia		Belgium	8		
Israel	17	Sweden	8	**Central America**	
China	16	Netherlands	6	British Virgin Isl.	1
Taiwan	9	Germany	5	Saint Kitts Isl.	1
Japan	6	Denmark	4		
South Korea	5	Greece	4	**South America**	
Malasya	4	Finland	3	Brazil	24
Singapore	3	Azores	2	Chile	5
Thailand	3	Norway	2	Galapagos	1
U. Arab Emirates	3	Croatia	1	Venezuela	1
India	2	Northern Ireland	1		
Indonesia	2	Poland	1	**Oceania**	
Saudi Arabia	2	Slovenia	1	Australia	68
Iran	1			New Zealand	8
Jordan	1	**Eurasia**		Guam Isl.	1
Maldivas	1	Turkey	5	N. Marianas Isl.	1
Philippines	1	Russia	2	Tasmania	1

Supplementary Material III. Number of studies per type of material in artificial aquatic habitats.

Experimental Habitat		**Coastal Defence**	
Concrete	95	Breakwater	31
Plate or Disk	45	Seawall	30
Tyres	19	Shark Net	2
Reef Ball®	18	Bars	1
Rocks	17		
Steel	16	**Aquaculture**	
Artificial Aquatic Plants	13	Fish Farming	5
Platstic	13	Mussel Farming	3
Special Concrete Blocks	10	Offshore Fish Farming	3
Others	8	Oyster Farming	3
Not Specified	7	Algae Farming	1
PVC	7	Offshore Mussel Farming	1
Oyster Shell	6	Scallop Farming	1
Waste	6		
Traps	5	**Offshore Platforms**	
Tree	5	Offshore Wind Farm	19
Wood	4	Offshore Oil and/or Gas Plataforms	18
Bamboo	2		
Geotextile Containers	2	Marine Renewable Energy Devices	7
Iron Statues	2		
Metal Net	2	Decommissioned Offshore Oil and/or Gas Platforms	6
Floating structure	2		
Gabions	1	Oil Terminal	5
Foam	1		
Ceramic	1	**Wreck**	
ECOncrete®	1	Intentional	21
		Not Specified	18
Marina		Unintentional	4
Fixed	24		
Floating	21		
Mooring	1		

Supplementary Material IV. Number of studies per sampling method in artificial aquatic habitats.

Acoustic Telemetry	11	Dive Operators Data	3
Collection of Individuals		Interview and/or Questionnaire	14
Angling	10	Mark and Recapture	4
Clove Oil	1	*Sediment Samples*	
Dynamite	1	Hapscore Sampler	1
Electric Fishing	1	Core Sample	12
Fyke Net	1	Peterson Grab	1
Gill Net	21	Van Veen Grab	8
Manual	18	Sonar	8
Rotenone	1	Structure Removal	63
Scuba Diving	15	Underwater Photography	35
Seine Net	2	Underwater Video	18
SMURFS	1	BRUV	5
Snorkeling	2	*Visual Census*	
Spear Fishing	1	Not diving	55
Trammel Net	2	Manned Submersible Vehicle	3
Traps	9	Remotely Operated Vehicle	6
Trawling Net	6	Scuba Diving	107
Zooplankton Net	4	Snorkeling	12

Supplementary Material V. Countries and protected areas where artificial aquatic habitats studies have been conducted.

Australia	**Indonesia**
Batemans Marine Park	Komodo National Park
Halifax Park Aquatic Reserve	
	Italy
Barbados	Marine Protected Area of Baia
Folkestone Marine Reserve	Marine Protected Area of Miramare
Brazil	**Northern Ireland**
Abrolhos National Marine Park	Marine Protected Area of Strangford Lough
China	**Spain**
Marine Special Areas in Hong Kong	Bay of Biscay
Marine Special Areas in Hong Kong	Cabrera Archipelago National Park
Marine Protected Area of Ping Island	Marine Protected Area of Palma Bay
Marine Protected Area of Port Shelter	Marine Reserve at Tabarca
Yan Chau Tong Marine Park	
	USA
France	Florida Keys National Marine Sanctuary
Golfe-Juan Marine Protected Area	Florida Keys National Marine Sanctuary
Marine Protected Area of Vallauris-Golfe Juan	Flower Garden Banks National Marine Sanctuary

CHAPTER 2

EXPLORING THE USE OF ARTIFICIAL HABITATS BY FISH IN NEOTROPICAL RESERVOIRS: IS THERE ANY PATTERN?

Exploring the use of artificial habitats by fish in Neotropical reservoirs: is there any pattern?

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Abstract

The use of artificial habitats as a tool for management and conservation of lentic freshwater ecosystems has been most studied in North America, and there, Centrarchidae are the dominant species in such habitats. Few studies have evaluated the use of artificial habitats by fish in Neotropical reservoirs. In addition, contrary to the north temperate ecosystems, Cichlidae species appear to be prevalent on artificial habitats in Neotropical reservoirs. In modern times, due to the rapid increase of non-native fish introductions globally, it is expected that more and more of these two fish families will be coexisting in the same environments. In this sense, the aim of this study was to evaluate the use of artificial (ceramic pipes and trees) and semi-natural (rocks and non-structured) habitats by fish, in two Neotropical reservoirs where Cichlidae and Centrarchidae species co-occur. The results showed that, small individuals (< 80 mm) of Cichlidae and Centrarchidae dominated the use of the habitats. However, the abundance and composition of species varied according to the habitat. In both studied reservoirs, native and non-native Cichlidae appear not to co-occur with Centrarchidae species in the same habitat, indicating some type of habitat partitioning between these two families. Ceramic pipes were mostly used by native Cichlidae, while trees seem to be preferred by Centrarchidae. This study discusses the applicability of artificial habitats in Neotropical reservoirs and highlights the importance of considering the kind of habitat and the origin of species associated with it, in

order to prevent invasive species from benefiting from the deployment of artificial structures in freshwater systems.

Keywords: Artificial reef, Artificial structures, Colonization, Ecological succession, Freshwater ecology, Fish assemblage, Lentic ecosystems, Successional changes.

Introduction

The anthropogenic transformation of freshwater lotic ecosystems into lentic environments for reservoirs construction has increased worldwide (Zarfl et al. 2015). Unfortunately, despite the many facilities provided to modern human life, such as water supply and power generation, modifications resulted from reservoirs construction cause drastic changes in the biotic and abiotic structure of these aquatic ecosystems (Rosenberg 2000). The impoundment of a river makes the physical environment less heterogeneous, by modifying the water dynamics and destroying shelters, breeding and feeding sites, changing the composition and structure of the fish assemblage (Agostinho et al. 2004). Such alterations facilitate the introduction (Vitule et al. 2009) and establishment of non-native species (Jonhson et al. 2008), consequently, leading to the homogenization of fish fauna in these environments (Clavero and Hermoso 2011, Vitule et al. 2012, Daga et al. 2015).

The use of artificial habitats as a tool for management and conservation of aquatic environments has been studied mostly in marine ecosystems (see Chapter I). In freshwater, some studies were performed to evaluate the effect of artificial structures on the attraction and catches of fish, especially in North America (Bolding et al. 2004). In temperate lentic environments, Centrarchidae species dominate the use of submerged artificial structures (Graham 1992, Rold et al. 1996, Magnelia et al. 2008, Daugherty et al. 2014). However, factors such as material, size, and complexity of structures (Johnson and Lynch 1992, Wills et al. 2004), as well as depth and temperature (Walters et al. 1991), may influence the use of these artificial habitats by freshwater fish.

Despite the great fish diversity in the Neotropical freshwater environments (Toussaint et al. 2016, Vitule et al. 2017) there are many increasing conservation problems, in particular dams constructions and fish invasions (Pelicice et al. 2017). Unfortunately, just four studies have evaluated the effects of artificial structures as fish habitat in reservoirs in such region (i.e.,

Freitas and Petrere 2001, Freitas et al. 2005, Santos et al. 2008, 2011). Contrasting with temperate northern regions, which are dominated by Centrarchidae, there seems to be a dominance of Cichlidae species associated with artificial habitats in Neotropical reservoirs (Santos et al. 2008). The use of artificial habitats by fish in reservoirs depends on the composition and abundance of local species, as well as, on the availability of natural habitats (Bolding et al. 2004). Nevertheless, Santos et al. (2011) proposed as conceptual model that, as Centrarchidae in temperate systems, Cichlidae would be the prevalent group of fish associated to artificial structures in Neotropical reservoirs.

Although there seems to be a direct relationship between species diversity and habitat heterogeneity (Freitas et al. 2005), it is important to consider the origin of species (i.e., native or non-native) associated with artificial habitats (Santos et al. 2008), since artificial structures can act as stepping stones or even corridors for some aquatic invasive species (Mineur et al. 2012). The conceptual model of Santos et al. (2011) is the only one to outline general biogeographic patterns on the use of artificial structures by fish in reservoirs. However, due to the increasing global flow of goods and people, and introductions of non-native species (Lockwood et al. 2007), it is expected that more and more of these two fish families will be coexisting in the same environment.

In Brazil, Centrarchidae, mostly represented by the largemouth bass (*Micropterus salmoides*) was introduced to several reservoirs, mainly in the south and southeast regions (Godoy 1954). Such species is already established, with self-sustaining populations, being considered an invasive species at several locations (Garcia et al. 2014, Ribeiro et al. 2015, Daga et al. 2016, Oliveira et al. 2016). This family is currently found coexisting with Cichlidae in several southern reservoirs, represented mainly by the native *Geophagus brasiliensis* and the non-native Tilapias *Coptodon rendalli* and *Oreochromis niloticus* (Daga et al. 2015, 2016). That is the case in some reservoirs including Piraquara I and Passauna around Curitiba.

In front of the growing global process of non-native fish introductions (Chamberlain 1947, Cambray 2003), such reservoirs around Curitiba, offer an ideal experimental model to formally test the use of artificial structures by fish, expanding the theoretical conceptual model proposed by Santos et al. (2011), due to the sympatric occurrence of Centrarchidae and Cichlidae members. Based on this perspective, the present study aims to explore the use of habitats by fish, in order to test if the Cichlidae species dominance on artificial structures is a general pattern for Neotropical reservoirs, even in the co-occurrence with Centrarchidae species, and if it is true, also verify what is the origin (i.e., native or non-native) of the prevalent and abundant groups.

Material and methods

Study Area

The study was carried out in two water supply reservoirs around Curitiba City, Paraná State, Southern Brazil (Fig. 1). The Piraquara I reservoir (25°30'17" S; 49°01'31" W) is a 3.3 km^2 impoundment, located 920 m above mean sea level. This reservoir was filled in 1979 and presents a mean discharge of 3 m^3/s, mean depth of 7 m, maximum depth of 23 m and is 6 km long. The Passauna reservoir (25°31'40" S, 49°23'16" W) is a 11 km^2 impoundment, located 900 m above mean sea level. This reservoir was filled in 1990 and presents a mean discharge of 1.5 m^3/s, mean depth of 9.4 m, maximum depth of 16 m and is 9.3 km long (Andreoli 2003). Both reservoirs are located in the Upper Iguaçu River Basin, in the Iguaçu Ecoregion (FEOW 2018) and are administered by the Sanitation Company of Paraná (SANEPAR), providing water to more than one million people. The water level fluctuation was negligible during the study period (< 0.4 m) in both reservoirs, and human activities such as swimming, vessels and fishing were not allowed at the time of the study.

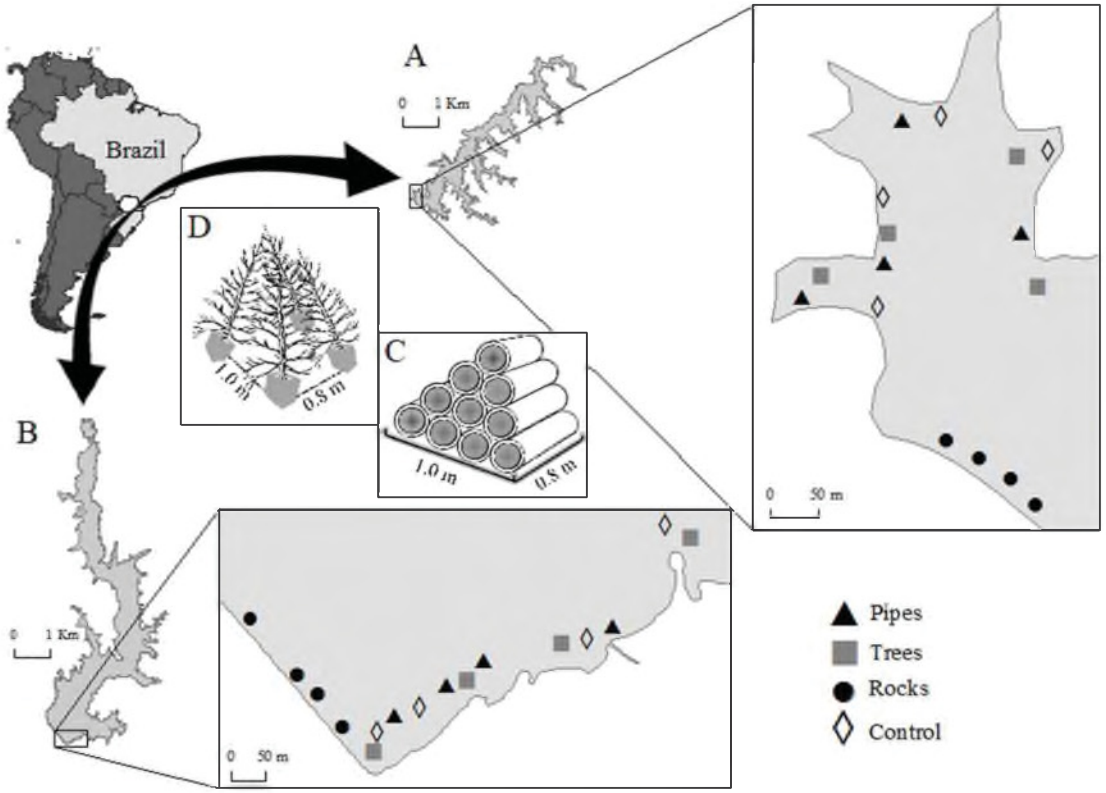


FIG. 1. Study area: Piraquara I (A) and Passauna (B) Reservoirs (Brazil), and the sites where the habitats were located in each reservoir. The final pyramidal arrangement of the pipes (C) and trees (D) artificial habitats.

Field Experimental Design

Four different habitats (two artificial named pipes and trees; and two semi-natural named rocks and control) were sampled in order to evaluate their use by fish. The artificial habitats *Pipes* was built by piling up 0.8 m long x 0.25 m diameter ceramic pipes in a pyramidal frame (10 pipes in a 4:3:2:1; base-to-top arrangement; idem used by Santos et al. 2011, Fig. 1C). The artificial habitat *Trees* was composed by four individuals of the invasive pine trees (*Pinus taeda*) (1.0 m high), each one attached to a concrete ballast, arranged in a rectangular base (1.0 x 0.8 m) with one tree in each corner, and tied all together by the top, in a pyramidal frame (Fig. 1D). The *Rocks* habitat was formed by four or five submerged rocks (between 0.5 and 0.8 m in diameter) already deposited to assist in the structural stabilization of the dam riprap, demarcated in a rectangular base (1.0 X 0.8 m). Non-structured habitats were demarcated directly on the substrate of the reservoirs devoid of any submerged structure in a

rectangular base (1.0 X 0.8 m) and named as *control*. A total of 16 habitats (four replicates per treatment) were built or delimited in each reservoir.

Sampling sites comprised an area near to the dam, constantly monitored, at an average depth of 3 m. In general areas near to the dam present higher water transparency than others parts of the reservoir, allowing comparisons between different types of reservoirs. The sampling sites were devoid of natural vegetation or other submerged structures around, and the substrate was composed of clay and sand. A minimal distance of 30 m was maintained between sites, in order to avoid interference among treatments. The arrangement of treatments was randomly assigned, and each received a float attached to a ballast to facilitate detection. The habitats were deployed or delimited between July 07 and 17 in the Piraquara I reservoir and between August 15 and 25 in the Passauna reservoir of 2015, by scuba divers.

Samplings

Underwater visual census was used to analyse the use of habitats by fish: The visual census was performed by a scuba diver following Santos et al. (2008). Each habitat was surveyed for about five minutes. All fish observed until 1 m away from the structures or delimited areas were considered associated with the habitats. All fish associated with the habitats were identified, counted, and recorded on an underwater writing tablet. The total length (Lt) of each individual was visually estimated, comparing fish size with adjacent objects of known size. Due to difficult identification and counting of small fish, only individuals larger than 30 mm in total length were considered. The only fish that were not identified to the specific level were Tilapias, due to difficulties in differentiating the two species that occur in our study. Therefore, the term *Tilapia* used thereinafter refers to individuals of the species *Coptodon rendalli* and *Oreochromis niloticus*, which are both non-native and co-occur in both reservoirs.

The samplings began in August and September of 2015 at the Piraquara I and Passauna reservoirs, respectively, about 30 days after the habitats establishment. Each reservoir was

sampled bimonthly (one reservoir per month, alternately) until January 2017, totalizing nine samplings, through 17 months period, in each. The order in which habitats were surveyed was randomized in each sampling event. The visual census survey was performed between 10:00 AM and 3:00 PM to optimize the visibility. Temperature of the water was measured at all sampling sites.

Data analyses

For general exploratory analyses, each reservoir was analysed independently and the species were arranged into broader taxonomic groups according to origin (i.e., native or non-native) (see Table 1). Analyses began with transforming the total abundance values of each taxonomic group in each visual census survey with log (X + 1) and then constructing a similarity matrix using the Bray-Curtis index (Bray and Curtis 1957) for each reservoir. The next step was to submit these matrixes to a permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) to test the hypothesis concerning the influence of different factors on the fish abundance. The analytical design tested the factors: habitat (four levels; Fixed) and month (nine levels; Fixed). All tests were permutated 9,999 times under a reduced model (Anderson et al. 2008). For statistically significant differences ($P \leq 0.05$), *post-hoc* pairwise comparisons were performed under 9,999 permutations to check for possible differences between pairs.

Using the same similarity matrices, Non-Metric Multidimensional Scaling and Unweighted Pair Group Method with Arithmetic Mean (NMDS – UPGMA) were conducted for each reservoir, to visualize the groupings formed as profiled against habitat factor (Clarke and Gorley 2006). Utilizing the total abundance values, bubbles were superimposed on the image obtained from the NMDS ordination plot, indicating the contribution of each taxonomic group in the clustering of habitats.

In order to verify possible temporal patterns in the use of habitats by fish, the mean number of individuals of each taxonomic group was evaluated per month, along with the mean water temperature, in each sampled habitat. Fish were also evaluated for their Lt, in order to find possible patterns in the use of the habitats by the distinct size classes. Based on the Lt, all individuals were arbitrarily grouped into six size classes (31 -79; 80-129; 130-179; 180-229; 230-279 and >280 mm).

Results

Overall, 940 and 1,303 individuals were observed in the sampled habitats in Piraquara I and Passauna reservoirs, respectively (Table 1). At each reservoir, seven fish species were recorded, being four species (*G. brasiliensis*, Tilapia, *M. salmoides* and *Astyanax bifasciatus*) common and prevalent to both. *Geophagus brasiliensis* was the most abundant species, and along with Tilapia and *M. salmoides* accounted for more than 93% of total abundance in both reservoirs. Rocks presented the highest mean species richness in both reservoirs (Fig. 2). Considering taxonomic groups, in both reservoirs, native Cichlidae showed greater mean abundance of individuals in pipes and control, and Centrarchidae in trees. In rocks, the highest mean individual's abundance were of non-native and native Cichlidae in Piraquara I and Passauna reservoirs, respectively (Fig. 2).

TABLE 1. Total abundance of individuals observed by habitat in each reservoir.

Taxonomic group Fish species	Total Abundance							
	Piraquara I				Passauna			
	Pipes	Trees	Rocks	Control	Pipes	Trees	Rocks	Control
Native Cichlidae								
Geophagus brasiliensis	182	38	103	67	600	56	157	53
Cichlasoma facetum			1					
Non-native Cichlidae*								
Tilapia *	12	31	218	11	68	7	112	9
Centrarchidae*								
*Micropterus salmoides**	29	79	84	25	128	71	36	1
Characidae								
Astyanax bifasciatus			53				2	
Oligosarcus longirostris			4					
Others								
Hoplias malabaricus		1	1	1				

Hypostomus derbyi					1			
Hypostomus myersi					1			
Rhamdia quelen					1			
Total	223	149	464	104	799	134	307	63

* *Non-native species*

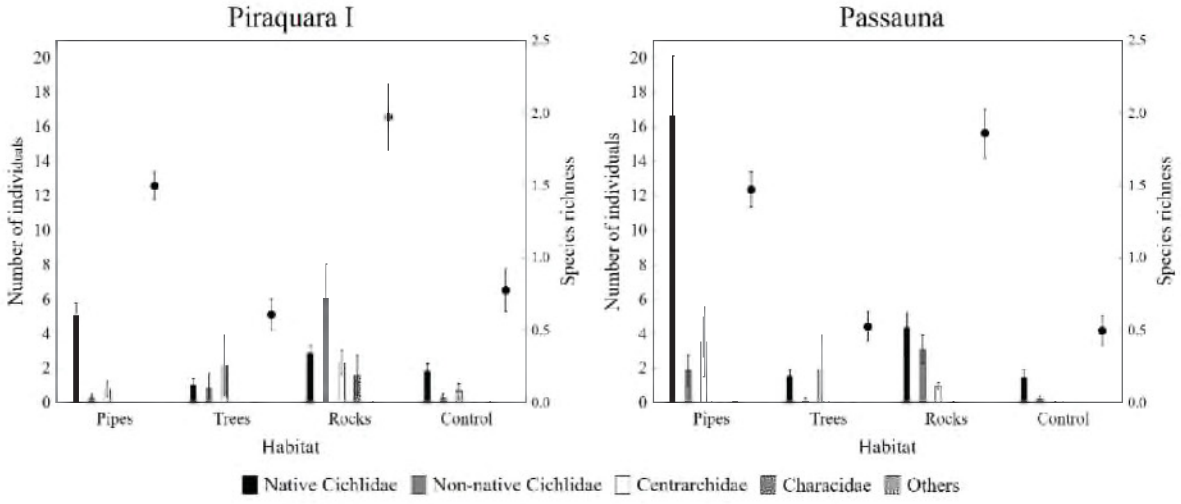


FIG. 2. Mean number of individuals by taxonomic group and mean number of species, associated to each kind of habitat in Piraquara I and Passauna reservoirs.

The PERMANOVA indicated that the taxonomic groups abundance were influenced by habitat, month and by the interaction between these two factors in both studied reservoirs (Table 2). However, in both reservoirs, the effect (*Pseudo-f*) of habitat was much higher than the other factors, indicating a greater relevance of this factor on fish abundance (Table 2). Based on these results, the PERMANOVA pairwise test for habitat indicated that fish abundance differed significantly between almost all treatments, with the exception of pipes versus control in the Piraquara I, and trees versus control in both reservoirs (Supplementary Material I).

TABLE 2. Results of the PERMANOVA analysis for taxonomic groups' abundance associated to the sampled habitats, through visual census.

Reservoir	Factor	d.f.	*SS*	*Pseudo-f*	*p(perm)*	Perms
Piraquara	Habitat	3	32720	10.277	0.0001	9936
	Month	8	30239	3.5618	0.0001	9923
	HabitatxMonth	21	48971	2.1973	0.0001	9886
Passauna	Habitat	3	31113	9.4619	0.0001	9955
	Month	8	36757	4.1919	0.0002	9919
	HabitatxMonth	22	52291	2.1685	0.0005	9877

It was possible to distinguish distinct grouping in the NMDS, indicating differences in the use of habitats among taxonomic groups (Fig. 3), corroborating the PERMANOVA results. Although native Cichlidae were present in all habitats, they were more abundant in pipes, especially in the Passauna Reservoir. Non-native Cichlidae were more abundant in rocks, showing an occurrence overlap with native Cichlidae in some habitats. Centrarchidae seem to use more trees habitats, and in general, they did not co-occur with Cichlids. Characidae and other fish used the studied habitats only sporadically, and the former just occurred in rocks (Fig. 4).

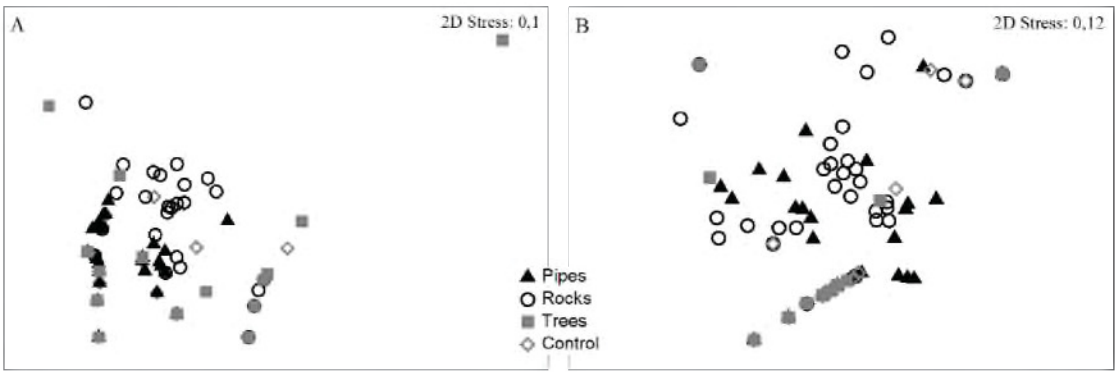


FIG. 3. Non-metric multidimensional scaling (NMDS) ordination of total abundance of taxonomic groups by habitats. (A) Piraquara I and (B) Passauna reservoirs.

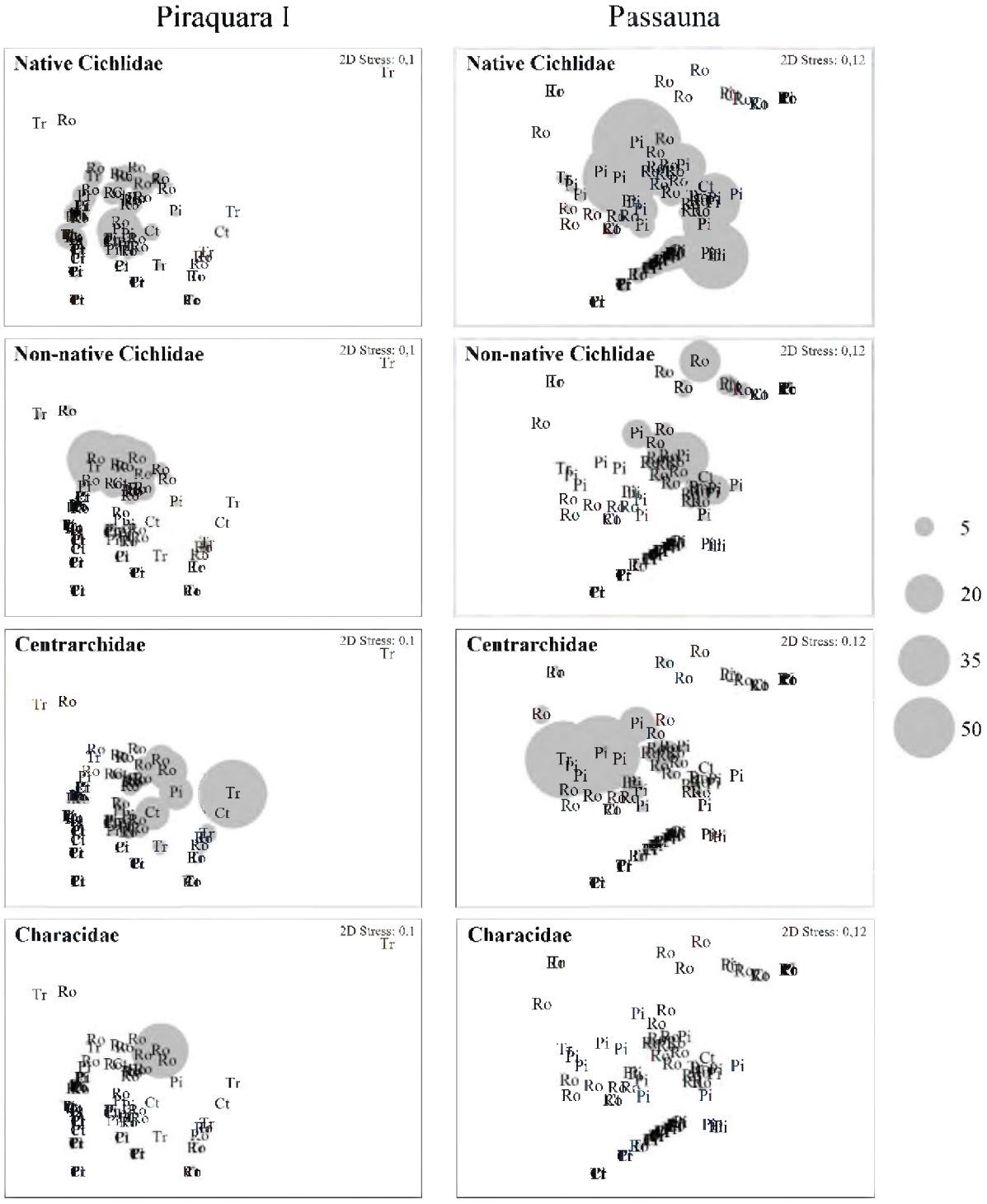


FIG. 4. Non-metric multidimensional scaling (NMDS) ordination of total abundance of taxonomic groups by habitats (Pi-pipes; Tr-trees; Ro-Rocks; Ct-control) in each studied reservoir. Bubbles size indicate the total abundance of each taxonomic group. Graphics are not displayed for Others taxonomic group due to the low number of individuals observed in this category (see Table 1).

The temporal analysis revealed the dominance of native Cichlidae in pipes during all sampling months in both reservoirs (Supplementary Material II). In the trees, a large number of Centrarchidae individuals occurred in November and December. Non-native and native

Cichlidae were the most abundant groups in rocks, mainly from September to March, and Characidae occurred especially from October to December. Even in low abundance, native Cichlidae was the most observed group in control habitats in most of the sampled months. In general, the greatest abundances of individuals were observed in the months of higher water temperature (Supplementary Material II).

Regarding size class, it was observed the dominance of native Cichlidae smaller than 130 mm Lt in pipes and Centrarchidae smaller than 80 mm Lt in trees, in both reservoirs. On the other hand, a larger number of native Cichlidae, between 80 and 130 mm Lt, and non-native Cichlidae and Centrarchidae of all size classes were recorded in rocks. Control habitats were more commonly used by native Cichlidae of different sizes (between 30 and 230 mm Lt), and sporadically by large individuals (>280 mm Lt) of non-native Cichlidae and Centrarchidae (Supplementary Material III and IV).

Discussion

The conceptual model proposed by Santos et al. (2011) (i.e., dominance of Centrarchidae in North America and Cichlidae in South America) reflects the original biogeographic distribution of the two main fish families associated with artificial habitats in lentic environments (Nelson et al. 2016). Centrarchidae is endemic to North America and the fossil record indicates that origin (Eocene *ca* 56 - 34 Ma) and diversification of this group occurred exclusively on this continent (Near and Koppelman 2009). In contrast, Cichlidae has a wide distribution in southern continental regions, including South and Central America, Africa, Arabia, Israel, Syria, Iran and India (Chakrabarty 2004). New evidence indicates that Cichlidae's origin occurred on the Palaeocene (*ca* 65 - 57 Ma), after the fragmentation of the ancient Gondwana super-continent, rather than before, as previously assumed (Friedman et al. 2013). However, the rupture of natural biogeographic barriers promoted by human assisted

species introductions has led to the coexistence of these two families in different parts of the world, such as South America (Daga et al. 2015), Africa (Ellender and Weyl 2014) and Asia (Matsuzaki et al. 2013).

Overall, both studied reservoirs presented a similar pattern regarding the abundance of the species. The native Cichlidae, *G. brasiliensis*, an abundant and widely spread species throughout freshwater environments in Brazil (Lowe-Mcconnell 1991), was prevalent in both reservoirs. Other important species were: the non-native Cichlidae, Tilapia, the most introduced non-native species in freshwater ecosystems in Brazil (Padial et al. 2017), especially for aquaculture purpose (Lima et al. 2016, Lima Jr. et al. 2018); and the Centrarchidae, *M. salmoides*, a top predator, considered one of the 100 worst invasive species on the planet (Lowe et al. 2000). However, the group's abundance varied according to the kind of habitat.

Pipes were most used by juvenile native Cichlidae in both reservoirs, indicating that it can be useful as shelter for smaller individuals (< 80 mm Lt) of *G. brasiliensis.* Other studies have already indicated that artificial habitats can potentially increase the juvenile survival by providing refuges against predators (Bolding et al. 2004, Santos et al. 2008). Comparing different materials Santos et al. (2011) verified that ceramic pipes concentrated more individuals and biomass than concrete and PVC pipes. However, as noted in the Passauna Reservoir, pipes can also be used by non-native juveniles, albeit to a lesser extent. Mixed shoals of native and non-native Cichlidae (< 80 mm Lt) were observed in the pipes, especially during the summer, indicating some degree of interaction between these fish. Interestingly, while juvenile Cichlidae appear to use more the interstices inside the pipes, the juvenile Centrarchidae tended to stay around the pipes (F. A. Frehse, *personal observation*).

Differently of the pipes, in the trees artificial habitat, Centrarchidae was the most abundant group in both reservoirs, especially juveniles of largemouth bass (< 80 mm Lt). Several studies in the North America have reported the dominance of Centrarchidae species

associated with trees, logs and brushes deployed intentionally in reservoirs and lakes, suggesting, the suitability of such kind of habitats, for this group (Johnson and Lynch 1992, Rold et al. 1996, Wills et al. 2004, Daugherty et al. 2014). Although trees do not provide small interstitial spaces, depending on the arrangement, they can create shade and darkness for concealment (Bolding et al. 2004). Most occurrences of *M. salmoides* were recorded in late spring (November) and early summer (December), coinciding with the end of the reproductive period for this species (Rodriguez-Sánchez et al. 2009, Taylor and Weyl 2017). In fact, nests and parental care were observed for *M. salmoides* in trees habitats during this period.

The rocks, a characteristic reservoir habitat, presented the highest mean number of species in both reservoirs, indicating a greater diversity of fish using this kind of habitat. This may be because rocks constitute a very structured and multiscale continuous habitat (about 200 m length and 6 m depth), colonized since the reservoir creation. Characidae species, a common fish family in Neotropical freshwater ecosystems (Lowe-Mcconnell 1991), were only observed in rocks during the summer. Also, in the summer, were observed in both reservoirs, a high abundance of adult individuals of *G. brasiliensis* (> 80 mm Lt) with nests. This, like other Cichlidae species, displays parental care (Nelson et al. 2016), with both sexes caring for the offspring. In this sense, small interstices between the rocks seem to provide cover for the fry of *G. brasiliensis*; moreover, more complex habitats may reduce intraspecific aggressiveness in this species (Kadry and Barreto 2010). Besides that, the rocks appear to be more used by large individuals (> 80 mm Lt) of non-native Cichlidae and Centrarchidae, indicating that this habitat can be used as feeding area for these non-native species.

In both studied reservoirs, the lowest abundances of individuals were recorded in control habitats. However, differently of Santos et al. (2011), which recorded only two fish individuals in unstructured habitats during 14 months of survey in Itaipu reservoir, in the present study, a considerable number of individuals (104 in Piraquara I and 63 in Passauna) were observed in

control habitats. This difference may be due to the size of the reservoirs, Itaipu is a 1,350 km^2 impoundment with about 150 km long, while the Piraquara I and Passuana reservoirs are at least 15 times smaller. Another reason may be simply differences in the life characteristics of the species present in these reservoirs. The most abundant species in control was the native Cichlidae, *G. brasiliensis*. This is a territorialist and resident species (Paraguassú et al. 2005), and in fact, some individuals of *G. brasiliensis* present in unstructured habitats did not leave the habitat, even in the presence of the diver. In contrast, non-native Cichlidae and Centrarchidae seemed to use these unstructured habitats only in transit, and even in low abundances, different size class individuals, especially adults, were observed, indicating that such habitat may not be favourable for juvenile individuals (<80 mm Lt), probably due to exposure to predators.

The use of artificial habitats by fish in freshwater can vary temporally, according to habitat age (Wilbur 1978), season (Daugherty et al. 2014) or even period of day (Moring and Nicholson 1994, Harris et al. 2017). Santos et al. (2011) verified that the first species to colonize artificial habitats in a Neotropical reservoir were rapidly maturing and short lived fish (e.g., Characidae), being later replaced by intermediate size species with parental care and fewer offspring (e.g., Loricaridae). However, such a pattern of species succession was not in the current study. Characidae species were only observed during summer and just three individuals of Loricariidae were recorded only in Piraquara I reservoir. However, nocturnal species, such as Siluriformes and Loricariidae, may be using the artificial habitats during non-sampled periods. Overall, throughout the whole study period, the dominant species associated with artificial habitats were the three most abundant species in these reservoirs (i.e., Cichlidae and Centrarchidae), usually with the abundance of individuals directly related to water temperature.

Artificial habitats can be important tools in the management and conservation of reservoirs, since such structures can potentially increase local abundance and diversity of fish

species. However, some important points related to the applicability and effectiveness of artificial habitats should be taken into account. Organic structures, such as trees and wood, have a short useful life in water (Rogers and Bergersen 1999), needing to be replaced after months or few years. In the present study after four months, all trees had already lost the leaves. On the other hand, ceramic pipes are stable structures that can last for many years, but they might be hazardous to boat passage in reservoirs where water level variations are common, besides being aesthetically inadequate when exposed out of the water (Bolding et al. 2004). Another important aspect is the deployment of artificial structures that will favour native rather than non-native species. In this sense, studies and simulations prior to deployment, as well as post-deployment monitoring, are essential to evaluate the species that will use these habitats.

In conclusion, the present study demonstrated the dominance of Cichlidae and Centrarchidae species, especially small individuals (< 80 mm Lt), associated with the artificial and natural habitats in Neotropical reservoirs. Interestingly, both native and non-native Cichlidae appear not to co-occur with Centrarchidae, indicating a habitat partitioning between these two families. However, the composition and abundance of species varied according to the kind of habitat. Considering artificial habitats, pipes appear to be more suitable for native Cichlidae, while trees seem to be preferred by Centrarchidae. In this sense, the results highlight the importance of considering, besides the kind of habitat, the origin of species associated with it, in order to prevent that invasive species benefiting from the deployment of artificial structures in freshwater systems.

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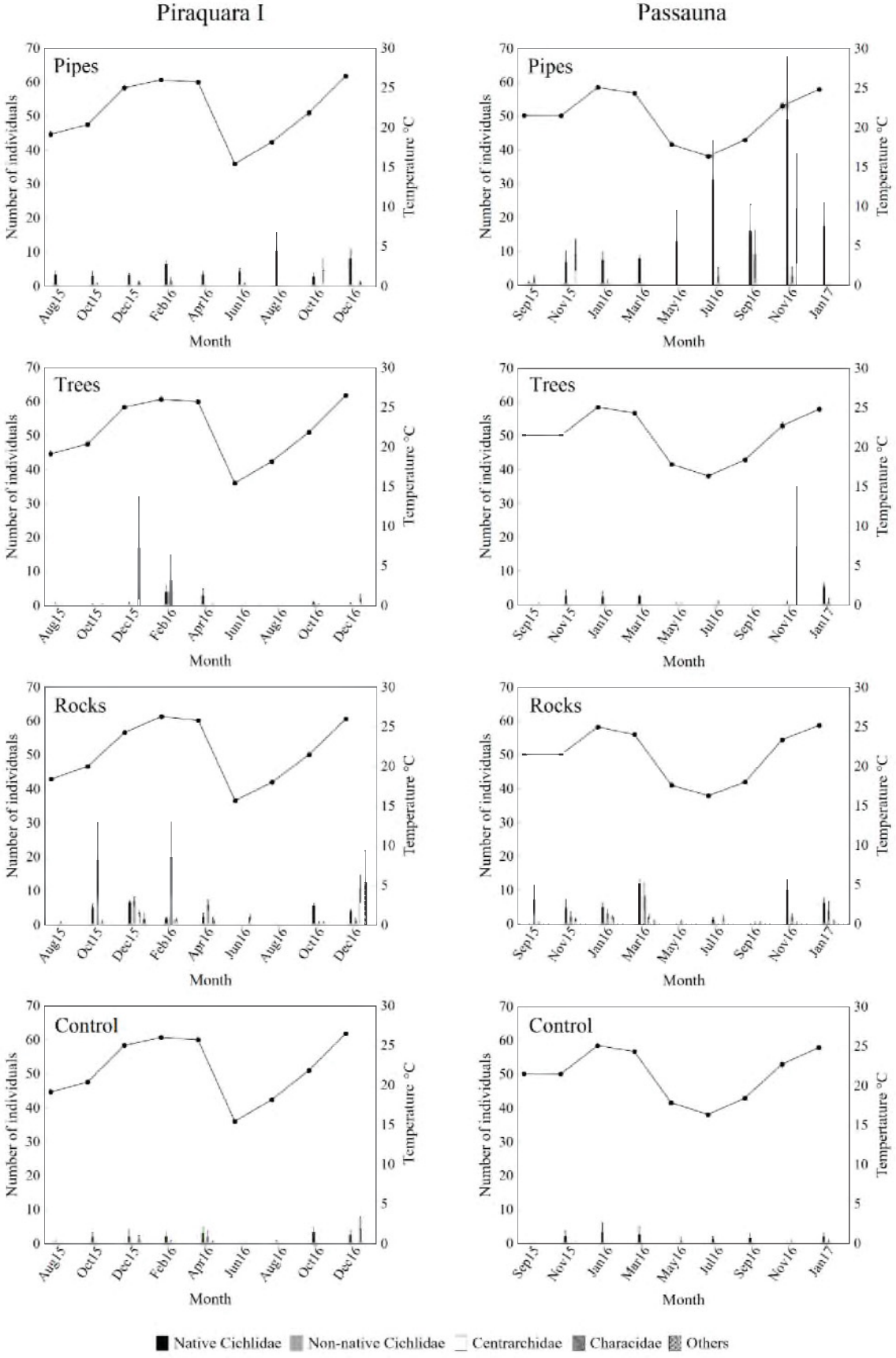
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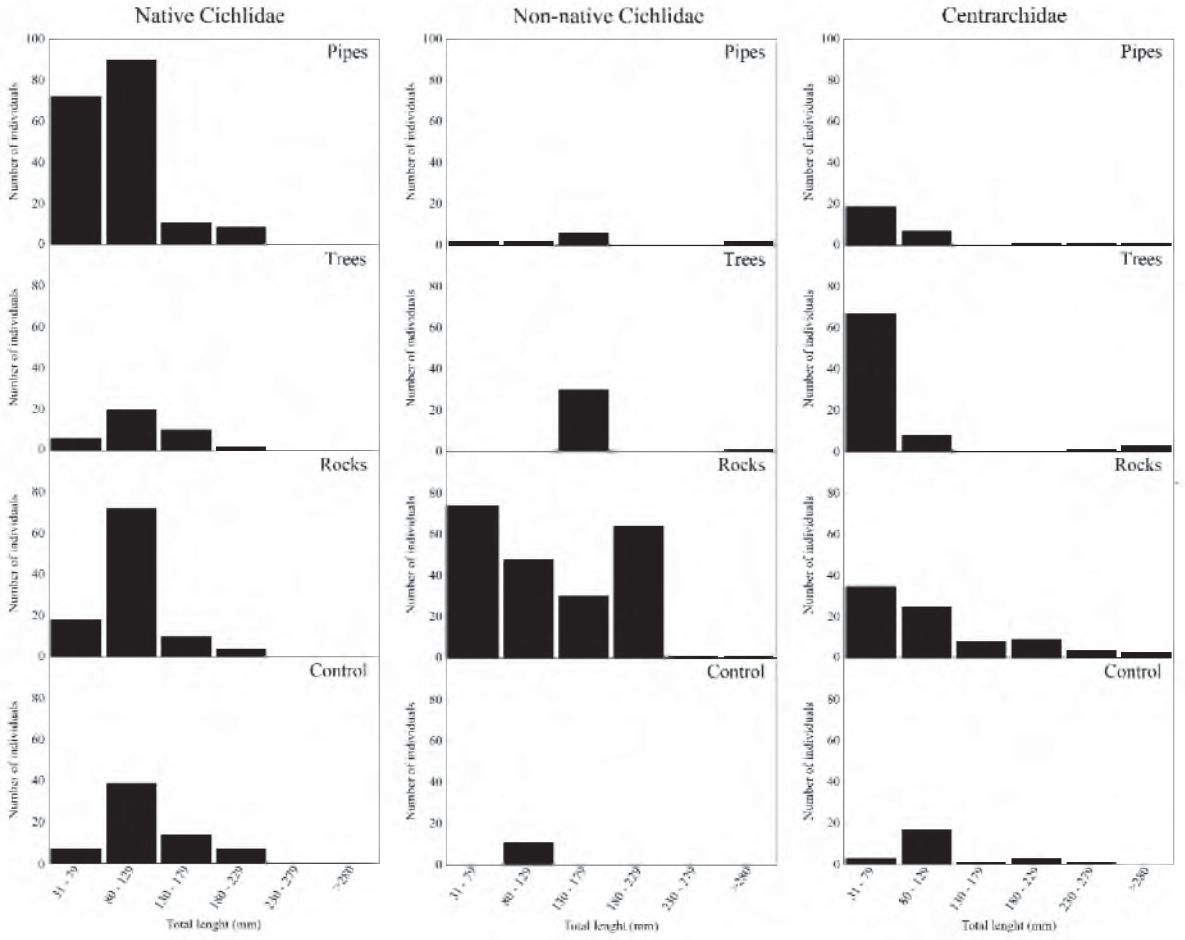
Supplementary Material I. Results of the pairwise test between habitats in each reservoir with significant differences in bold.

Groups	Piraquara I		Passauna	
	T	*p(perm)*	*T*	*p(perm)*
Pipes x Rocks	4.8083	**0.0001**	3.3021	**0.0002**
Pipes x Trees	2.6529	**0.001**	1.7852	**0.0219**
Pipes x Control	1.0278	0.3704	1.9412	**0.0154**
Rocks x Trees	2.1703	**0.0059**	3.2114	**0.0001**
Rocks x Control	3.0154	**0.0001**	2.6331	**0.0014**
Trees x Control	1.2655	0.179	0.8151	0.5588

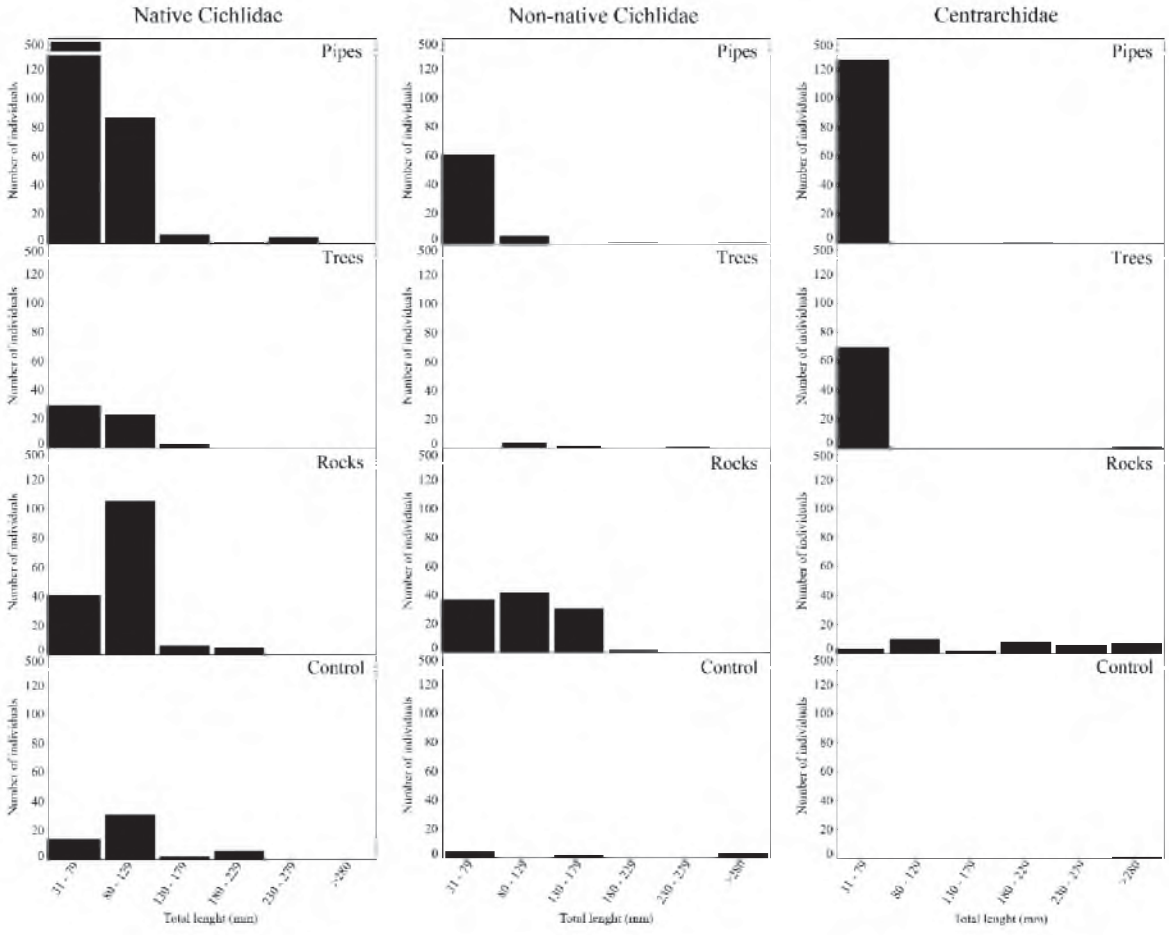
Supplementary Material II. Mean number of individuals of each taxonomic group per month for each kind of habitat. The black dots correspond to the mean water temperature per month for each habitat.



Supplementary Material III. Number of individuals by size classes of the main taxonomic groups associated with each kind of habitat in the Piraquara I reservoir.



Supplementary Material IV. Number of individuals by size classes of the main taxonomic groups associated with each kind of habitat in the Passauna reservoir.



CHAPTER 3

COMPARISON OF VISUAL CENSUS AND UNDERWATER VIDEO FOR FISH SAMPLING IN NEOTROPICAL RESERVOIRS

Comparison of visual census and underwater video for fish sampling in Neotropical reservoirs

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Abstract

Freshwater fish are involved in many ecosystem services provided by reservoirs, and because of this, they have been the focus of several studies on these ecosystems. Different techniques have been employed to fish surveys in reservoirs, however, for specific issues like the use of habitats, visual census (VC), through snorkeling or scuba diving, has been the mostly used method. Despite the increasing applicability of underwater video (UWV) systems for fish sampling in different aquatic ecosystems, such technology has not been tested in reservoirs. This study aimed to compare the fish species number, composition, and size class recorded through VC and UWV in two Neotropical reservoirs. The total number of species recorded by both sampling methods was similar, even though UWV recorded higher mean number of species per sample than VC, in both reservoirs. In general, VC was more efficient in recording cryptic and resident species, while UWV, was effective for detecting highly mobile species. Considering size classes, VC was more efficient than UWV in detecting smaller individuals (i.e., 31-79 mm total length). The potential and limitations of the analyzed sampling methods are discussed. Although observational sampling methods depend on water transparency, UWV can be considered a complementary and promising technique for fish surveys in shallow areas of reservoirs.

Keywords: artificial habitats; fish assemblage; freshwater ecosystems; non-destructive methods; observational survey; subaquatic cameras.

Introduction

Water reservoirs are human modified environments, through the impoundment of rivers (Agostinho et al., 2007). Information about these freshwater ecosystems is important to support management and conservation measures, in order to maintain the ecosystem services provided by such environments (e.g., water supply, irrigation, power generation, fishery, aquaculture, and recreational activities) (Postel & Carpenter, 1997; Dudgeon et al., 2006). For many of these services, fish are particularly important (Holmlund & Hammer, 1999) and have been the focus of several studies (e.g., Fernando & Holcik, 1991; Agostinho et al., 2008; 2016; Pelicice et al., 2016). A variety of capture techniques have been employed to evaluate fish assemblages in reservoirs, such as gill, seine and fyke nets (Gido et al., 2000; Booth & Potts, 2006; Prchalová et al., 2010), traps (Pelicice et al., 2005), angling (Ribeiro et al., 2015), and electro fishing (Dumont & Dennis, 1997). However, for more specific issues, such as, habitat use, intra and inter-specific interactions and fish behavior, the most common sampling method adopted is direct observation through visual census (VC), by snorkeling (Wills et al., 2004; Santos et al., 2008; 2011a) or scuba diving (Schulze et al., 2004; Santos et al., 2011b).

VC is a rapid and non-destructive sampling method, which provides a broad set of variables and adequate levels of replication (Lowry et al., 2012). However, despite being widely adopted, VC has some limitations (water transparence, bottom time, maximum depth, cold-water exposure) and bias (influence of diver on behavior of some species and the diver's experience and training level) (Williams et al., 2006; Assis et al., 2013). In the last two decades, underwater video (UWV) techniques have been increasingly applied to observe fish and habitats as an alternative method, especially in marine ecosystems (Harasti et al., 2015). Novel technologies related to cameras, battery life, and data storage have expanded the use of such technology for biological data collection (Whitmarsh et al., 2016). UWV systems have been used to sample different environments and depths (Mallet & Pelletier, 2014), through a wide

variety of configurations such as single-videos (Ebner & Morgan, 2013; Ellender et al., 2012), stereo-videos (Watson et al., 2010; Boutros et al., 2015), baited videos (Wills & Babcock, 2000; Harvey et al., 2007; Heagney et al., 2007), towed videos (Mallet & Pelletier, 2014), diver-operated videos (Cruz et al., 2008), and remote operated vehicles (Bryan et al., 2012; Ajemian et al., 2015).

Some studies have compared the abundance and/or richness of fish recorded through VC and UWV in coral reefs (Longo & Floeter, 2012; Mallet et al., 2014), rocky reefs (Colton & Swearer, 2010; Assis et al., 2013), estuaries (Lowry et al., 2012), freshwater streams (Ebner et al., 2015) and rivers (Schmid et al., 2017). The main conclusion in comparing studies is that distinct methods might obtain different results depending on the kind of ecosystem that is under observation. Despite the increasing applicability of UWV for fish surveys, no studies have evaluated its effectiveness in reservoirs. In this sense, the present study aims to evaluate the value of using VC versus UWV for observing the fish assemblage in Neotropical reservoirs. The main interest was two folded: (*i*) comparing the number and composition of species detected by each technique adopted and (*ii*) comparing which technique performs better in detecting specific size classes.

Material and methods

Study Area

The study was carried out in two water supply reservoirs of Curitiba City, Paraná State, Southern Brazil: Piraquara I (25°30'17" S; 49°01'31" W) and Passauna (25°31'40" S; 49°23'16" W) reservoirs (for more details see *Study Area* in chapter 2).

Field Experimental Design and Samplings

In order to compare the effectiveness of VC and UWV in detecting fish, four kinds of habitats (two artificial: pipes and trees; and two semi-natural: rocks and control) were sampled in each reservoir (for more details see *Field Experimental Design* in chapter 2).

All fish until 1 m away from the structures or delimited areas were considered associated with the habitats. The VC was performed by a scuba diver following Santos et al. (2008), each survey lasted five minutes. All fish associated with the habitats were identified, counted and recorded on an underwater writing tablet. The UWV was performed through a single-video system, with GoPro® cameras (Hero 4) placed on a base previously installed in front of each sampling site ("L" shaped metal rod, 1.2 m long and 0.5 m high). The placement and removal of the cameras were performed through free diving and each site was filmed during 30 minutes. Posteriorly, the videos were analyzed using the free software VLC (www.videolan.org). Fish were identified to the lowest possible taxonomic level and, when necessary, taxonomic specialists were consulted. The maximum number (MaxN) of individuals of a species in a single frame was used to estimate the relative abundance of the species (Cappo et al., 2004; Whitmarsh et al., 2016) in each video survey.

In order to minimize biases, all dive surveys and videos analyses were conducted by the same experienced researcher, and in both sampling methods (VC and UWV), the total length (Lt) of fish were estimated comparing the fish size with adjacent objects of known size and distances (e.g., pipes, trees, rocks, metal rod supporting the camera, delimitated areas). Due to difficult identification and counting of small fish, only individuals larger than 30 mm in total length were considered. The only fish that were not identified to the specific level were Tilapias, due to difficulties in differentiate the two species that occur in our study. Therefore, the term *Tilapia* used thereinafter refers to individuals of the species *Coptodon rendalli* and *Oreochromis niloticus*, which are both non-native invaders and co-occur in both reservoirs.

The samplings began in August and September 2015 at the Piraquara I and Passauna reservoirs, respectively, about 30 days after the habitats establishment. Each reservoir was sampled bimonthly (one reservoir per month, alternately) until January 2017, providing nine samplings events in a 17-month period, in each. The VC and UWC were performed on subsequent different days in order to avoid interference between the methods. The order of each habitats survey was randomized in each sampling event. Both VC an UWV were performed between 10.00 - 15.00 hours to optimize the visibility. Temperature, transparency (through a Secchi disk) and pH of the water were measured at all sampling sites (Table 1).

Data analyses

For general exploratory analyses, each reservoir was analyzed independently. The frequency of occurrence (%FO) was given as the percentage of all surveys occasions in which a particular species was recorded by each sampling method (VC and UWV). A factorial analysis of variance (ANOVA) was applied to the species abundance data to test for possible differences in the mean number of species recorded in each kind of habitat by the two sampling methods (VC and UWV). When interactions between sampling method and habitat were found, a Tukey HSD *post hoc* test was applied to the data to check for differences among levels.

Fish were also evaluated for their Lt, to find possible detection patterns of UVC and UWV of individuals according to their size classes. Based on the Lt, all individuals were arbitrarily grouped into six size classes (31-79; 80-129; 130-179; 180-229; 230-279; and >280 mm). Then, a factorial ANOVA was applied to test for possible differences in the mean number of individuals recorded into each size class throughout the study period. The analyses were performed using the software Statistica 12 (StatSoft Inc., 2014).

TABLE 1 Mean values and range (in parentheses) of environmental variables taken bimonthly, August 2015–January 2017, at sampling sites in Piraquara I and Passauna reservoirs, Southern Brazil

Environmental variables	Piraquara I		Passauna	
	VC	UWV	VC	UWV
Transparency (m)	2.83 (3.4-1.5)	2.91 (3.0-1.8)	2.37 (3.4-1.5)	2.40 (3.0-1.8)
Temperature (°C)	22.13 (26.6-15.1)	21.98 (26.6-15.4)	21.43 (25.3-16.1)	21.38 (25.4-15.9)

pH	7.14 (7.5-6.5)	7.13 (7.7-6.6)	8.05 (8.7-6.2)	8.04 (8.7-7.2)

Results

A total of 144 samples were obtained from each sampling method adopted in each reservoir. Fish were detected on 103 (72%) occasions through VC and 140 (97%) through UWV in the Piraquara I reservoir, and on 101 (70%) occasions through VC and 135 (94%) through UWV in the Passauna reservoir. Overall, in the Piraquara I reservoir 940 individuals of seven species were recorded through VC and 995 individuals of eight species through UWV. Likewise, in the Passauna reservoir, 1,303 and 1,033 individuals, of seven species, were recorded through VC and UWV, respectively (Table 2). Four species (*Geophagus brasiliensis*, Tilapia, *Micropterus salmoides*, and *Astyanax bifasciatus*) were recorded by both sampling methods in both reservoirs. *Cichlasoma facetum, Hypostomus derbyi,* and *Hypostomus myersi* were recorded only through VC, while *Australoheros kaaygua*, and *Corydoras ehrhardti* were recorded only through UWV (Table 2). *Geophagus brasiliensis*, Tilapia, and *M. salmoides* were the only species with frequency of occurrence higher than 20% in both reservoirs, being more frequently recorded through UWV than VC, especially Tilapias and *M. salmoides* (Table 2).

TABLE 2 Total abundance of individuals (N) and frequency of occurrence (%*FO*) of fish species recorded through visual census (VC) and underwater video (UWV) in each reservoir

	Piraquara I				Passauna			
	VC		UWV		VC		UWV	
	N	*%FO*	N	*%FO*	N	*%FO*	N	*%FO*
Cichlidae								
Geophagus brasiliensis	390	61.1	274	81.3	866	56.9	155	56.9
Australoheros kaaygua			1	0.7				
Cichlasoma facetum	1	0.7						
Tilapia*	272	22.9	395	62.5	196	27.8	465	74.3
Centrarchidae								
*Micropterus salmoides**	217	32.6	204	52.1	236	21.5	371	45.8
Characidae								
Astyanax bifasciatus	53	2.1	89	13.2	2	0.7	30	4.9
Oligosarcus longirostris	4	0.7	29	8.3			10	4.9
Callichthyidae								
Corydoras ehrhardti			2	1.4				
Erythrinidae								
Hoplias malabaricus	3	2.1	1	0.7			1	0.7
Loricariidae								
Hypostomus derbyi					1	0.7		

Hypostomus myersi				1	0.7	
Heptapteridae						
Rhamdia quelen				1	0.7	1	0.7
Total	940	995	1303		1033	

* *Non-native species*

The number of recorded species varied according to the sampling method and habitat in both reservoirs (Figure 1). The ANOVA detected interaction between the sampling method and habitat in the Piraquara I reservoir ($F_{3,280}$ =8.843, p<0.0001). The Tukey HSD test indicated significant differences among all habitats when considering the number of species recorded by each method, except for pipes (p=0.6259). In the Passauna reservoir the number of species varied according to the sampling method ($F_{1,280}$ =64.282, p<0.0001) and habitat ($F_{3,280}$ =38.027, p<0.0001) considered.

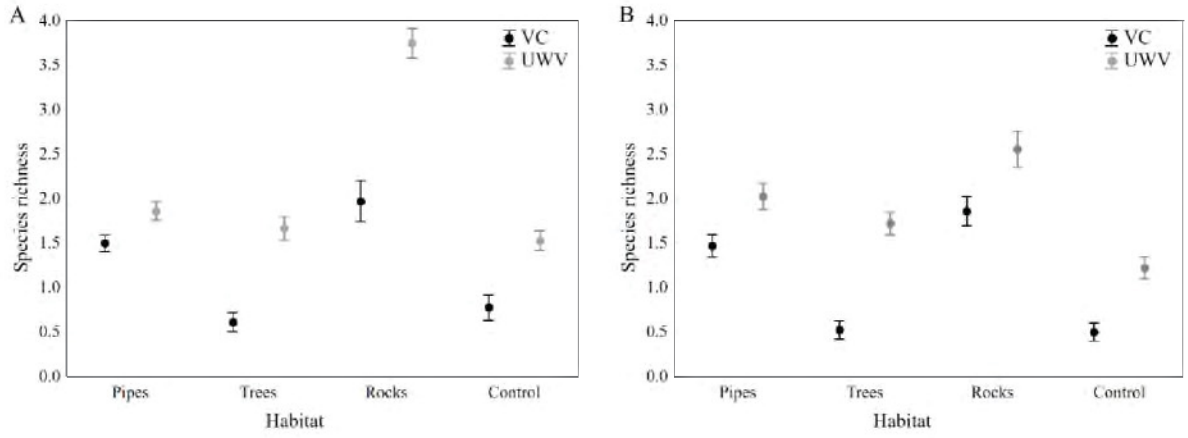


FIGURE 1 Mean number of species recorded through visual census (VC) and underwater video (UWV), in each kind of habitat in Piraquara I (A) and Passauna (B) reservoirs

The number of individuals varied according to the size class and the sampling method in both reservoirs (Figure 2). The ANOVA detected significant interaction between the size class and the sampling method for both reservoirs, Piraquara I ($F_{5,96}$ =5.071, p=0.0003) and Passauna ($F_{5,96}$ =2.953, p=0.0159). The Tukey HSD test indicated that, in both reservoirs, only in the smallest size class (31-79 mm Lt) there was significant differences among the sampling methods, Piraquara I (p=0.0085) and Passauna (p=0.0389).

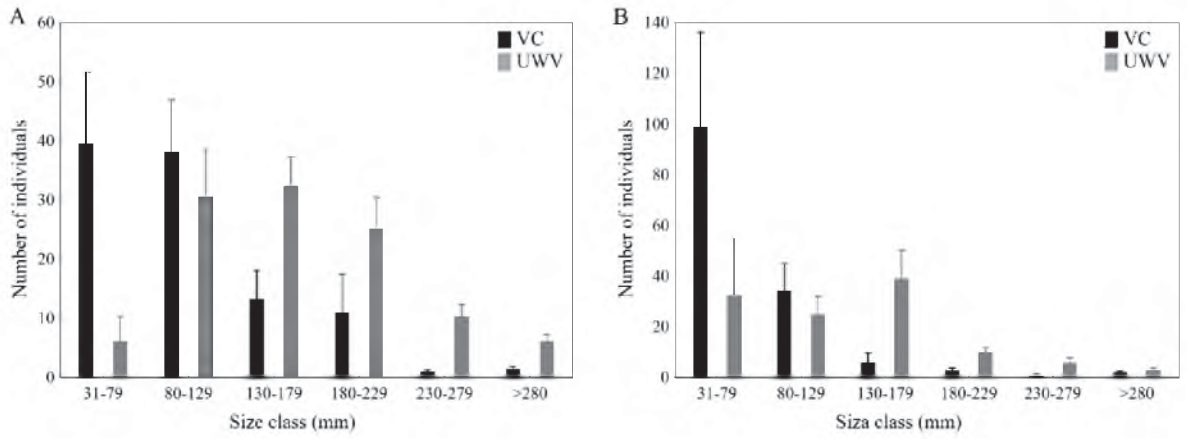


FIGURE 2 Mean number of individuals recorded through visual census (VC) and underwater video (UWV), in each size class in Piraquara I (A) and Passauna (B) reservoirs

Discussion

The present study found that UWV is a complementary and alternative method for fish sampling in different kind of habitats in reservoirs. Although the total number of individuals and species recorded through VC and UWV was similar in both reservoirs, differences were observed regarding the mean number of species and size classes of individuals recorded by the different sampling methods. Similarly, Ebner & Morgan (2013) demonstrated that UWV techniques associated with other conventional fish sampling methods increase the capacity of surveying freshwater fish communities.

The UWV detected fish more frequently and higher mean number of species than VC in both reservoirs. This may be due to the effect of the presence of the diver (Dickens et al., 2011), or simply because UWV lasted more than VC surveys (30 and 5 minutes, respectively), increasing the chance of detecting more species or individuals. The ability to perform longer surveys seems to be an advantage of UWV in relation to VC for fish sampling. Nevertheless, it is important to consider the time taken to perform the video analyses (Mallet et al., 2014; Ebner et al., 2015). Ellender et al. (2012) verified that less than 25 minutes was enough to estimate with accuracy the MaxN of two fish species in headwaters streams. Similarly, Ebner & Morgan (2013) found that most of species recorded through UWV in waterholes of a freshwater river

were detected in less than 10 minutes of shooting. Although it was not the aim of this study to determine the ideal soak time of the cameras, 30 minutes seems to be a suitable time for fish sampling in reservoirs.

Geophagus brasiliensis, was frequently recorded by both sampling methods, however, VC recorded a greater abundance of individuals of this species than UWV. On the other hand, UWV recorded greater occurrence and abundance of *M. salmoides*, Tilapia, *Astyanax bifasciatus*, and *Oligosarcus longirostris* than VC. These differences in the abundance estimation between the sampling methods are probably related to the ecological niche and behaviour of the species (Lowry et al., 2012). For example, many individuals of *G. brasilensis*, a territorialist species (Kadry & Barreto, 2010), occupied the interstices of some habitats, especially pipes, and were not detected by the camera. Conversely, some mobile species such as *M. salmoides*, Tilapia, *A. bifasciatus*, and *O. longirostris*, seem to present aversion to the diver' presence, being less detected by VC than UWV. *Hypostomus derbyi* and *H. myersi*, two bottom structures associated species, were only recorded through VC. Lowry et al. (2012) indicated that the VC is more appropriate for sampling cryptic species associated with structures. However, interestingly, *C. ehrhardti*, another cryptic bottom species, was detected only through UWV. *Hoplias malabaricus* and *R. quelen*, two common species in Neotropical freshwater environments (Lowe-Mcconnell, 1991), were recorded sporadically by both sampling methods.

The VC was more efficient than UWV in recording smaller individuals (i.e., 31-79 mm in Lt), and although not statistically significant, the opposite seems to be a trend for larger individuals (i.e., > 130 mm in Lt) (Figure 2). Similar pattern regarding size of fish recorded through VC and UWV was identified in estuaries (Lowry et al., 2012) and coral reefs (Mallet et al., 2014). The efficiency of VC in sampling small fish is probably related with the ability of the diver in exploring microhabitats that may be imperceptible through the cameras (Lowry et

al., 2012). On the other hand, larger fish seem to flee in the presence of the diver. Although stereo-video systems seem to provide a more accurate estimate of fish length than VC (Harvey et al., 2001) and single-video systems (Harvey et al., 2002), some precautions were taken in order to minimize biases associated with the sampling methods used in this study (see Materials and Methods). Moreover, stereo-video systems demand more financial resources (e.g., more cameras, specific software to video analyses, and specialized gear for retrieval) and time (to set-up before and during surveys) (Whitmarsh et al., 2016), which sometimes make their use infeasible in small-scale research projects.

The applicability of both observational sampling methods used in this study are dependent on water transparency (Mallet & Pelletier, 2014). Shallow areas (< 3 m depth) near to the dam are most appropriate for fish sampling through the VC and UWV in reservoirs, for usually presenting higher water transparency (Straskraba et al., 1993). Although it is recognized, that all fish sampling techniques present limitations and biases (Assis et al., 2013), UWV was able to survey different habitats demanding reduced field effort. In addition, UWV proved to be efficient in detecting and estimating the abundance of invasive fish species (e.g., Tilapia and *M. salmoides*), which are usually underestimated by conventional sampling methods in reservoirs (Ribeiro et al., 2015). In this sense, other UWV techniques can be potentially explored in reservoirs, such as, night surveys, baited, and towed videos.

In conclusion, UWV proved to be a potential tool for rapid fish survey in shallow areas of reservoirs. Although UWV present restrictions regarding water transparency and the detection of small cryptic species, it was able to estimate the abundance of mobile fish species more effectively than VC. Considering that no single sampling technique can outperform a combination of methods (Ebner & Morgan, 2013), UWV can be considered a complementary technique for fish surveys in reservoirs.

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