BÁRBARA ANGÉLIO QUIRINO

# Aquatic macrophytes as drivers of multiple aspects of fish community:
trophic, functional and taxonomic fish responses in tropical and
temperate freshwater ecosystems

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutora em Ecologia e Limnologia e aprovada pela Comissão Julgadora composta pelos membros:

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Local de defesa: Anfiteatro Prof. "Keshiyu Nakatani", Nupélia, Bloco G-90, *campus* da Universidade Estadual de Maringá.

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*"Sonho que se sonha só*
*É só um sonho que se sonha só*
*Mas sonho que se sonha junto é realidade"*

**(Música de Raul Seixas)**

## Macrófitas aquáticas como preditoras de múltiplos aspectos da comunidade de peixes: respostas tróficas, funcionais e taxonômicas de peixes de ecossitemas de água doce tropicais e temperados

### RESUMO

As macrófitas são amplamente reconhecidas por aumentarem a complexidade do *habitat* e exercerem papel relevante na estruturação das comunidades em ecossistemas aquáticos. Investigou-se como a densidade, cobertura e diversidade de macrófitas podem influenciar múltiplos aspectos da comunidade de peixes, incluindo diversidade alfa e beta funcional, eficiência de forrageamento, amplitude e sobreposição de nicho trófico, abundância e biomassa de total de peixes, bem como de determinadas espécies e tamanhos de peixes. Utilizou-se 30 bancos de macrófitas amostrados na zona litorânea de um rio tropical, bem como, um conjunto de dados de 88 lagos rasos temperados. Neste ultimo caso, foram consideradas duas escalas espaciais (lago e pontos dentro do lago), visto que geralmente há uma relação negativa entre peixes e a cobertura de macrófitas, e essa relação pode ser mais evidente na escala de ponto dentro do lago, enquanto o efeito é atenuado na escala de lago. Os resultados evidenciaram que as macrófitas podem selecionar características funcionais das comunidades de peixes e influenciar sua diversidade funcional alfa e beta. Os maiores valores de diversidade alfa funcional ocorreram em densidades intermediárias e elevada diversidade de macrófitas. A variação na densidade de macrófitas foi fortemente responsável pelas variações na diversidade beta funcional. Os peixes apresentaram respostas tróficas gerais à variação dos atributos das macrófitas, mas estas respostas também dependeram dos hábitos alimentares dos peixes, com a amplitude de nicho dos herbívoros respondendo de forma oposta à amplitude de onívoros. O aumento na densidade de macrófitas levou a um aumento no consumo de vegetais superiores pelos peixes e na eficiência de forrageamento, e reduziu a amplitude do nicho trófico. A sobreposição de nicho de peixes foi maior nos níveis intermediários de densidade de macrófitas. As respostas dos peixes dependeram da identidade da espécie de peixe, produtividade da lagoa e profundidade da água. Quanto mais profundos e eutróficos eram os lagos, mais os peixes tenderam a ocupar pontos cobertos por macrófitas dentro de um lago. Mediante os resultados, ressaltamos como as plantas aquáticas podem afetar a comunidade de peixes por influenciarem na natação, disponibilidade de alimento e interações entre as espécies, e que a relação entre peixe e macrófita pode variar de acordo com a escala do estudo e as características dos peixes considerados. A aplicação dos resultados aqui obtidos tem grande potencial de auxílio em projetos de manejo e conservação da biodiversidade, e na escolha das melhores configurações de bancos de macrófitas para a manutenção da ictiofauna.

**Palavras-chave:** Diversidade beta. Traços funcionais. Nicho trófico. Guilda Trófica. Estruturação da assembleia de peixes.

**Aquatic macrophytes as drivers of multiple aspects of fish community:** trophic, functional and taxonomic fish responses in tropical and temperate freshwater ecosystems

*ABSTRACT*

Macrophytes are widely recognized for increasing habitat complexity and driving a relevant role in structuring communities in aquatic ecosystems. We investigated how the density, coverage and diversity of macrophytes can influence multiple aspects of fish community, including functional alpha and beta diversity, foraging efficiency, trophic niche breadth and overlap, total abundance and biomass of fish, as well as of certain species and sizes of fish. We used 30 macrophyte stands sampled in the littoral zone of a tropical river, as well as a dataset of 88 shallow temperate lakes. In the latter case, two spatial scales were considered (lake and points within a lake), since there is usually a negative relationship between fish and macrophyte coverage, and this relationship may be more evident at the point scale, while the effect is attenuated at the lake scale. The results showed that macrophytes can select functional characteristics of fish communities and influence their alpha and beta functional diversity. The highest values of functional alpha diversity occurred in intermediate macrophyte density and high macrophyte diversity. The variation in macrophyte density was strongly responsible for variations in functional beta diversity. Fish showed general trophic responses to the variation in macrophyte attributes, but these responses also depended on fish feeding habits, with herbivores niche breadth responding in the opposite way to omnivores. The increase in macrophyte density led to an increase in fish consumption of higher plants and foraging efficiency, and reduced trophic niche breadth. Fish niche overlap was higher at intermediate levels of macrophyte density. Fish responses depended on fish species identity, lake productivity and water depth. The deeper and more eutrophic the lakes, the more fish tended to occupy macrophyte-covered points within a lake. The results emphasize how aquatic plants can affect the fish community by influencing swimming, food availability and interactions between species, and that the relationship between fish and macrophytes can vary according to the scale of the study and the fish characteristics. The application of the results obtained here has great potential to help in projects of management and conservation of biodiversity, and in the choice of the best configurations of macrophyte stands for the maintenance of ichthyofauna.

*Keywords*: Beta diversity. Functional traits. Trophic niche. Trophic guild. Fish assemblage structuring.

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## 1  GENERAL INTRODUCTION

Aquatic macrophytes are widely recognized by providing multiple ecosystem services, especially increasing habitat complexity in freshwaters  (Jeppesen et al. 1998; Thomaz 2021). These plants affect the spatial distribution of several organisms, such as periphyton, invertebrates and fish, because they provide spawning substrate, nursery area, refuge against predators, and food (Thomaz and Cunha 2010). The coverage, density and diversity of aquatic vegetation are important factors in structuring fish assemblages (Tonn and Magnuson 1982), and fish can actively select aquatic macrophytes as habitat based on their stem density (Gotceitas 1990). Generally, studies have shown that fish prefer areas with intermediate levels of macrophyte density (Dibble 1996; Agostinho et al. 2007; Cunha et al. 2019). This habitat selection by fish may be related to the fact that low-density macrophyte stands may not provide efficient as shelter and do not support a good amount of food resources (Choi et al. 2014), while excessive macrophyte density led to chemical (e.g., hypoxia) and physical restrictions for fish (Miranda and Hodges 2000). Thus, along a gradient of macrophyte density, fish tend to avoid staying in macrophyte stands with both extreme conditions (Dibble 1996).

Generally, there is a greater availability of macroinvertebrates (which are important food for fish) living in association with macrophytes with finely divided leaves (Taniguchi et al. 2003; Mormul et al. 2011), or in macrophyte stands with various growth forms (Brown et al. 1988), and with greater biomass and density (Warfe and Barmuta 2006; Thomaz et al. 2008). Macrophyte stands with a greater plant diversity also tend to support greater density and richness of invertebrates (Choi et al. 2016). In this sense, food consumption by fish may increase and their trophic niche breadth may become broaden with macrophyte coverage, abundance or diversity (Middaugh et al. 2013; Vejříková et al. 2017; Nohner et al. 2018; Yofukuji et al. 2021; Aleixo et al. 2022). However, because the difficulties to move and visualize prey in stands with extreme macrophyte density, the predator-prey encounter is reduced and fish tend to present a low foraging efficiency in this condition (Savino and Stein 1982; Sammons and Maceina 2006; Liversage et al. 2017). Thus, macrophytes may shape trophic niche variation of fish (Vejříková et al. 2017) and this effect may change according to species ability to explore submerged structures of aquatic plants. Some fish can show a better foraging efficiency because they are more adapted to swim through the macrophyte structures than others (Priyadarshana et al. 2001). In addition, greater diet segregation and then reduced competition for resources can be found in abundant macrophyte vegetation compared to poor macrophyte coverage (Vejříková et al. 2017; Eloranta et al. 2017).

Besides changing foraging efficiency of fish, dense macropyte stands can influence other fish characteristcs, for example, limiting their size and body shape (Cunha et al. 2019), which means that macrophyte can act as an environmental filter (Zobel 1997) for fish community, selecting fish species and functional traits. In addition, there is a strong association between fish beta diversity and the structural complexity provided by macrophytes, with a replacement of species in a sparse macrophyte stand to completely different species in a stand of dense vegetation (Cunha et al. 2019). As the gradient of macrophyte density ranges from stands with relatively few submerged structures to stands with high structural complexity and numerous small interstices, it is expected a reduction in fish size along the gradient of vegetation density (Cunha et al., 2019). This response can be explained because large species generally require larger open spaces (Yeager and Hovel 2017). In this context, depending on the stem density, some macrophyte stands can contribute more or less with fish beta diversity, including functional beta diversity, since they can present unique or more common fish species, and functional traits.

Considering that macrophytes can represent different degrees of complexity at different scales, from patches of plants to single natural macrophyte leaves (Thomaz and Cunha 2010; Kovalenko et al. 2012), fish-macrophyte relationship may also vary with the spatial scale. Since fish can actively select the macrophyte patches according to their offer of refuge and feeding ground (Gotceitas and Colgan 1989), they are supposedly more closely related to near macrophyte patch where they were sampled (e.g., point in a lake scale), but this response may not be the same at the whole water body scale (e.g., lake scale). Fish catches can also be related to other lake characteristics, such as area, depth and productivity (Mehner et al. 2005; Brucet et al. 2013). Larger and deeper lakes usually present greater fish richness and abundance, because the greater stability and presence of more microhabitats (Olin et al. 2002; Drakou et al. 2009; Emmrich et al. 2011; Brucet et al. 2013). Regarding productivity, the eutrophication generally leads to higher total fish biomass and density (Jeppesen et al. 2000; Brucet et al. 2013; Yu et al. 2021).

This thesis consists of three papers assessing the fish-macrophyte relationship, but evaluating distinct responses of fish community. Two papers were conducted in 30 macrophyte stands sampled along a river in Brazil and one paper was conducted in 88 lakes distributed across Denmark. In the first paper we verified whether the density and diversity of aquatic macrophyte acts as an environmental filter selecting functional traits in fish communities, thereby influencing their functional alpha and beta diversity. In the second one, we investigated if the variations in macrophyte density and diversity can influence the consumption of particular

food items by fish, affecting each trophic guild differently, as well as the trophic aspects of fish community in general. Finally, in the third paper, we used an extensive field dataset that encompasses the Danish territory extent to determine how are fish abundance and biomass, fish sizes and particular fish species related to submerged macrophyte coverage in lakes with different trophic states (chlorophyll *a*), lake area and lake depth, and how these relationships vary across two scales (lake scale and point scale).

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## 2 MACROPHYTE STAND COMPLEXITY EXPLAINS THE FUNCTIONAL α AND β DIVERSITY OF FISH IN A TROPICAL RIVER-FLOODPLAIN

**ABSTRACT**

Aquatic macrophytes increase habitat complexity and influence the structure of fish communities. We investigated relations between macrophyte stand complexity and functional alpha and beta diversity of fish. We sampled fish and plants in 30 macrophyte stands with differences in density and diversity in the Paraná River floodplain. The functional alpha diversity, measured as functional richness index (FRic), was calculated for each macrophyte stand. The functional beta diversity was examined using pairwise Jaccard dissimilarity as well as its turnover and nestedness components. We also partitioned functional beta diversity into contributions of single sites to overall beta diversity (LCBD) aiming to assess its relationship to macrophyte stand characteristics. We then used beta regressions and generalized dissimilarity modeling (GDM) to examine diversity patterns. As we expected, the greatest FRic occurred in stands with intermediate macrophyte density and high macrophyte diversity. The functional beta diversity responded mainly to the variation in macrophyte density, but the turnover component increased slowly at the beginning of the gradient (low density) and, after a certain point, it started to increase more rapidly. The stands that contributed the most to the functional beta diversity (higher LCBD values) were those with low and high FRic and, consequently, with lower and higher macrophyte density, as well as lowest macrophyte diversity. Our findings highlight the role of macrophytes as environmental filters that select the traits in fish communities, and the variation in fish traits is probably a result of factors such as food availability, shelter from predators and physical space for locomotion.

*Keywords:* functional richness, traits, habitat complexity, metacommunity, ecological uniqueness

# A COMPLEXIDADE DO BANCO DE MACRÓFITAS EXPLICA A DIVERSIDADE α E β FUNCIONAL DE PEIXES EM UM RIO DE PLANÍCIE TROPICAL

## RESUMO

As macrófitas aquáticas aumentam a complexidade do habitat e influenciam a estrutura das comunidades de peixes. Foram investigadas as relações entre a complexidade do banco de macrófitas e a diversidade alfa e beta funcional de peixes. Foram amostrados peixes e plantas em 30 bancos de macrófitas com diferenças de densidade e diversidade na planície de inundação do rio Paraná. A diversidade alfa funcional, medida com o índice de riqueza funcional (FRic), foi calculada para cada banco de macrófitas. A diversidade beta funcional foi examinada usando a dissimilaridade de Jaccard, bem como seus componentes de turnover e aninhamento. A diversidade beta funcional também foi particionada em contribuições de locais únicos para a diversidade beta geral (LCBD) com o objetivo de avaliar sua relação com as características do banco de macrófitas. Em seguida, foram utilizadas regressões beta e modelos de dissimilaridade generalizados (GDM) para examinar os padrões de diversidade. Como esperado, o maior FRic ocorreu em bancos com densidade de macrófitas intermediária e diversidade de macrófitas elevada. A diversidade beta funcional respondeu principalmente à variação da densidade de macrófitas, mas o componente turnover aumentou lentamente no início do gradiente (baixa densidade) e, após certo ponto, passou a aumentar mais rapidamente. Os bancos que mais contribuíram para a diversidade beta funcional (maiores valores de LCBD) foram aqueles com baixa e alta FRic e, consequentemente, com menor e maior densidade de macrófitas, assim como a menor diversidade de macrófitas. Os resultados destacam o papel das macrófitas como filtros ambientais que selecionam as características das comunidades de peixes, e a variação nas características dos peixes é provavelmente resultado de fatores como disponibilidade de alimento, abrigo contra predadores e espaço físico para locomoção.

**Palavras-chave:** riqueza funcional, traços, complexidade de habitat, metacomunidade, singularidade ecológica

**2.1 Introduction**

Quantifying how biodiversity responds to environmental conditions is a major goal of ecological research. Approaches based on functional traits, including morphological, physiological or ecological traits, have been highlighted as tools to detect such responses (Carvalho and Tejerina-Garro 2015; Lechêne et al. 2018). This is because species vary in their resource use, and their abundances and distributions rely on their functional characteristics, which directly affect the species-specific fitness (Violle et al. 2007).

Recently, assessing the functional structure of ecological communities has been made possible by the use of indices based on the position of species in the functional space (Mason et al. 2005; Villéger et al. 2008). Although an increase in functional diversity is expected with an increase in taxonomic diversity, measures that consider the functional differences of species may be more sensitive to environmental gradients and, therefore, more directly related to the functioning and maintenance of ecosystem processes than traditional measures of taxonomic diversity (Luck et al. 2009; Villéger et al. 2017). Some species share equivalent functional traits, resulting in a low niche differentiation between them and, thus, they are selected in a similar way by environmental filters (Villéger et al. 2013). Moreover, high values of functional diversity are expected to be found in high-diversity communities, which enhance ecosystem functioning through greater niche efficiency (Díaz and Cabido 2001).

In addition to the functional richness of the local community (i.e., functional alpha diversity), another component of diversity that responds to environmental variation is related to site-by-site dissimilarities in the functional traits of communities (i.e., functional beta diversity) (Villéger et al. 2011). Functional beta diversity depicts more about trait-environment associations across space or time, incorporating species-specific functional traits in their estimates (Swenson 2011; Villéger et al. 2013). Moreover, functional beta diversity can be decomposed into turnover (i.e., when local assemblages have low overlap in the functional space) and nestedness (i.e., when a local assemblage fills only a portion of the functional space of another assemblage) (Villéger et al. 2013). Another interesting aspect of functional beta diversity that may be associated with environmental gradients, but that is still little explored in the functional context (but see Tolonen et al. 2018), is the Local Contribution to Beta Diversity (LCBD; Legendre and De Cáceres 2013). This attribute allows us to infer the degree of ecological uniqueness of each site (Legendre and De Cáceres 2013). Sites with high LCBD values may correspond to areas with unique ecological conditions, which may in turn have unique species combinations (Silva et al. 2018). Similarly, through employing the functional

beta diversity approach, high LCBD values could be associated with the presence of unique traits or a low number of traits in local communities.

Sets of functional traits in a given location are primarily determined by environmental filtering, which selects traits that facilitate the persistence of species under conditions imposed by the environment (Zobel 1997). These environmental filters determine the structure of the local community (Keddy 1992). Habitat complexity is seen as a key driving factor that structures communities and is usually positively related to taxonomic alpha diversity (MacArthur and MacArthur 1961) and functional alpha diversity (Göthe et al. 2017; Richardson et al. 2017). In addition, the variation in habitat complexity between different locations may be responsible for inducing spatial changes in ecological communities and, consequently, increase the functional beta diversity (Braghin et al. 2018; Peláez and Pavanelli 2019). Among the explanations underlying these findings is the greater availability of food and refugia in more complex habitats, which may also allow the decoupling of predator-prey interactions (Kovalenko et al. 2012).

There is no consensus regarding the definition of "habitat complexity", and the way in which it is measured differs widely among studies (e.g., McCoy and Bell 1991). Actually, this is a multi-faceted entity, encompassing different characteristics of habitat structure that exert different influences on organisms (Tokeshi and Arakaki 2012). That is the reason why it is important to cover as many facets of habitat complexity as possible. For instance, Tokeshi and Arakaki (2012) recognized at least five main components to habitat complexity, including the density and diversity of structural physical elements. Aquatic macrophytes are known to increase habitat complexity in freshwater ecosystems and provide spawning, refugia and foraging areas for fish (Agostinho et al. 2007). Previous studies have reported that many fish species that use macrophyte stands have sedentary behavior, inhabiting the interior of the stands where the complexity levels are higher. Meanwhile, other species are more active and generally occupy the stand's edge, where the complexity is less and allows greater swimming activity (Lopes et al. 2015). Increased vegetation density can lead to a decrease in the size of fish that colonize dense macrophyte stands (Cunha et al. 2019). At the same time, there is a strong association between fish beta diversity and the structural complexity gradient provided by macrophytes, especially for species turnover (Cunha et al. 2019). The density and diversity of aquatic vegetation (two facets of the habitat complexity) are important factors in structuring fish assemblages (Tonn and Magnuson 1982), since different architectures and growth forms of aquatic plants can affect the food resource availability. For instance, macrophyte stands with a greater plant diversity support greater density and richness of invertebrates (Choi et al. 2016),

leading in turn to greater fish species richness. Thus, aquatic plants influence the distributions of fish.

Despite the consensus on the positive effect of habitat complexity on biodiversity (Ortega et al. 2018), studies have shown that fish alpha diversity is higher at intermediate levels of macrophyte density (e.g., Tokeshi and Arakaki 2012; Cunha et al. 2019). Very densely-structured macrophytes can be harmful to foraging, making it difficult for fish to move and detect prey (Crowder and Cooper 1982; Warfe and Barmuta 2006). In addition, they may sometimes intensify aquatic hypoxia (Miranda and Hodges 2000; Cunha et al. 2019). Conversely, low-density macrophyte stands may not be as efficient as shelter and support fewer food resources (Choi et al. 2014). However, these studies have assessed fish taxonomic diversity, while the effects of macrophyte complexity on functional alpha and beta diversity remain inadequately understood.

In this study, we evaluated whether the structural complexity of aquatic macrophyte stands acts as an environmental filter selecting functional traits in fish communities. In this way, four predictions were formulated: *i)* the functional alpha diversity of fish (measured as functional richness - FRic) shows higher values in macrophyte stands with intermediate density, whereas it shows a positive relationship with the taxonomic diversity of aquatic macrophytes; *ii)* the total functional beta diversity and its components (turnover and nestedness) are influenced by the variation in the density and diversity of macrophytes; *iii)* LCBD decreases with increasing fish FRic. This last prediction considers the pattern already observed for the taxonomic richness-LCBD relationship (Legendre and De Cáceres 2013; Heino and Grönroos 2017; Silva et al. 2018); *iv)* as a consequence of prediction *iii*, we expect the macrophyte stands that contribute the most to the increase in the functional beta diversity (higher LCBD values) have low macrophyte diversity and extremes of macrophyte density (low and high).

## 2.2 Methods

### 2.2.1 Study area

This study was carried out in the Upper Paraná River floodplain, the last dam-free stretch of this river in Brazil, considered a key area for biodiversity conservation (Agostinho and Zalewski 1996). Samplings were performed in the Baía River (22º 43'23'' S, 53º17'25'' W – Fig. 1), a ca. 70 km long tributary of the Paraná River. The Baía River has low current velocity,

which is propitious for the colonization of large aquatic plant stands dominated by *Eichhornia azurea* (Sw.) Kunth, *Cyperus* spp., *Eichhornia crassipes* (Mart.) Solms., *Salvinia* spp. and *Polygonum* spp. (Carvalho et al. 2013). The sampled area supports macrophyte stands in almost its entire perimeter and a great diversity of plants (Thomaz et al. 2004) and fish (Lopes et al. 2015).
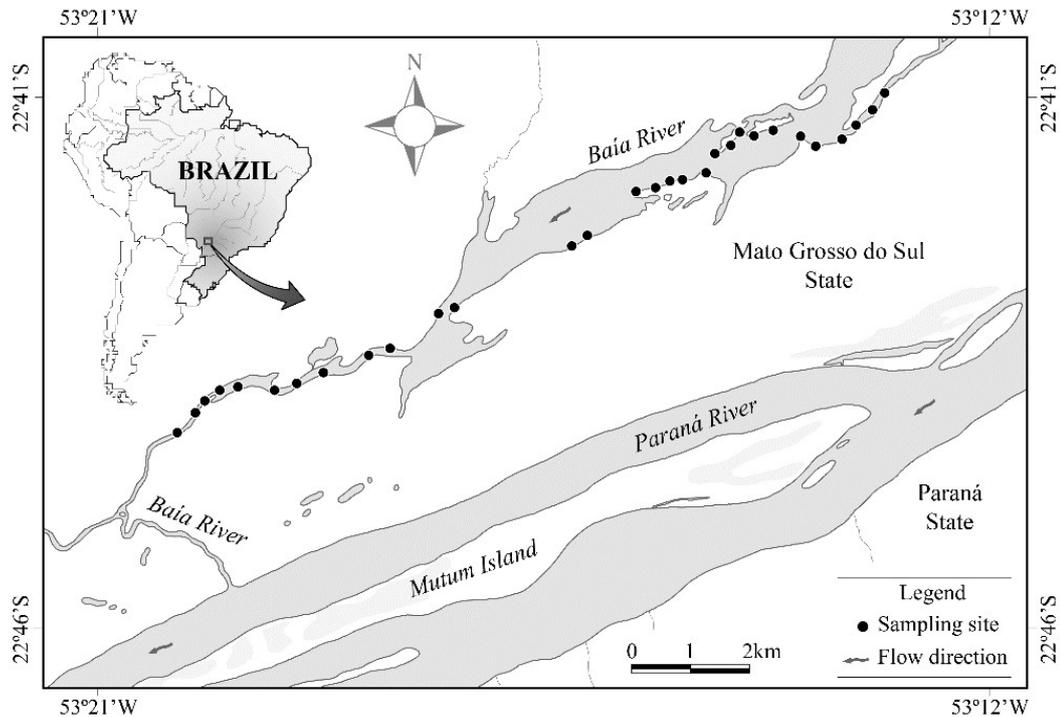


**Fig. 1** Map of the study area on the Baía River, showing the sampling locations (black dots). Geographic coordinate system based on the EPSG 4618

2.2.2 Sampling

Field surveys were carried out in August 2018 in the period of low water (Online resource Fig. S1), when the lower hydrometric level increases the importance of aquatic plants as a habitat for fish. This is a consequence of decreased aquatic area, water volume and depth, which tend to isolate environments and concentrate organisms within biotic communities, increasing interactions among organisms (Fitzgerald et al. 2017). The low water period is also conducive to measuring beta diversity because the lower connectivity among habitats may result in strong associations between species composition and local environmental conditions (Lansac-Tôha et al. 2016), which makes communities more unique and increases the possibility of enhanced between-local communities variation. In contrast, during flood periods, heavy rains promote

the raising of the water level, thereby intensifying the connectivity and similarity (biotic and abiotic) among habitats (Thomaz et al. 2004).

We sampled 30 macrophyte stands (Fig. 1) along a 13.7 km long stretch in the Rio Baía. The geographic position of each sampled stand was obtained with a GPS (Datum WGS-84). We applied the following four criteria to select the stands: they should (1) be separated by at least 350 m from the nearest stand sampled to prevent spatial dependency; (2) present a suitable depth (≥0.5 m) for sampling; (3) have the presence of *Eichhornia azurea*, one of the most abundant aquatic macrophytes in the Upper Paraná River floodplain (Souza et al. 2017); and (4) show different macrophyte biomass and diversity, which was visually assessed in the field (Online resource Fig. S1) and subsequently confirmed in the laboratory. It is noteworthy that there were no macrophyte stands or no suitable depth for sampling in some stretches, which explains the great distances between some stands (Fig. 1). Fish and macrophytes were simultaneously sampled.

We collected the fish using plexiglass traps that have advantages in investigating the relationship between fish and macrophytes, especially because they do not alter habitat conditions during the capture procedure, since the trap is composed mainly of transparent material (e.g., Dibble and Pelicice 2010). Although the trap effectiveness might be influenced by plant density that affects fish mobility, its influence also occurs in environments devoid of vegetation. Thus, it can be assumed that sample effort reflects the fish abundance of stands (Dibble and Pelicice 2010). In order to maximize the sampling effort, we placed three traps in each stand. These were positioned 4 m inside the stands, to minimize the effect of species that transit between the open area and the area structured by macrophytes. We placed the traps at 0700 and checked for fish every 8 hours, totaling a 24-hour sampling effort. All captured fish were anesthetized with clove oil and fixed in 4% formaldehyde. Subsequently, they were identified according to Ota et al. (2018).

In light of the importance of estimating various metrics of habitat complexity (Tokeshi and Arakaki 2012), we evaluated two facets of habitat complexity: the density and the diversity of aquatic macrophytes. After removing the fish traps, we collected the macrophytes using a 0.5 m × 0.5 m square and all plant material contained to a depth of 0.5 m was removed. The plants were identified to species (Lorenzi 2000; Pott and Pott 2000), washed and dried to constant dry weight in an oven at 60°C (adapted from Wetzel and Likens 1991). To estimate the habitat complexity provided by macrophytes, the sum of the biomass of all species (total biomass) in each stand was used as a predictor variable in the models, with the density expressed as g DW m$^{-3}$. The use of macrophyte biomass as a complexity indicator is based on the fact that

a greater biomass in general is related to a greater number of underwater structures, including roots stems and rhizomes. To calculate the macrophyte diversity in each stand (a second complexity indicator), we used the exponential of Shannon-Wiener index (Jost 2007), considering the biomass of each species to evaluate the relative abundance values. The exponential of Shannon is recommended because it indicates the effective number of species, allowing linear and more intuitive comparisons (Jost 2007), and providing a greater gradient of diversity values compared to the Shannon index. To characterize the abiotic conditions in each macrophyte stand, we measured temperature (°C), pH, conductivity (µS/cm) and dissolved oxygen (mg/L) (YSI digital meters) in the water subsurface near the traps. The measurements were taken at the installation and at each inspection of the traps, and the data were combined in an average for each sampled stand (Online resource Table S1).

### 2.2.3 Functional traits

To characterize the functional traits of fish species, we adapted the method proposed by Winemiller et al. (2015), who suggested using five basic niche dimensions. Among the suggested dimensions, we gathered information on "life history" (fertilization, parental care and spawning) and "trophic niche" (trophic guild), and we added "ecomorphology" (body shape and maximum total length) traits, as this feature is intimately related to the use of habitats structured by aquatic macrophytes (Prado et al. 2016) (Online resource Table S2, S3). The information was obtained from the individuals captured in this study (total length and trophic guild when possible – see Online resource Table S4 for details on diet analysis) or from literature. When not available for the species, we used information available for the genus (see Online resource Table S3).

### 2.2.4 Data analysis

To assess alpha and beta functional diversity, a functional space was first constructed with a PCoA (Villéger et al. 2008), using the Gower distance between species, which is suitable for mixed data (a matrix with numerical and categorical data) (Gower 1966). Prior to the PCoA calculation, we assigned weights to the functional traits, in order to equate the three functional dimensions used in this study (life history, trophic niche and ecomorphology). Thus, each trait that represents life history (parental care, spawning type and fertilization type) received a

weight of 0.3333, the trophic guild, which represents the niche dimension, received a weight of 1, while the ecomorphological data (TL max and body shape) were weighted 0.5 each. According to the method proposed by Maire et al. (2015), a high-quality functional space needs to show congruence between the initial functional distance (Gower distance) and the standardized distances in the new space (generated by PCoA). Therefore, using the mean squared deviation (mSD) between the distances of the PCoA, the best functional space was the one based on four dimensions, which presented the mSD of only 0.0012 (Maire et al. 2015) (Online resource Fig. S3).

From the volume of multidimensional space occupied by the species, we calculated the functional richness index (FRic), subsequently used as a measure of functional alpha diversity, and the functional beta diversity. FRic represents the volume of multidimensional space filled by the species of the assemblage of interest, and increases with the presence of extreme traits (Mason et al. 2005). The index was calculated in the R environment (R Core Team, 2019) using the function "dbFD" of the *FD* package (Laliberté and Legendre 2010). We used the square-root transformation to account for negative eigenvalues present in this analysis (Legendre 2014). Beta diversity was estimated pairwise among macrophyte stands, using the Jaccard dissimilarity index between species, as initially proposed by Baselga (2010) for taxonomic beta diversity and subsequently adapted by Villéger et al. (2011) for functional beta diversity. Thus, the beta functional diversity between two assemblages is equivalent to the portion of the non-shared functional space in relation to the total filled functional space. The total functional beta diversity (FBD-total) was partitioned into turnover (FBD-turn) and nestedness components (FBD-nest), according to Villéger et al. (2013). The calculations were performed using the function "functional.beta.pair" of *betapart* package (Baselga et al. 2019). Finally, the local contribution to functional beta diversity (LCBD; Legendre and De Cáceres 2013) of each stand was also investigated for the three components of beta diversity generated in the previous step, using the function "LCBD.comp" of *adespatial* package (Dray et al. 2019).

In order to investigate the relationship between the fish functional richness index (FRic – response variable) and the density and diversity of aquatic macrophytes, we performed beta regressions (Ferrari and Cribari-Neto 2004), an appropriate analysis for continuous response variables that are restricted between zero and one. Considering that the geographical distance between stands may drive some variation in fish assemblages (Lansac-Tôha et al. 2019), we used Moran's I correlograms (Legendre and Legendre 2012) to check if the control for spatial autocorrelation bias was required, which could somehow inflate the significance of each predictor in beta regression analysis. According to this test, FRic did not present bias related to

spatial autocorrelation structures (p = 0.92). In addition, as the dissolved oxygen (that ranges from 4.07 to 9.43 mg/L) can also influence the fish community (Cunha et al. 2019), we included this variable as an additional regressor for the precision parameter and overlapped it on a dispersion submodel. To assess the non-linear relationship (prediction *i*), we modeled FRic according to the macrophyte density based on a polynomial model from first to third order. The third order term was used to allow the model's curve to lower after the peak. Models with different polynomials were evaluated by likelihood ratio tests, with the smaller model (least number of polynomials) being chosen when no significant differences were found. Beta regressions were performed using the function "betareg" of *betareg* package with "logit" as link function in the mean model (mu) and "log" in the precision model (phi) (Zeileis et al. 2019) and the likelihood test using the "lrtest" function of *lmtest* package (Zeileis and Hothorn 2002).

To test the prediction (*ii*) and predict patterns in functional beta diversity of fish across the aquatic macrophyte complexity gradient, we used Generalized Dissimilarity Modelling (GDM). The main advantages of GDM, in comparison with other essentially linear approaches often used to relate community responses to environmental gradient (e.g. Lichstein 2007), is that it allows pairwise between-site compositional dissimilarity to be modelled as a non-linear function of environmental distance between plot pairs (Ferrier et al. 2007). In ecological data with high levels of beta diversity, the rate of compositional turnover along environmental gradients is rarely constant but rather varies among different parts of the gradient, showing in most cases a curvilinear rather than linear relationship with the predictor variables (Ferrier et al. 2007). The response curves derived from the GDM model provide an indication of the total amount of variation explained by a predictor variable and also the rate of change of compositional turnover along a gradient, using an iterative maximum-likelihood estimation and I-splines (Ferrier et al. 2007). The slope of the I-splines curves indicates the rate of biological change along the gradient while the height of each I-spline represents the total amount of variation associated with the variable, holding all other variables constant. In this case, the functional dissimilarity matrices (FBD-total, FBD-turn and FBD-nest) were used as response variables, and as inter-site distances of predictor variables based on values of density and diversity of macrophytes, dissolved oxygen, as well as geographical distance between sites. The importance value of individual variables can be estimated from the sum of the I-spline coefficients (Fitzpatrick et al. 2013). Consequently, each I-spline is a partial regression fit and thus represents a Partial Ecological Distance related to the contribution of individual variables. Using the function "formatsitepair" from the R-package *gdm* (Manion et al. 2019), we built a site-pair table required to fitting a GDM, which in turn, was performed using the "gdm"

function from the same R-package. Finally, using the "gdm.varImp" function from this package we quantified model significance and variable importance of GDM through 999 permutations.

In order to assess the relationships between LCBD of each component of functional beta diversity (FBD-total, FBD-turn and FBD-nest) with FRic (prediction *iii*), and with the density and diversity of macrophytes (prediction *iv*), we also used beta regressions. For both predictions, we modeled third order polynomials with the predictive variables FRic and macrophyte density, respectively, and in the latter case, dissolved oxygen was also added in a dispersion sub-model. Despite not expecting a hump-shaped relationship between LCBD and FRic, visual inspections showed non-linear relationships, so polynomial regression models were also performed. In both predictions, we performed the same procedure adopted for prediction *i*, using likelihood ratio tests for model selection. As well as FRic, none of the LCBD components presented bias related to spatial autocorrelation structures according to Moran's I correlograms (LCBD FDB-total, p = 0.99; LCBD FDB-turn, p = 0.48; LCBD FDB-nest, p = 0.75).

## 2.3  Results

### 2.3.1 Data description

In the 30 macrophyte stands sampled, a total of 17 species of plants were recorded (Online resource Table S5), and the richness ranged from one to 12 species per quadrat. The macrophyte density ranged from 366 to 7.066 g DW m$^{-3}$, while the exponential of Shannon ranged from 1 (monospecific stands) to 4.65. Twenty-five fish species were identified (Online resource Table S3), totaling 4,648 individuals. Fish taxonomic richness ranged from 6 to 15 species. The LT max of species ranged from 23 to 89 mm, with most species (15 species, 4,176 individuals) belonging to the Characidae family (Characiformes), which mostly includes small-sized species. However, young individuals of medium-sized species were also captured, including species such as *Leporinus lacustris* Campos 1945*, Hoplias mbigua* Azpelicueta et al. 2015 (Characiformes), *Crenicichla britskii* Kullander 1982 (Cichliformes) and *Brachyhypopomus gauderio* Giora and Malabarba 2009 (Gymnotiformes).

2.3.2 Functional alpha diversity

The alpha functional diversity of fish, measured with the functional richness index (FRic), ranged from 0.001 to 0.465 resulting in a mean value of 0.131. The relationship between FRic and macrophyte density was best described by a third order polynomial relationship (Online resource Table S6). The multiple regression model showed moderate predictive power, explaining approximately 44.8% of the FRic variation (Pseudo-$R^2$ = 0.448). FRic showed a significantly hump-shaped response to macrophyte density, increasing to approximately 1000 g DW m$^{-3}$ (or 3.1 in log scale) and then decreasing (Fig. 2a). Differently, FRic responded positively to the macrophyte diversity (Table 1, Fig. 2b).

**Table 1** Results of beta regression analysis for functional richness (FRic) of fish caught along a gradient of aquatic macrophytes

|  | Estimate | SE | Z | *P* |
|---|---|---|---|---|
| (Intercept) | -2.72 | 0.25 | -10.66 | < 0.0001 |
| Density | -1.02 | 0.93 | -1.1 | 0.27 |
| Density$^2$ | 1.48 | 0.79 | 1.86 | 0.06 |
| Density$^3$ | 2.24 | 0.79 | 2.83 | 0.0046 |
| Exp. Shannon index | 0.27 | 0.10 | 2.6 | 0.0094 |
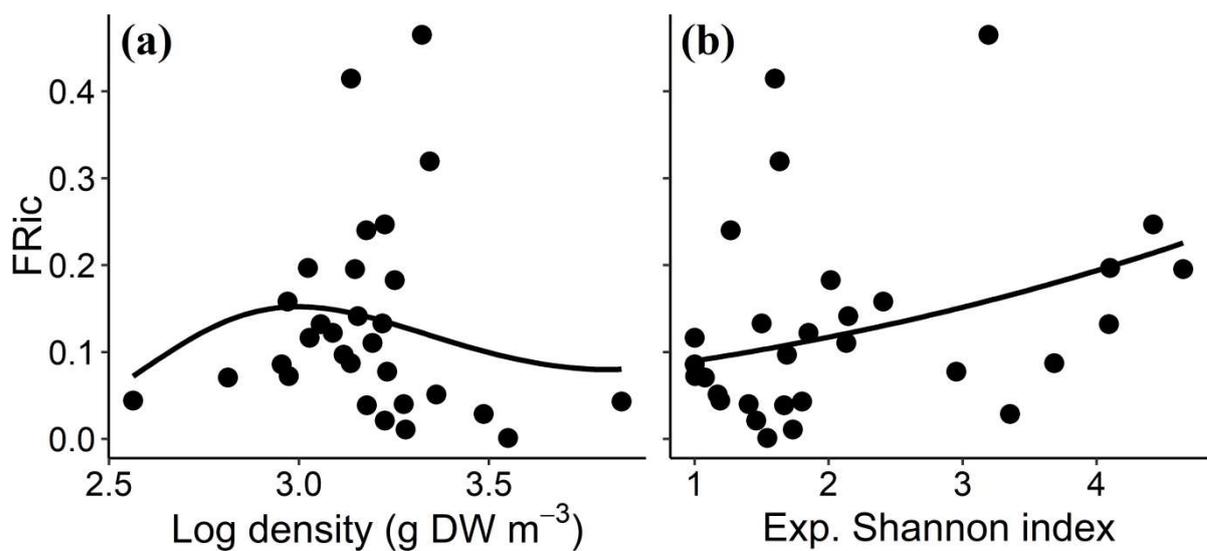


**Fig. 2** Relationship between fish functional richness (FRic) and macrophyte density (**a**) and diversity (**b**). Note log scale used for macrophyte density

The total functional beta diversity (FBD-total) was high (mean = 0.81) and had a greater contribution of FBD-turn (mean = 0.52) compared to FBD-nest (mean = 0.29) (Online resource Fig. S4). The FBD-total was mainly affected by the macrophyte density gradient (Table 2), and the response of the fitted functions (GDM-fitted I-splines) showed an almost linear relationship along the gradient (Fig. 3a). Macrophyte diversity, geographical distance and dissolved oxygen showed only weak or null relationships with FBD-total (Table 2, Fig. 3b, Online resource Fig. S5).

**Table 2** Summaries of the GDM models for each component of beta diversity of fish caught along a gradient of aquatic macrophytes stands

|  | Components of functional beta diversity | | |
|---|---|---|---|
|  | **FBD-total** | **FBD-turn** | **FBD-nest** |
| Model deviance | 55.5 | 207.4 | 188.9 |
| Explained (%) | 18.6 | 9.86 | 0.81 |
| (Intercept) | 1.17 | 0.52 | 0.25 |
| *P-value* | 0.02 | 0.01 | 0.64 |
| Variable impacts: |  |  |  |
|   Geographical distance | 0.78 | 0.00 | 23.82 |
|   Dissolved oxygen | 0.00 | 1.49 | 16.37 |
|   Density | 93.33 | 72.19 | 0.00 |
|   Exp. Shannon index | 4.10 | 23.11 | 39.22 |
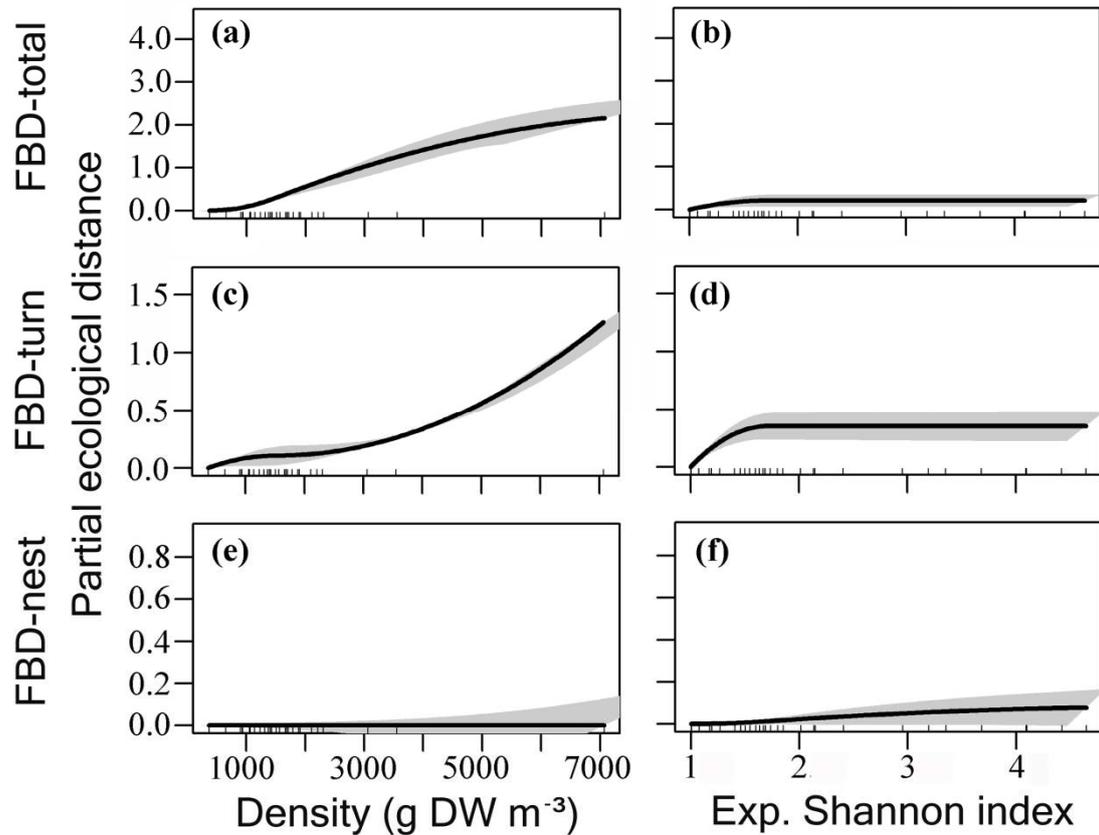
**Fig. 3** Plots of I-splines of the predictor variables (black) and confidence intervals from bootstrapping (grey) for each component of beta diversity of fish along plant density (**a**, **c**, and **e**) and diversity (**b**, **d** and **f**) gradients. The maximum height reached by each curve indicates the total amount of functional dissimilarity associated with that variable, holding all other variables constant. The rug on the bottom of the plot indicates the distribution of the raw data

The FBD-turn component was also mainly explained by the variation in the macrophyte density, but part of its variation was also attributed to the macrophyte diversity (Table 2). The density showed an increasing curvilinear relationship, where low values along the density gradient had little effect on the turnover, but from ca. 4000 gDW it grew exponentially (Fig. 3c). The relationship between FBD-turn and macrophyte diversity showed a different pattern, reaching a plateau after 1.8 of Shannon exponential (Fig. 3d). Geographical distance and dissolved oxygen also did not influence the FBD-turn (Table 2, Online resource Fig. S5). No significant relationship was found between the FBD-nest component and the predictor variables (Table 2, Fig. 3e, f).

2.3.3 Relationship between LCBD and fish functional richness

FRic was significantly related to the three components of the LCBD (Table 3). The relationship between LCBD FBD-total and FRic was best described by a second order polynomial relationship (Table 3a; Online resource Table S7). FRic showed high predictive power, explaining approximately 83% of the LCBD FBD-total variation (Pseudo-$R^2$ = 0.827). The LCBD FBD-total tends to decrease with the increase in FRic up to approximately 0.3 units, from where the LCBD FBD-total increases with the increase in FRic (Fig. 4a). The relationship between LCBD FBD-turn and FRic was best described by a third order polynomial model (Table 3b; Online resource Table S7). FRic explained approximately 79% of the LCBD FBD-turn variation (Pseudo-$R^2$ = 0.795), with this component slightly increasing up to the point where FRic reached 0.1 unit, and subsequently decreased successively (Fig. 4c). Finally, the relationship between LCBD FBD-nest and FRic was best described by a third order polynomial relationship (Table 3c, Online resource Table S7). Compared to the other two components, FRic had less predictive power, explaining approximately 55% of the variation of LCBD FBD-nest (Pseudo-$R^2$ = 0.553). LCBD FBD-nest tends to decrease with an increase in FRic up to about 0.1 units, from where the LCBD FBD-nest increases with an increase in FRic (Fig. 4e). Hence, stands with high LCBD FBD-turn values generally showed low LCBD FBD-nest values, and the inverse pattern between these variables was confirmed with a Pearson correlation (-0.76; $P$<0.0001). It was also possible to observe that even neighboring stands can have distinct fish functional richness and completely opposite contributions to the beta diversity (Fig. 4b, d, f).

**Table 3** Results of beta regression analysis for local contributions to beta diversity (LCBD), with fish functional richness (FRic) used as predictor variable

|  | Estimate | SE | Z | *P* |
|---|---|---|---|---|
| **(a) LCBD FBD-total** |  |  |  |  |
| (Intercept) | -3.38 | 0.01 | -237.58 | < 0.0001 |
| FRic | -0.58 | 0.08 | -7.63 | < 0.0001 |
| $FRic^2$ | 0.72 | 0.08 | 9.39 | < 0.0001 |
| **(b) LCBD FBD-turn** |  |  |  |  |
| (Intercept) | -3.48 | 0.06 | -29.98 | < 0.0001 |
| FRic | -3.13 | 0.47 | -6.58 | < 0.0001 |
| $FRic^2$ | -0.42 | 0.40 | -1.05 | 0.29 |
| $FRic^3$ | 1.18 | 0.37 | 3.20 | 0.0014 |
| **(c) LCBD FBD-nest** |  |  |  |  |
| (Intercept) | -3.50 | 0.09 | -37.61 | < 0.0001 |
| FRic | 1.42 | 0.33 | 4.32 | < 0.0001 |
| $FRic^2$ | 1.91 | 0.39 | 4.93 | < 0.0001 |
| $FRic^3$ | -1.99 | 0.36 | -5.48 | < 0.0001 |
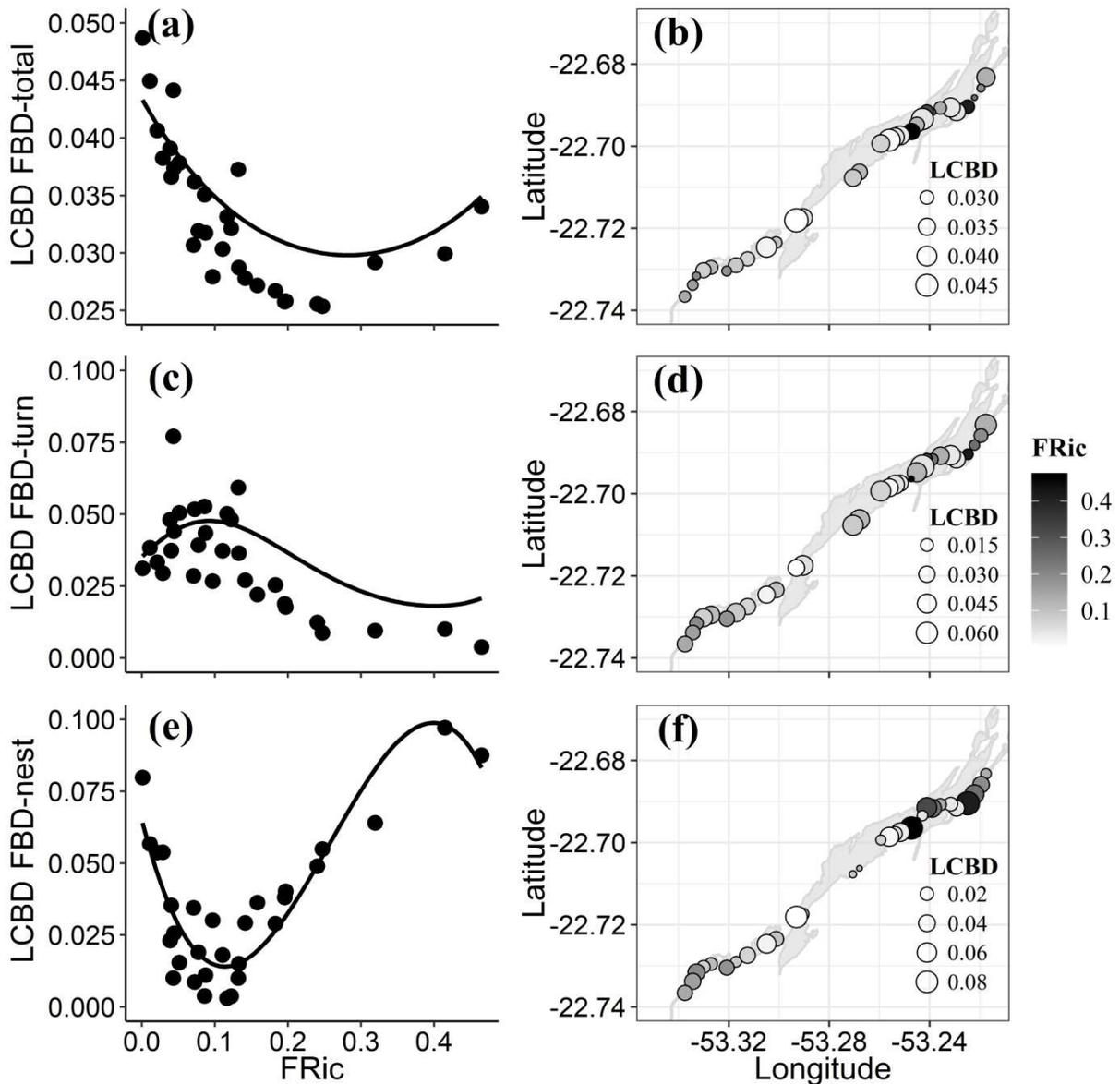
**Fig. 4** Relation between local contribution to fish beta diversity (LCBD) and fish functional richness (FRic; **a**, **c** and **e**), and spatial variation of LCBD (**b**, **d** and **f**). In **b**, **d**, and **f** circle sizes are proportional to the LCBD value at that site. The gray scale indicates the increase of FRic. Note log scale used for macrophyte density

2.3.4 Relationship between LCBD and macrophyte stand complexity

Beta regressions showed that the LCBD FBD-total was significantly related to macrophyte density (third order polynomial relationship; Online resource Table S8) and macrophyte diversity, with these predictors explaining approximately 44.9% of the variation in LCBD FBD-total (Pseudo-$R^2$ = 0.449; Table 4, Fig. 5a, b). Regarding the macrophyte density gradient, the FBD-total LCBD decreased up to approximately 1000 g DW $m^{-3}$ (or 3.1 in log scale), from where it increased again. The macrophyte diversity had a negative effect on LCBD

FBD-total. Thus, stands with either the lowest or more commonly the highest macrophyte densities are the most distinct in terms of the functional space of the fish community, being the stands that most contribute to the regional variability of the functional traits of communities. On the other hand, stands with greater macrophyte diversity are more similar in terms of the functional space of the fish assemblage, thereby being those that least contribute to the regional variability of the functional traits of this community.

LCBD FBD-turn was better explained by the first order model, with 9.3% explanatory power (Online resource Table S8), but with a negative and significant effect only for the macrophyte diversity (Table 4, Fig. 5c, d). LCBD FBD-nest showed a hump-shaped relationship with macrophyte density (Online resource Table S8), and no relation with macrophyte diversity. This model explained 23.8% of variation (Pseudo-$R^2$ = 0.238; Table 4, Fig. 5e, f).

**Table 4** Results of beta regression analysis for local contribution to beta diversity (LCBD), with macrophyte density and macrophyte diversity used as predictor variables

|  | Estimate | SE | Z | *P* |
|---|---|---|---|---|
| **(a) LCBD FBD-total** | | | | |
| (Intercept) | -3.26 | 0.06 | -58.02 | < 0.0001 |
| Density | 0.48 | 0.14 | 3.38 | < 0.0008 |
| Density$^2$ | -0.10 | 0.13 | -0.78 | 0.43 |
| Density$^3$ | -0.35 | 0.11 | -3.08 | 0.0021 |
| Exp. Shannon index | -0.06 | 0.02 | -2.29 | 0.02 |
| **(b) LCBD FBD-turn** | | | | |
| (Intercept) | -2.82 | 0.17 | -16.59 | < 0.0001 |
| Density | < -0.0001 | < 0.0001 | -0.33 | 0.74 |
| Exp. Shannon index | -0.24 | 0.07 | -3.28 | 0.001 |
| **(c) LCBD FBD-nest** | | | | |
| (Intercept) | -3.69 | 0.26 | -14.09 | < 0.0001 |
| Density | 0.73 | 0.72 | 1.02 | 0.31 |
| Density$^2$ | -2.04 | 0.66 | -3.11 | 0.0019 |
| Exp. Shannon index | 0.12 | 0.10 | 1.16 | 0.25 |
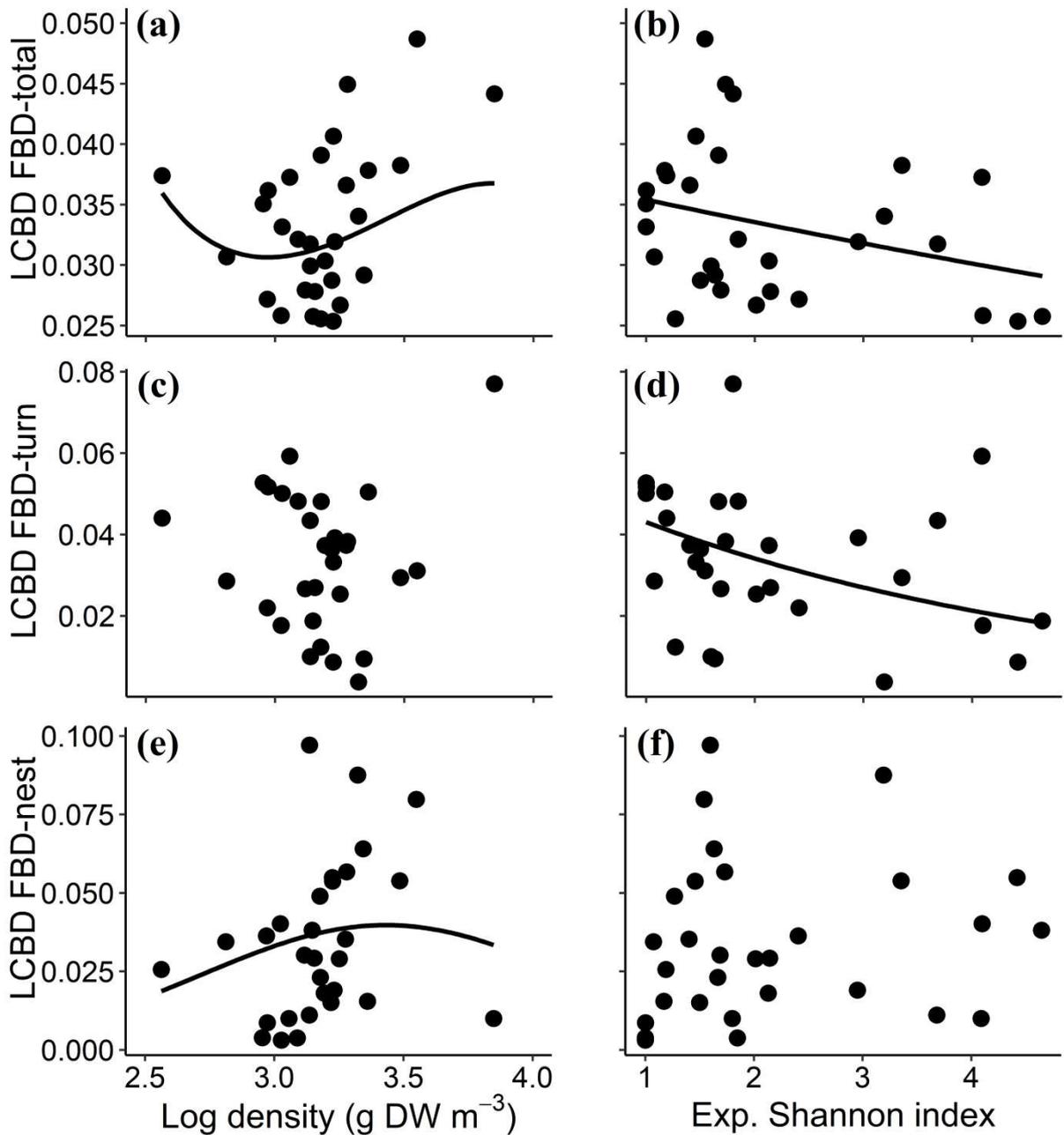
**Fig. 5** Relationship between local contribution to beta diversity (LCBD) for each component of functional beta diversity and macrophyte density (**a**, **c** and **e**) or diversity (**b**, **d** and **f**)

LCBD FBD-turn was better explained by the first order model, with 9.3% explanatory power (Online resource Table S8), but with a negative and significant effect only for the macrophyte diversity (Table 4, Fig. 5c, d). LCBD FBD-nest showed a hump-shaped relationship with macrophyte density (Online resource Table S8), and no relation with macrophyte diversity. This model explained 23.8% of variation (Pseudo-R² = 0.238; Table 4, Fig. 5e, f).

**2.4 Discussion**

Our findings support the hypothesis that the habitat complexity gradient provided by aquatic macrophytes acts as an environmental filter for the functional structuring of fish assemblages, based on examination of both alpha and beta diversity. It is widely known that environmental conditions filter species from the regional species pool according to their functional traits (e.g. morphological, physiological or life history) (Zobel 1997; Peláez and Pavanelli 2019), and that habitat complexity is one of the main factors that explains the structuring of ecological communities (Kovalenko et al. 2012; Ortega et al. 2018). In our study, habitat complexity was assessed through macrophyte density and diversity, both of which influenced the filtering of fish functional traits. However, they had different effects on fish communities, indicating the action of different processes.

The fish functional richness (FRic) showed a unimodal response to the increase in macrophyte density. This was an expected pattern, since it has also been observed for taxonomic diversity (Tokeshi and Arakaki 2012; Cunha et al. 2019). Low FRic in lower-density stands can be explained by the low availability of physical structures, which reduces the availability of refuges favoring predation. In fact, predation is exerted mainly by larger limnetic fish and the risk is higher in less complex habitats (Santos et al. 2009). Dense macrophyte stands, in turn, can reduce FRic since very complex structures may provide only small interstitial spaces that make it difficult for fish to move (Crowder and Cooper 1982; Warfe and Barmuta 2006). In this case, despite the increase in food availability provided by the greater macrophyte complexity, there is a decrease in the foraging efficiency of fish due to the inaccessibility and increase of physical refuges for prey (Schultz and Dibble 2012; Clemente et al. 2019). It should be noted that stands with extremely high density can select specific traits that facilitate locomotion and foraging in limited spaces. For example, this was the case of the largest species caught *Brachyhypopomus gauderio*, a species that is compressed fusiform in shape and uses electricity to capture prey animals.

Considering that the FRic index reflects the functional space filled by species, emphasizing the most extreme traits (Villéger et al. 2008), the addition of rare species has relevant contributions to the functional structure of ecological communities (Mouillot et al. 2013). Indeed, the stands with the highest functional richness (stands with intermediate macrophyte density) were the ones with the highest taxonomic richness and, consequently, incorporated rare species with specific traits. For example, *Crenicichla bristkii*, that shows parental care, had 50% of its total occurrence in the three highest FRic stands (Online resource

Table S3). It is worth emphasizing that reproductive features are important for demographic responses to environmental variation (Winemiller et al. 2015), and many species find a refuge environment against predation for adults and young fish as well as eggs within macrophyte stands (Bulla et al. 2011). In this regard, intermediate plant densities can facilitate parental care since even if the environment is structured, it does not limit the movements of fish. Another important aspect for fish movements at intermediate macrophyte densities may be greater maneuverability, since the ability to swim among macrophytes is fundamental for efficient foraging (Manatunge et al. 2000). Therefore, not too dense stands can increase the fish FRic by allowing the presence of different feeding strategies, while still providing shelter against predation.

In agreement with our prediction, macrophyte diversity positively influenced fish FRic. This result is expected because the macrophyte species vary in some characteristics, such as physical architecture, as well as how they may alter the water chemistry (Dionne and Folt 1991; Miranda and Hodges 2000), enabling the selection of different fish traits. The effects of plant identity on fish FRic can be direct or indirect, given that fish exhibit trait variability, such as body size and feeding behavior (Schultz and Dibble 2012). In this sense, body size can directly affect fish locomotion among stands of different plant species, while feeding behavior is indirectly influenced by prey distribution, which in turn is affected by the plant identity. Important prey species, such as zooplankton, also show preferences for different macrophyte species (Zeng et al. 2017), which can affect the choice of different macrophytes by fish with different feeding strategies. For example, stands with higher macrophyte richness accommodate greater zooplankton diversity (Choi et al. 2016), what may enhance the coexistence of fish with distinct feeding habits.

The macrophyte complexity gradient also explained the variation in fish beta diversity. Heterogeneous environments, which include changes in the habitat physical structure, generally increase beta diversity values (Petsch et al. 2017). This is because different habitats may filter distinct traits. Depending on the degree of organism dispersal between sites, different proportions of turnover and nestedness components are expected (Gianuca et al. 2017). Here, we observed a greater contribution of FBD-turn to the between-site traits variation. This suggests that dispersal between stands, even in very close ones, is not great enough to the point of promoting the functional traits homogenization by mass effects. Rather, it is sufficient to cause the filtering of traits according to the environmental conditions imposed by differences in habitat complexity (Gianuca et al. 2017). The fish taxonomic turnover along the macrophyte density gradient is due to species-specific responses to environmental conditions, which leads

to selective colonization and extinction (Cunha et al. 2019). The dominance of turnover also indicates that stands with the lowest FRic are not a subset of highest FRic stands, showing the clear contribution of the macrophyte complexity gradient to the turnover of functional traits. Therefore, the fish functional nestedness may not respond to directional processes, including macrophyte density (Cunha et al. 2019) and diversity, which would explain the fact that both FBD-nest and LCBD FBD-nest did not show significant or showed weak relationships with the complexity variables evaluated. This has a direct implication for conservation and management, since macrophyte stands with different complexities are necessary to preserve a greater variety of functional traits.

As we expected, the functional similarity of fish assemblages decreased along the macrophyte density gradient, being responsible for most of the FBD-Total and FBD-Turn variation (see Table 2). This suggests that macrophyte stands with different densities have different environmental filters that select specific traits, which consequently generates communities that are almost totally different functionally. Moreover, functional variations occur throughout the entire gradient, except in low density stands that showed little variation (see Fig. 4). Low levels of macrophyte density, besides exposing more fish to the risk of predation, show low functional richness, which can contribute to a functional similarity among fish communities. This could explain why the traits turnover occurs only above certain density values.

The FBD-turn component was also related to the variation in macrophyte diversity up to a certain threshold. This result may be related to the different types of shelters and food resources provided by low- and high-diversity stands. As mentioned above, more diverse stands support greater diversity of shelter and food, since they are composed of macrophytes with different growth forms (see Online resource Table S5). For example, free-floating and submerged macrophytes can differently influence water physical and chemical features, as well as contribute to roles of bottom-up or top-down mechanisms (Meerhoff et al. 2003). This allows the presence of common traits of species between these sites. In contrast, less diverse stands with the dominance of a certain species plant can reduce the possibilities of coexistence between fish species possessing different traits, contributing to the dissimilarity between the communities. However, it is important to highlight that diverse macrophyte stands do not necessarily support a great variety of life forms, since some species could possess similar morphology.

The degree of local contribution to beta diversity was variable, with macrophyte stands being singular or common within the environmental gradient (Legendre and De Cáceres 2013).

The FBD-total and FBD-turn LCBD components showed a U-shaped relationship with FRic. Thus, although we expected a negative relationship, which has already been shown in several previous studies on taxonomic diversity (Legendre and De Cáceres 2013; Heino and Grönroos 2017; Silva et al. 2018), we showed that the functional LCBD values tend to rise again above a given FRic value. Hence, sites with high and low FRic contribute significantly to the fish functional beta diversity. This shows that the functional richness alone is not always a good indicator to be used in biodiversity assessment and conservation planning. Species-rich assemblages can usually host functionally redundant species (Mouillot et al. 2013) and, in this case, they do not contribute as much to beta diversity as species-poor assemblages, which nevertheless have species with unique traits. This may be the case mainly for LCBD-turn, since the higher levels of FRic reflected low contributions to the substitution of traits. However, diverse communities also include species with traits that are absent in other sites, as discussed for stands of intermediate macrophyte density, which increases the local contribution to beta diversity, as we observed mainly for the FBD-nest component (see Fig. 5e). These results emphasize the importance of conserving both sites with high and low functional richness. Sites with high LCBD values may be valuable for guiding biodiversity conservation, as they present ecological uniqueness (Legendre and De Cáceres 2013).

Ecologically unique sites may support an unusual species combination, probably reflecting special environmental conditions (Legendre and De Cáceres 2013). Our results showed that, following our expectation, stands of lower and higher macrophyte density contributed more to beta diversity (LCBD FBD-total). Thus, despite presenting low FRic, macrophyte stands with extreme densities filter traits that contributed to increase the fish functional dissimilarity. For example, *Aphyocharax dentatus* Eigenmann and Kennedy 1903 is the only species captured that has a prognathous mouth. This trait facilitates predation at the water surface (Hahn et al. 2000), which could explain its presence only in a low density macrophyte stand where the water surface is more available. This inference suggests that rare species often perform unique ecosystem functions and strongly affect differences in functional diversity (Toussaint et al. 2016; Violle et al. 2017). In contrast, LCBD FBD-nest showed a different pattern, with the highest values observed in intermediate densities. This occurs because the majority of macrophyte stands with intermediate density showed high FRic values. Consequently, stands with lower FRic tend to be subsets of intermediate density stands. Regarding macrophyte diversity, less diverse stands contributed more to beta diversity, indicating that these sites are also of utmost importance for community variation, supporting traits not present in more diverse stands. Although less diverse stands have lower FRic, they

possibly have species with specific traits that can facilitate occurrence at sites with low diversity of resources. These results are in agreement with our prediction that stands with low FRic present greater singularity in relation to the other places in a region.

The spatial extent of the studied area could have been a limiting factor, since spatially close sites tend to show high similarity, both environmental and biological (Nekola and White 1999). However, the results showed that the geographical distance between the stands did not explain the fish beta diversity patterns (see Tab. 2). Even neighboring sites had functionally distinct assemblages and contributed to fish functional beta diversity in an opposite way, indicating that nearby stands may represent different environmental conditions (see Fig. 5). Similarly, a study based on taxonomic data in the same floodplain also verified that the environmental heterogeneity provided by the presence of macrophytes was responsible for variations in fish assemblage similarity in lentic environments (Granzotti et al. 2019). Peláez and Pavanelli (2019) found that environmental factors were more important to explain the fish functional dissimilarity in a Neotropical river, while spatial factors explained the taxonomic dissimilarity, which suggests that these aspects of beta diversity are influenced by different processes. As well as the geographical location, oxygen is one of the main factors that influence the fish distribution (Agostinho et al. 2007). However, none of the sampled stands had limiting values for dissolved oxygen. Consequently, dissolved oxygen and its variation between sampling stands were not related to functional diversity (see Table 2), indicating that this factor was not crucial for the functional community structure along the studied gradient. In fact, in macrophyte stands, the distribution of fish species may be more dependent on which plant species dominates the stand than on limnological variables, such as oxygen and temperature (Sánchez-Botero et al. 2008).

In conclusion, the macrophyte stand complexity played a major role in the filtering of fish functional traits. Macrophyte density affected the functional alpha diversity of fish in a non-linear way, with the highest values occurring in intermediate densities, while macrophyte diversity showed a positive effect. The variation in macrophyte density was the main factor responsible for the variations in functional beta diversity. The sites that contributed most to functional beta diversity had low and high FRic values and, consequently, the lowest and highest plant densities, as well as low plant diversity. Considering that functional diversity is crucial to sustain different ecosystem services (Luck et al. 2009), understanding which physical configuration of aquatic macrophyte stands (i.e. density and diversity) favors high fish functional diversity can help in management and conservation strategies. Thus, our study increases the knowledge of the importance of aquatic gradients for the distribution of littoral

fish. This also contributes to the understanding of the functioning of floodplain ecosystems, which are characterized by the importance of these plants.

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# 3 AQUATIC MACROPHYTES SHAPE THE FORAGING EFFICIENCY, TROPHIC NICHE BREADTH, AND OVERLAP AMONG SMALL FISHES IN A NEOTROPICAL RIVER, BRAZIL

## ABSTRACT

Aquatic macrophytes are generally recognized to mediate fish-prey interactions. We assessed how consumption of particular food categories by fish, foraging efficiency, trophic niche breadth, and niche overlap of fish respond to a gradient of macrophyte density and diversity. We sampled small fishes and macrophytes in 30 stands distributed over the littoral zone of Baía River, Brazil. Increasing macrophyte density (from 366 to 7,066 grams of dry weight/$m^3$) favored herbivory and increased fish foraging efficiency. In general, fish reduced their trophic niche breadth along the gradient of macrophyte density while niche overlap among species increased until a certain extent of plant density when species started to partition the niche more strongly. Despite the general patterns, different responses were observed according to the trophic guild considered, with omnivorous and herbivorous fish generally showing opposite responses. Macrophyte diversity was negatively related to the consumption of higher plants by fish but positively related to the consumption of insects. Fish density and diversity were positively related to the trophic niche overlap of fish. Therefore, in addition to the presumable effects of macrophytes on resources availability and prey encounter rates, the trophic relationships of fish among macrophyte stands are dependent on trophic guilds and interspecific competition.

**Keywords:** Fish diet. Trophic ecology. Feeding habit. Habitat complexity

# MACRÓFITAS AQUÁTICAS MOLDAM A EFICIÊNCIA DE FORRAGEAMENTO, AMPLITUDE E SOBREPOSIÇÃO DE NICHO TRÓFICO ENTRE PEQUENOS PEIXES EM UM RIO NEOTROPICAL, BRASIL

## RESUMO

Foi avaliado como o consumo de determinadas categorias de alimentos, a eficiência de forrageamento, a amplitude e sobreposição de nicho trófico dos peixes respondem a um gradiente de densidade e diversidade de macrófitas. Foram amostrados pequenos peixes e macrófitas em 30 bancos distribuídos ao longo da região litorânea do rio Baía, Brasil. O aumento da densidade de macrófitas favoreceu a herbivoria e aumentou a eficiência de forrageamento dos peixes. Em geral, os peixes reduziram a amplitude do nicho trófico ao longo do gradiente de densidade de macrófitas, enquanto a sobreposição de nicho entre as espécies aumentou até certo ponto da densidade de plantas, quando as espécies começaram a particionar o nicho mais fortemente. Apesar dos padrões gerais, foram observadas respostas diferentes de acordo com a guilda trófica considerada, com peixes onívoros e herbívoros geralmente apresentando respostas opostas. A diversidade de macrófitas foi negativamente relacionada com o consumo de plantas superiores pelos peixes, mas positivamente relacionada com o consumo de insetos. A densidade e diversidade de peixes foram positivamente relacionadas com a sobreposição de nicho trófico dos peixes. Portanto, além dos efeitos presumíveis das macrófitas sobre a disponibilidade de recursos e as taxas de encontro com as presas, as relações tróficas dos peixes entre os bancos de macrófitas são dependentes das guildas tróficas e da competição interespecífica.

**Palavras-chave:** Dieta dos peixes. Ecologia Trófica. Hábito alimentar. Complexidade de habitat.

## 3.1 Introduction

Aquatic macrophytes are recognized as one of the major components that provide structural complexity in freshwater environments (Jeppesen et al., 1998). Their submerged structures (i.e., stems, roots, and leaves) along with the occurrence of different life forms (i.e., submerged, free-floating, emergent, and epiphyte) lead to great diversity and stability of biotic communities (Thomaz & Cunha, 2010). These plants affect various processes, influencing the lake ecological state and water features (Jeppesen et al., 1998; Søndergaard et al., 2002; 2010), as well as supply multiple ecosystem services, such as erosion regulation, water purification, and habitat provision (Thomaz, 2021). Several organisms inhabit vegetated habitats, including invertebrates, fish, and other primary producers (such as algae) (Thomaz & Cunha, 2010). Due to the protection from predators and the food associated with macrophytes, littoral regions represent important spawning and feeding grounds for fish (Nakatani et al., 1997; Carniatto et al., 2020), and the plants stabilize the dynamic interaction between fish and their prey (Jeppesen et al., 1998; Pelicice & Agostinho, 2006; Aleixo et al., 2022).

For fish, in particular, the trophic niche is a dominant dimension mediating how species interact (Correa & Winemiller, 2014; Mateus et al., 2016; Carniatto et al., 2017). Two important feeding features for fish coexistence are the trophic niche breadth (the level of diet specialization) and the niche overlap (degree of diet similarity between species). Both can vary according to the competition and food availability, and they may increase where there is a great food supply (Abbey-Lee et al., 2013; Walker et al., 2013a), as in more complex macrophyte stands (Warfe & Barmuta, 2006). In habitats with high food availability and consequently less intense competition for resources, the maximum tolerable overlap may be greater (Pianka, 1974; Dukowska & Grzybkowska, 2014), while in cases where resources are scarce, a contraction of the trophic niche and consequently a reduction in the overlap are predicted according to the classical theory of competition for resources (MacArthur & Pianka, 1966; Pianka, 1974). However, there is no consensus regarding these responses, especially concerning the effects of habitat complexity provided by aquatic macrophytes, which may affect fish species interactions and shape trophic niche variation (Vejříková et al., 2017).

Macrophytes are colonized by various organisms consumed by fish, such as periphytic algae (Rodrigues et al., 2003; Schneck et al., 2011) and invertebrates (Warfe & Barmuta, 2006; Thomaz et al., 2008; Campos et al., 2021). Studies have shown that the richness, diversity, and abundance of macroinvertebrates increase in structurally more complex macrophytes, e.g., with finely divided leaves (Taniguchi et al. 2003; Mormul et al. 2011; Walker et al. 2013b), with

various growth forms (Brown et al., 1988), and with greater biomass and density (Warfe & Barmuta, 2006; Thomaz et al., 2008). Fish, in turn, are also able to select aquatic macrophytes based on plant characteristics such as stem density (Gotceitas, 1990). However, unlike their food resources, fish community attributes (e.g., richness and diversity) increase with plant density to an optimal density level above which they decline (Cunha et al., 2019; Quirino et al., 2021a). Several factors may explain this decline, such as 1) chemical restrictions due to oxygen depletion (Miranda & Hodges, 2000; Bunch et al., 2015); 2) physical restrictions found at higher levels of plant density, which generates adverse conditions for fish to move and visualize prey (Harrel & Dibble, 2001), limiting them by their size and body shape (Cunha et al., 2019); 3) reduced foraging efficiency, which is affected by plant architecture (Dibble & Harrel, 1997), macrophyte density and coverage (Savino & Stein, 1982; Sammons & Maceina, 2006; Liversage et al., 2017).

Fish that can easily swim through the macrophyte structures may show a better foraging efficiency (Priyadarshana et al., 2001). Generally, the food consumption may increase with macrophyte coverage due to a larger amount of food (Middaugh et al., 2013; Nohner et al., 2018) and their trophic niche breadth becomes broader in more abundant (Vejříková et al., 2017; Quirino et al., 2021b; Aleixo et al., 2022) and diverse vegetation (Yofukuji et al., 2021). However, in dense vegetation the encounter and attack prey decline, and fish can capture smaller amounts of food, thereby reducing their feeding efficiency (Crowder & Cooper 1982; Liversage et al. 2017). Thus, intermediate levels of macrophyte density have been recognized as optimal for small fishes (Grenouillet & Grenouillet, 2002). Regardless of the trophic niche breadth, a more pronounced diet segregation and hence a reduced interspecific resource competition has already been found in abundant macrophyte vegetation (Eloranta et al., 2017; Vejříková et al., 2017). However, a high degree of diet segregation among fish species may occur regardless of the macrophyte patch studied (Pelicice & Agostinho, 2006).

In addition, the availability of food items varies according to the plant density and composition in macrophyte stands, which can influence fish diets. For example, the predominant use of algae by some fish species in sites with low-complexity habitat (e.g., open areas) may be related to the higher abundance of phytoplankton in these areas, while in habitats with high-complexity habitat provided by macrophytes, zooplankton can be the most consumed resource (Dias et al., 2022). At the same time, the complex macrophyte stands provide refuge for certain prey, resulting in lower foraging efficiency and higher interindividual variability in the diet planktivorous fish (Jeppesen et al., 1998; Quirino et al., 2021b; Dias et al., 2022). Omnivorous fish, in turn, can have their individual specialization reduced in stands with low

macrophyte biomass due to the high competition in these sites (Cardozo et al., 2021). Moreover, they can increase the consumption of plant material and expand their trophic niche breadths with increased macrophyte biomass (Aleixo et al., 2022). Therefore, variation of macrophyte density and diversity interfere with the ingestion of different food items and hence, affects differently fish species belonging to distinct trophic guilds.

Fish may respond differently to the vegetation density (Lopes et al., 2015) and the trophic variation is strongly associated with species-specific responses, as it depends on their ability to partition the niche and to forage at different plant densities and diversities (Savino & Stein, 1982; Aleixo et al., 2022). However, in this study, we aimed to understand the general trophic responses of fish to changes in macrophyte community attributes. We hypothesized that variations in the density and diversity of aquatic macrophytes could affect different food items, and consequently affect each trophic guild differently. Specifically, we predicted that (1) plant consumption by fish increases with macrophyte density and decrease with macrophyte diversity, while the consumption of invertebrates has an inverse relationship (Fig. 1). In addition, we expected that the foraging efficiency, trophic niche breadth, and overlap among fish species will be greatest (2) at the intermediate levels of macrophyte density (resulting in a hump-shaped relationship – Fig. 2a) and (3) at the high levels of macrophyte diversity (resulting in a linear relationship – Fig. 2b). The first prediction is based on the greater availability of plants expected for denser macrophyte stands, while higher invertebrate density is expected in more diverse macrophyte stands (Choi et al. 2015). The second prediction is expected because although very densely structured macrophytes provide more food resources, they also lead to low foraging rates since the physical barriers can reduce mobility and visibility, making it difficult to find and handle prey (Crowder & Cooper, 1982; Warfe & Barmuta, 2006). The last prediction is based on the fact that invertebrate diversity increases with macrophyte diversity (Yofukuji et al., 2021), leading to the availability of more types of food resources, allowing the increase in the niche breadth and, consequently, greater diet overlap among species (Pianka, 1976). Finally, despite these general trend expectations, we also expected that (4) the foraging efficiency and niche breadth of distinct trophic guilds may respond differently to the variation in macrophyte density and diversity. As the trophic niche is also mediated by species interactions, we also considered fish community attributes (density, richness, and diversity) influencing their feeding aspects. We tested these predictions using 30 macrophytes stands sampled in the subtropical Paraná River floodplain in Brazil, which has a diverse macrophyte and fish community.
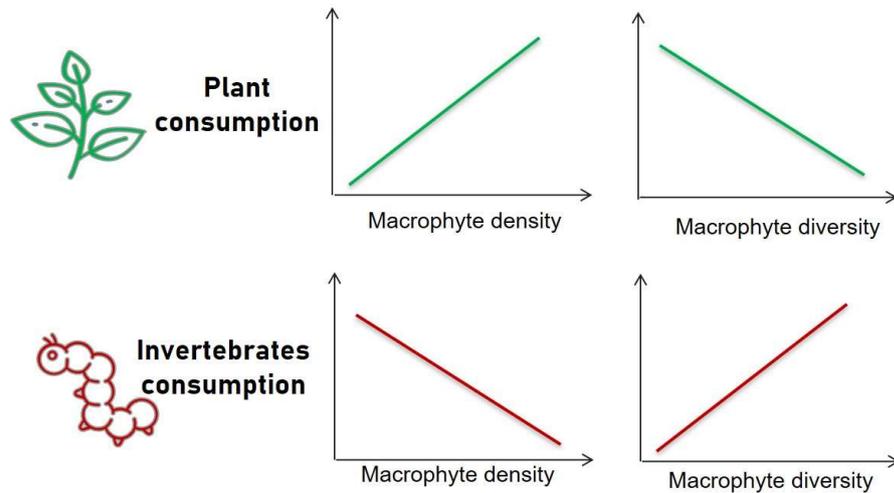
**Fig. 1.** Representation of prediction 1.
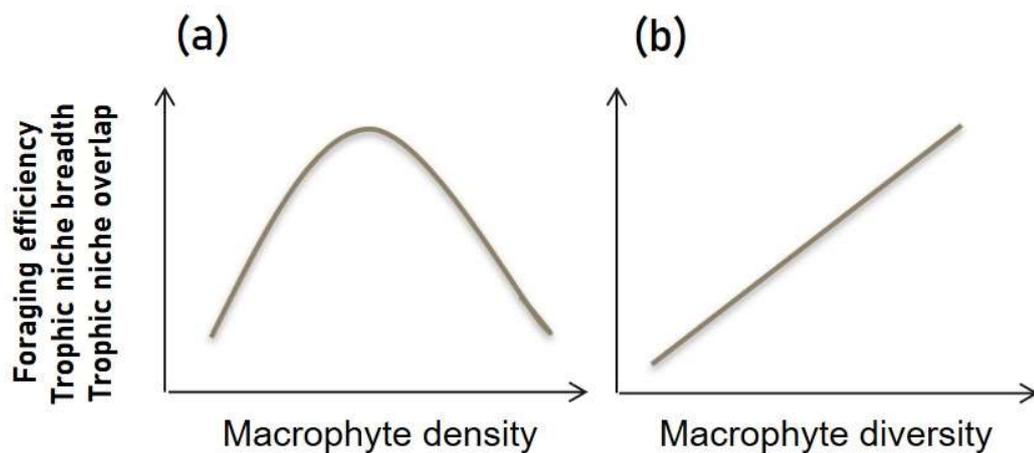


**Fig. 2.** Representation of predictions 2 (a) and 3 (b).

## 3.2 Methods

### 3.2.1 Study area

The study was conducted in the Upper Paraná River floodplain. The samplings were carried out in the Baía River (22º 43'23" S, 53º17'25" W – Fig. 3), a ~70 km long tributary that runs parallel to the Paraná River. This river has an average depth of 3.2 m and a low flow speed, ranging from 0.11 to 0.55 m s$^{-1}$ (Carvalho et al., 2013), which allows the establishment of large stands of aquatic macrophyte (Table S1), supporting a high richness of fish species (Table S2).
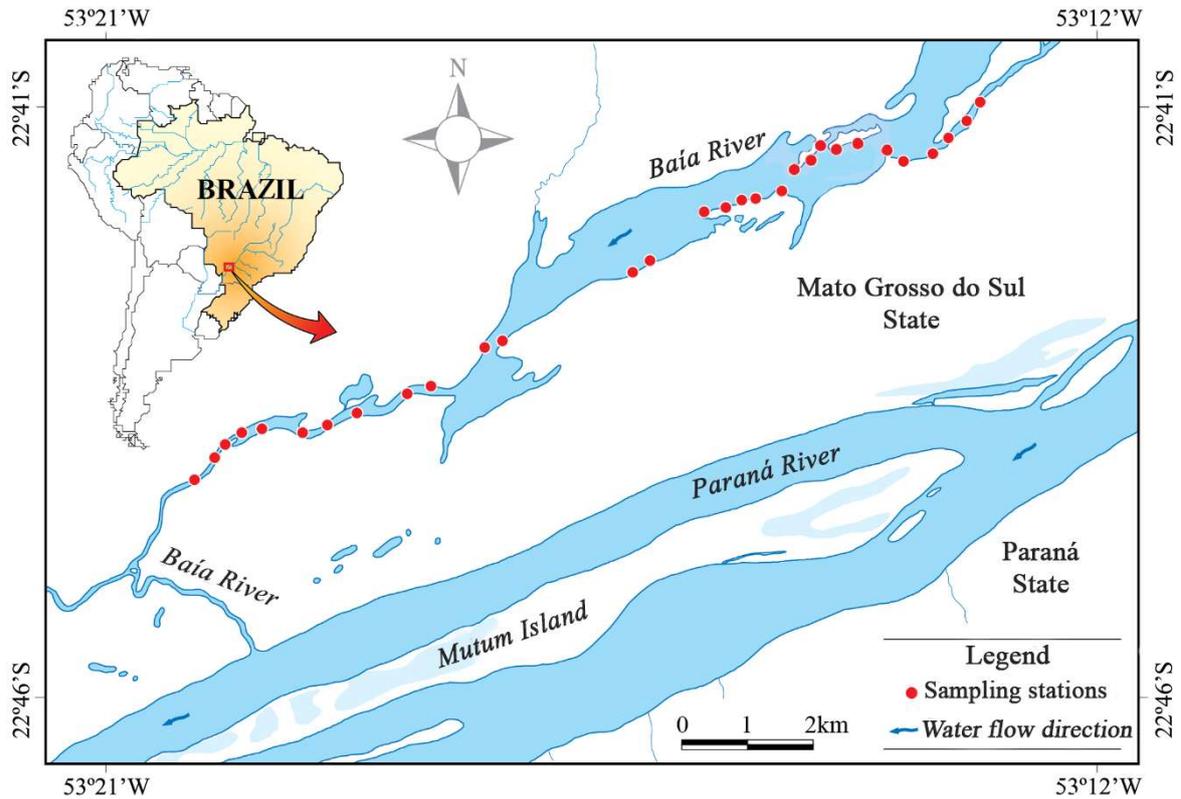
**Fig. 3** Map of the study area showing the sites (red points) where macrophyte stands were sampled in the Baía River (river-floodplain system of the Upper Paraná River, Brazil). EPSG: 4618.

3.2.2 Sampling

Fish and plants were sampled during the dry season (August 2018) in 30 macrophyte stands separated by at least 350 m and distributed over a 13.7 km long stretch of the littoral region of the Baía River (Fig. 3 – Quirino et al. 2021a). During low water periods, like the one during which we sampled, the floodplain habitats remain most dissimilar in terms of abiotic characteristics and communities (Thomaz et al., 2007), which tend to maximize fish interactions living in association with aquatic macrophyte (Gomes et al., 2012). Thus, great variability among habitats with a gradient of abiotic and biotic characteristics was found during this period, a propitious condition to test our hypotheses. We sampled macrophyte stands with different levels of plant density and diversity (Table S3), and these were assessed visually in the field observations and later confirmed with dry weight and exponential of Shannon index. Some stretches did not have macrophyte stands or stands with suitable depth to install the fish traps, explaining the great distances between some of the sampling points (Fig. 3).

Fish were collected by plexiglass traps (0.3m×0.3m×0.3m; Dibble & Pelicice, 2010; Cunha et al., 2019). In each macrophyte stand, we placed three traps inside the stands (~4 m from the border) to minimize the possibility of capturing pelagic species. We placed the traps at 7 a.m., and they were checked for fish and emptied every 8 hours for a total of 24 hours. The captured fish were anesthetized with clove oil according to ethical practices (Animal Use Ethics Committee of the State University of Maringá (CEUA/ UEM) - protocol number 5980040618) and fixed in 4% formaldehyde. Subsequently, we identified them to the species level (Ota et al., 2018 – Table S2). Fish density in each macrophyte stand was expressed as the number of individuals/3 traps/24h. Fish diversity was calculated using the exponential of Shannon Wiener Diversity Index $(\text{Exp}(\text{H}')) = \exp\left(-\sum_{i=1}^{n} pi \ \ln pi\right)$, in which $pi$ is the proportion of fish species $i$ and $n$ is the number of fish species found in each macrophyte stand. The exponential of Shannon indicates the effective number of species, providing a greater gradient of diversity values compared to the Shannon index (Jost, 2007). Fish richness (S) was considered as the total number of species per macrophyte stand.

After the last inspection of the traps, we sampled the macrophytes using one square of 0.5 m × 0.5 m per macrophyte stand, and all plant material to a depth of 0.5 m was removed, totaling a volume of $0.125m^3$. The macrophytes were separated, identified to species level (Lorenzi, 2000; Pott & Pott, 2000 – Table S1), washed, and dried out in an oven at 60°C until a constant dry weight (DW) to obtain the biomass of each species. Since we estimated the water volume, we pooled the biomass of all species and expressed it in grams of dry weight/$m^3$ (gDW/$m^3$) to estimate the macrophyte density in each stand. To calculate the macrophyte diversity in each stand, we used the exponential of Shannon-Wiener index (Exp (H'); Jost, 2007), considering the biomass of each species to evaluate the relative abundance values.

In the laboratory, we weighed, measured, and gutted the fish. After fish evisceration, the stomachs were visually assessed for the degree of stomach fullness and assigned to the categories: 0 = empty stomach; 1 = 1-25% of stomach volume occupied by food; 2 = 25-75%; and 3 = 75-100%. We analyzed the stomach content in stereoscopic and optical microscopes and identified the food items to the lowest possible taxonomic level (McCafferty, 1983; Elmoor-Loureiro, 1997). After identification, the 61 food items were quantified using a volumetric method (Hyslop, 1980), with a gridded dish in which the volume of the items was obtained in $mm^3$. These data were used for the later calculation of the niche breadth and overlap. Subsequently, food items were grouped into five categories: higher plants (macrophyte leaves and seeds), algae (Zygnematophyceae, Oedogoniophyceae, Bacillariophyceae, Cyanophyceae, and Rhodophyceae), insects (terrestrial and aquatic, totaling 29 taxa), other invertebrates

(including 20 taxa), and detritus-sediment, which were used to verify whether the consumption of these resources is associated with the macrophyte and fish community attributes (predictor variables).

According to the food categories, fish species were classified into four trophic guilds: herbivores (>60% of diet volume composed of higher plants and algae, totalizing three species), insectivores (>60% of aquatic or terrestrial insects, totalizing seven species), invertivores (>60% of other invertebrates than insects, totalizing 2 species), and omnivores (no food category over 60% of diet volume, totalizing 4 species) (Table S4).

### 3.2.3 Trophic variables

The foraging efficiency of each species in each macrophyte stand was assessed by the degree of stomach fullness (DF), expressed by the following equation: $DF = (N0*0) + (N1*1) + (N2*2) + (N3*3)/N$, where N0, N1, N2, and N3 correspond to the number of individuals with stomach fullness of 0, 1, 2, and 3, respectively, and $N$ is the total number of captured individuals of that species in a given macrophyte stand (Carniatto et al., 2020). To calculate the foraging efficiency, we used all the individuals captured, totaling 4,648 of 25 fish species. The consumption of each food category (higher plants, algae, insects, other invertebrates and detritus-sediment) was evaluated by calculating the volume percentage of each food category to each individual per macrophyte stand to prevent interference from the size or degree of stomach fullness. For this purpose, we used only the stomachs with food content, excluding empty ones, which totalized 1813 stomachs of 18 fish species.

The trophic niche breadth was evaluated through the mean distance from the centroid (DC) for each species in each macrophyte stand in a multidimensional space. For the calculation of DC, a PCoA was performed from a data matrix of the volume of food items (columns) by individuals (rows) using the Bray-Curtis distance. Then, the mean distances of individuals in relation to the centroid of their populations were calculated for each species in each macrophyte stand. Greater DC indicates that the diets of the individuals are more dissimilar and have a broader niche (Correa & Winemiller, 2014). The trophic niche overlap was calculated for each pair of species in each macrophyte stand using the Pianka's index (O): $O_{jk} = O_{kj} = \sum_i^n Pij - Pik / \sqrt{\sum_i^n Pij^2 \sum_i^n Pik^2}$, where $Pij$ and $Pik$ are the proportions (volume) of the $i^{th}$ food item used by $j^{th}$ and $k^{th}$ species. This index varies from 0 (no resource overlap) to 1 (complete overlap in resource use) (Pianka, 1974). For these analyses, we considered only those

species with at least two individuals with stomach contents per macrophyte stand and up to a maximum of 30 stomachs, resulting in a total of 1506 stomachs of the 12 fish species analyzed (Table S5).

Since the niche is a population metric and to reduce the influence of species composition in macrophyte stands, all the response variables were initially assessed by population (DF, food categories, and DC) or by population pairs (O) in each macrophyte stand. Subsequently, they were summarized into a mean (mean of each food category, mDF, mDC, and mO – Fig. 4). Hereby, we obtained a single value of each response variable per macrophyte stand, which allowed us to assess the general fish responses along the gradient of macrophyte density and diversity. However, herbivorous species (those that had consumed ≥60% of algae and higher plants) were excluded from the general mean of DC and O since their restricted diets presented an opposite trend, which eliminated the significant effect of the predictor variables. In addition, we also calculated a mean value of mDF and mDC to each trophic guild for each macrophyte stand to evaluate the patterns separately.
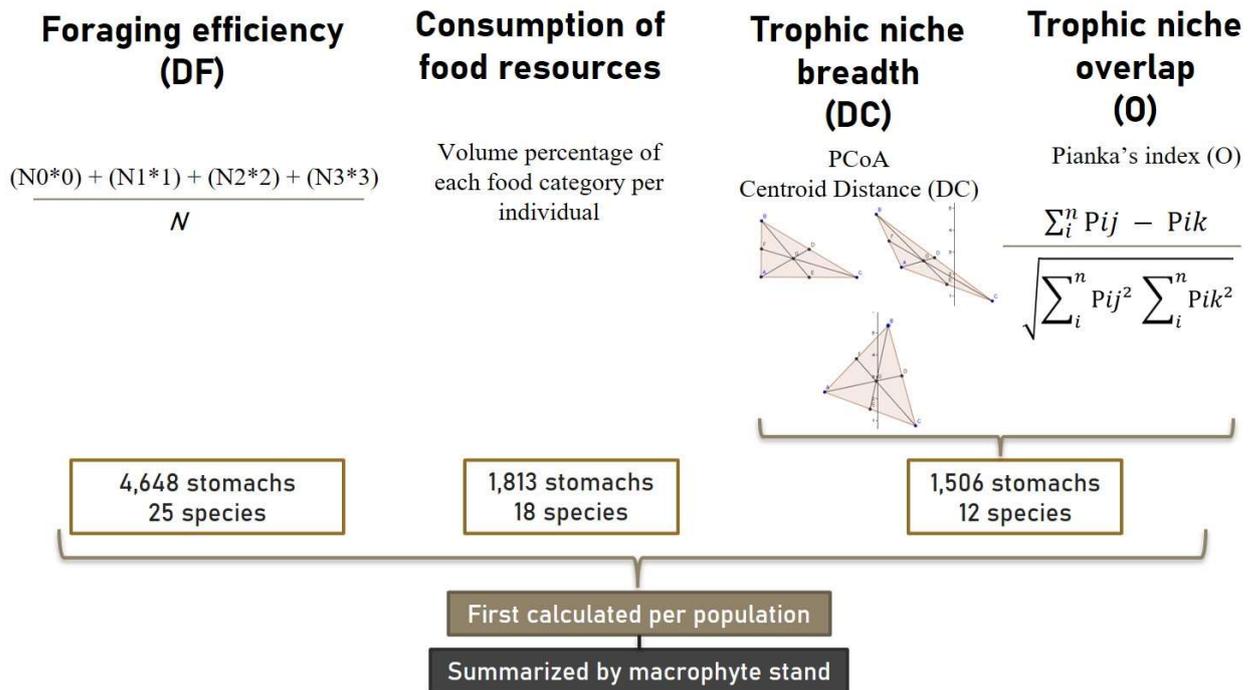


**Fig. 4.** A summary of the analytical approach.

3.2.4 Statistical analysis

To test the relationships between macrophyte attributes (density and diversity) and the trophic variables (mDF, mDC, mO and the ingestion of differente food items) we used polynomial

models from first to third order for macrophyte density. The third order term was used to assist lowering of the model's curve after the peak. Models with different polynomials were evaluated by likelihood ratio tests using the "lrtest" function of lmtest package (Zeileis & Hothorn, 2002). When no significant differences were found, we chose the model with the least number of polynomials (Table S6, S7, S8). Generalized Linear Models (GLMs) were performed to model the foraging efficiency (mDF) and the ingestion of different food items against macrophyte density and diversity as well as fish density, diversity, and richness using a Gaussian distribution with the "glm" function of the vegan package. Beta regressions (Ferrari & Cribari-Neto, 2004) were used to model trophic niche breadth (mDC) and overlap (mO) since these variables are continuous and constrained between 0 and 1, and they were performed using the function "betareg" of betareg package (Zeileis et al., 2022).

The models analyzing mDF and mDC responses were performed considering a general mean of all populations (except herbivores for mDC) and considering each trophic guild separately. To encompass the interspecific competition in all the models, which has a strong influence in the trophic niche (Hutchinson, 1978), we also used fish density, diversity, and richness as predictor variables besides macrophyte density and diversity. To model GLM, we checked and met the linearity, normality of residuals and homogeneity of variances. Possible spatial autocorrelation of response variables among macrophyte stands was assessed through Moran's I correlograms (Legendre & Legendre, 2012). According to this analysis, none of the response variables presented bias related to spatial autocorrelation structures. A significance level of p≤0.05 was used for all analyses, which were performed in the R environment (R Core Team, 2022).

## 3.3 Results

We recorded a total of 17 macrophyte species and 25 fish species during the sampling (Table S1). Considering the 30 macrophyte stands, plant density ranged from 366 to 7,066 g DW m$^{-3}$ (mean = 1,747.46 g DW m$^{-3}$), while plant diversity (exp(H')) ranged from 1 to 4.64 (mean = 2.16). Fish density ranged from 15 to 563 individuals per macrophyte stand (mean = 155 individuals) and fish richness ranged from 6 to 15 species (mean = 9 species), while fish diversity (exp(H')) ranged from 2.61 to 7.09 (mean = 4.61). The average total length of fish individuals captured along the 30 macrophyte stands varied between 29 mm and 42.4 mm (mean = 33.4 mm).

3.3.1 Foraging efficiency

Considering all individuals and species of fish captured, the mean of the stomach fullness degree (mDF) calculated for each macrophyte stand ranged from 0.60 to 1.99 (2.12±0.34). Considering each trophic guild separately, the averages of foraging efficiency were 1.61±0.65 for hebivores, 1.89±0.42 for insectivores, 1.55±0.35 for invertivores and 2.12±0.35 for omnivores. The relationship between foraging efficiency and macrophyte density was best described by a first order polynomial relationship (Table S5), explaining 32% (Nagelkerke $R^2$) of the variance. The generalized linear model showed that the amount of food consumed increased significantly with increasing macrophyte density, while the other predictor variables did not affect the foraging efficiency (Table 1; Fig. 5a). When considering mDF for trophic guilds separately, only the model for herbivores (Nagelkerke $R^2 = 0.34$) showed a significant (and positive) relationship with macrophyte density, with a range of mDF from 1 to 3 (Fig. 5b, Table 1). Omnivores also showed a positive, but not significant, trend (Nagelkerke $R^2 = 0.25$).

**Table 1** Results of generalized linear model for fish foraging efficiency assessed by the degree of stomach fullness (mDF) considering a general mean and a mean calculated for each trophic guild separately. Herb= herbivores; Omni= omnivores; Insect= insectivores; Invert= invertivores. * = statistically significant values.

| | General mean | | | | Trophic guilds | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | Herb | | Omni | | Insect | | Invert | |
| R² | | 0.32 | | | 0.35 | | 0.26 | | 0.16 | | 0.25 | |
| | Estimate | SE | t | *p* | t | p | t | p | t | p | t | p |
| (Intercept) | 0.68 | 0.31 | 2.20 | 0.04* | 0.21 | 0.83 | 4.57 | <0.01* | 3.31 | <0.01* | 2.43 | <0.01* |
| Macrophyte density | 0.00 | 0.00 | 3.17 | >0.01* | 2.13 | 0.04* | 1.42 | 0.16 | -0.90 | 0.37 | 0.53 | 0.66 |
| Macrophyte diversity | -0.04 | 0.05 | -0.67 | 0.51 | -0.13 | 0.90 | -0.73 | 0.33 | 0.22 | 0.83 | -0.09 | 0.99 |
| Fish density | -0.00 | 0.00 | -0.97 | 0.34 | -0.67 | 0.51 | 0.15 | 0.99 | 1.61 | 0.14 | 1.96 | 0.05 |
| Fish diversity | -0.01 | 0.07 | -0.24 | 0.81 | 0.86 | 0.40 | -1.03 | 0.29 | 1.05 | 0.40 | 1.86 | 0.07 |
| Fish richness | 0.05 | 0.05 | 0.94 | 0.35 | 0.56 | 0.58 | 1.31 | 0.17 | -1.00 | 0.38 | -1.54 | 0.11 |
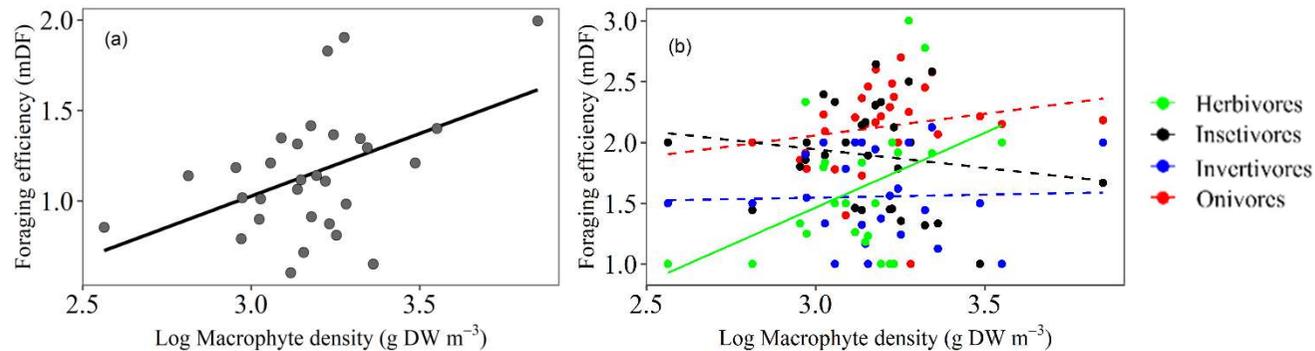


**Fig. 5** Fish foraging efficiency assessed by the degree of stomach fullness considering a general mean (a) and a mean calculated for each trophic guild separately (b) related to the only variable with a significant effect in the generalized linear model performed for the general mean and for at least some trophic guilds. Dashed lines mean non-significant models. Note that log scale was used for macrophyte density.

3.3.2 Food categories

Regarding food items ingested by the individuals along the macrophyte gradient, we found that plant density only influenced the consumption of higher plants, which increased with increasing macrophyte density (Table 2; Fig. 6). The consumption of higher plants, algae, and detritus was negatively and insect ingestion was positively correlated with macrophyte diversity. Fish richness was positively correlated with the consumption of higher plants and negatively with the consumption of other invertebrates (Table 2; Fig. 6).

**Table 2** Results of generalized linear models for the consumption of five food categories. * = statistically significant values.

| | Food items | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Higher plants** | | **Algae** | | **Insects** | | **Other invertebrates** | | **Detritus** | |
| $R^2$ | 0.43 | | 0.47 | | 0.29 | | 0.21 | | 0.42 | |
| | T | p | t | p | t | p | t | p | t | p |
| (Intercept) | 10.8 | <0.01* | 7.76 | <0.01* | 17.3 | <0.01* | 10.20 | <0.01* | 3.33 | <0.01* |
| Macrophyte density | 2.01 | 0.05* | -1.70 | 0.10 | -0.33 | 0.74 | -0.61 | 0.55 | 0.78 | 0.44 |
| Macrophyte diversity | -3.41 | <0.01* | -2.31 | 0.03* | 2.61 | <0.01* | 0.80 | 0.43 | -2.21 | 0.04* |
| Fish density | -1.72 | 0.10 | 0.76 | 0.45 | -0.32 | 0.75 | 1.76 | 0.10 | -0.02 | 0.98 |
| Fish diversity | 0.21 | 0.84 | -0.09 | 0.93 | -0.82 | 0.42 | 1.55 | 0.13 | -2.51 | 0.02* |
| Fish richness | 2.13 | 0.04* | 1.03 | 0.31 | -0.35 | 0.73 | -2.41 | 0.02* | 1.82 | 0.08 |
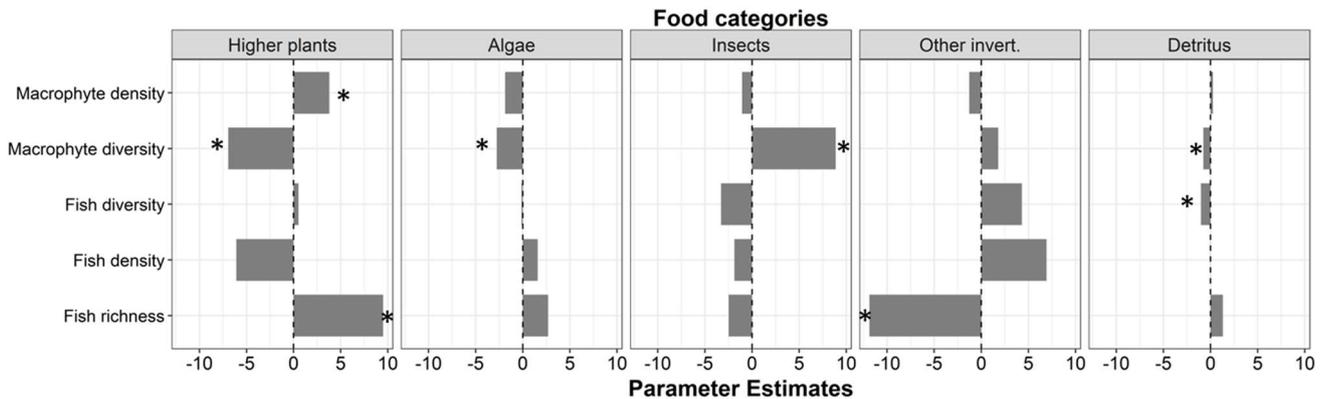


**Fig. 6** Parameter estimates of generalized linear models for the consumption of five food categories (higher plants, algae, insects, other invertebrates, and detritus) in relation to macrophyte and fish attributes. The dashed line represents the confidence interval. * = statistically significant values.

### 3.3.3 Trophic niche breadth

Trophic niche breadth, assessed by the mean distance from the centroid (mDC), ranged from 0.24 to 0.57 along the macrophyte stand sampled. The beta-regression model also revealed a moderate explained variance (Pseudo-$R^2$ = 0.30) with a first order polynomial relationship (Table S6), and the trophic niche breadth was negatively associated with macrophyte density but positively with fish diversity (Table 2; Fig. 7). When considering the mean trophic niche breadth for the guilds separately, omnivores (Pseudo-$R^2$ = 0.23) and herbivores (Pseudo-$R^2$ = 0.80) were the only guilds that showed significant relationships in the performed models, and they generally showed opposite trends. However, the only significant variables for herbivores were macrophyte diversity (positive relationship) and fish diversity (negative relationship), while for omnivores, macrophyte density had a negative relationship, in addition to fish diversity that was positively related to their trophic niche breadth (Fig. 7, Table S6).

**Table 2** Results of beta regression analysis for the trophic niche breadth assessed by the mean distance from the centroid (mDC) considering a general mean (except hebivores) and a mean calculated for each trophic guild separately. Herb= herbivores; Omni= omnivores; Insect= insectivores; Invert= invertivores. * = statistically significant values.

| | General mean | | | | Trophic guilds | | | | | | | |
| | | | | | Herb | | Omni | | Insect | | Invert | |
| | Estimate | SE | Z | p | z | p | z | p | Z | P | z | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Intercept) | -0.31 | 0.28 | -1.34 | 0.18 | 0.08 | 0.93 | -0.49 | 0.62 | -2.58 | 0.01* | -0.97 | 0.33 |
| Macrophyte density | -0.00 | 0.00 | -2.27 | 0.02* | -0.03 | 0.97 | -2.90 | <0.01* | -0.49 | 0.62 | -0.35 | 0.35 |
| Macrophyte diversity | 0.00 | 0.02 | 0.09 | 0.93 | 2.22 | 0.03* | 0.70 | 0.48 | 0.53 | 0.59 | -1.04 | 0.30 |
| Fish density | 0.00 | 0.00 | 0.68 | 0.50 | -1.63 | 0.10 | 1.74 | 0.08 | -0.51 | 0.61 | -1.40 | 0.16 |
| Fish diversity | 0.10 | 0.05 | 1.92 | 0.05* | -2.60 | 0.01* | 2.27 | <0.02* | 0.56 | 0.58 | -0.51 | 0.61 |
| Fish richness | -0.04 | 0.04 | -0.88 | 0.38 | -0.89 | 0.37 | -1.92 | 0.06 | 0.62 | 0.53 | 1.21 | 0.22 |
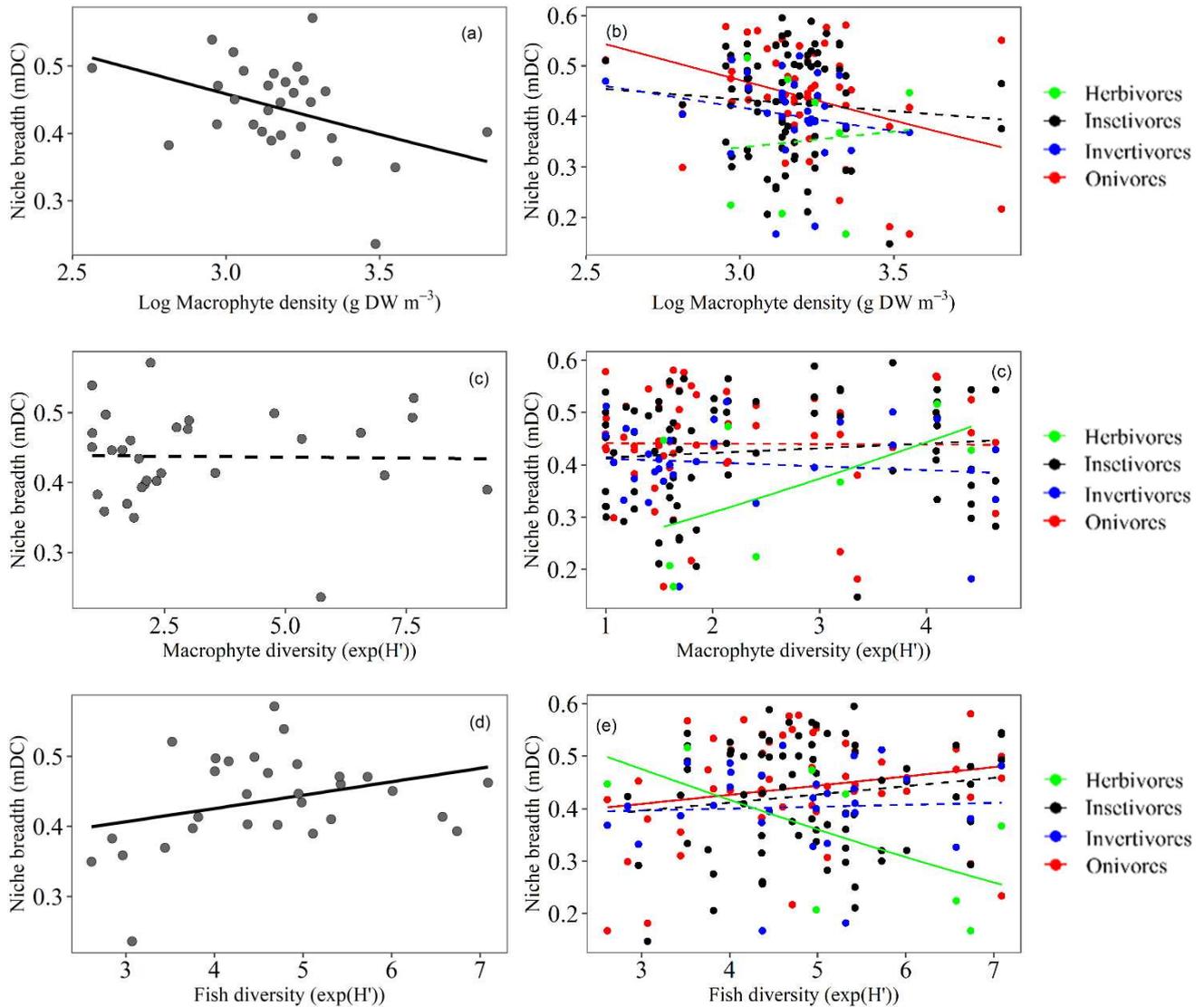
**Fig. 7** Trophic niche breadth, assessed by the mean of the distance from the centroid (mDC) considering a general mean - except hebivores (**a, c**) and a mean calculated for each trophic guild separately (**b, d, e**), related to the variables with a significant effect for at least some trophic guilds or for the general mean in the performed beta regression. Dashed lines mean non-significant models. Note that log scale was used for macrophyte density.

### 3.3.4 Trophic niche overlap

The mean value of the Pianka index (mO) ranged from 0.13 to 0.68. The beta regression showed that the trophic niche overlap was significantly related to macrophyte density (with a second order polynomial relationship; Table S7), fish density, and fish diversity, and the model explained approximately 40.3% of the variation (Pseudo-$R^2$ = 0.403; Table 3; Fig. 8). The trophic niche overlap increased slightly with macrophyte density, while it decreased with fish density and diversity.

**Table 3.** Results of beta regression analysis for trophic niche breadth, assessed by the Pianka's Index (mO) considering a general mean (except hebivores). * = statistically significant values.

|  | Estimate | SE | Z | P |
|---|---|---|---|---|
| (Intercept) | 0.48 | 0.62 | 0.77 | 0.43 |
| Macrophyte density | 0.97 | 0.73 | 1.34 | 0.18 |
| Macrophyte density$^2$ | -1.73 | 0.72 | -2.39 | 0.02* |
| Macrophyte diversity | -0.04 | 0.13 | -0.30 | 0.77 |
| Fish density | -0.00 | 0.00 | -2.26 | 0.02* |
| Fish diversity | -0.45 | 0.16 | -2.80 | <0.01* |
| Fish richness | 0.19 | 0.12 | 1.61 | 0.11 |
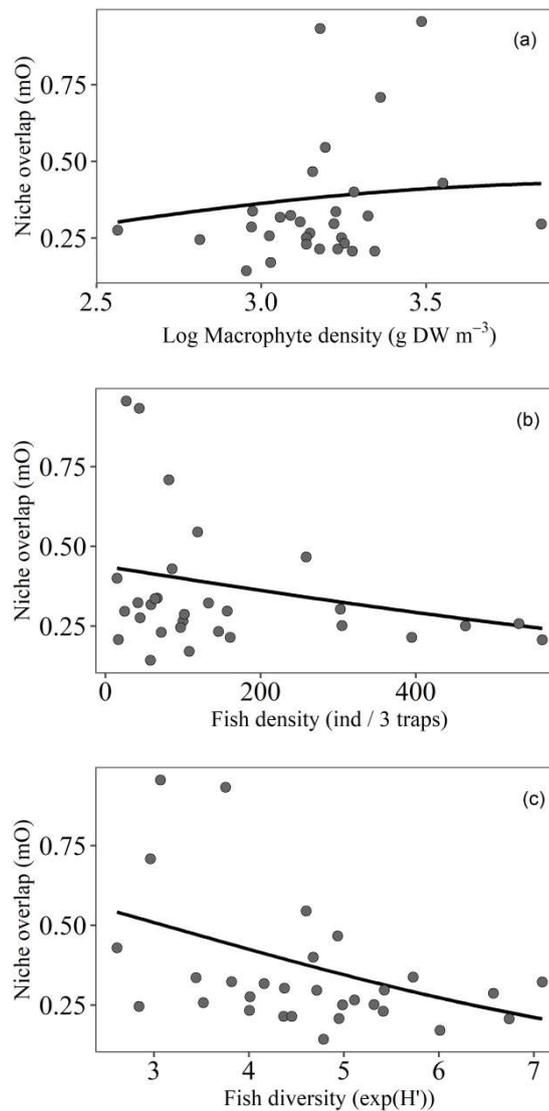


**Fig. 8** Trophic niche overlap assessed by the mean of Pianka's Index (mO) considering a general mean (except hebivores) related to macrophyte density (**a**), fish diversity (**b**), and fish density (**c**), the variables with a significant effect in the performed beta regression performed. Note that log scale was used for macrophyte density.

**3.4 Discussion**

Macrophyte density, widely known to provide habitat complexity and influence predator-prey interactions (Jeppesen et al., 1998; Thomaz & Cunha, 2010), was related to fish foraging efficiency, trophic niche breadth and overlap, as well as plant consumption by fish in the littoral region of Baía River, Brazil. In addition to density, the diversity of macrophytes present in the stands showed significant relationships with the trophic niche breadth of herbivores and the consumption of algae and detritus by fish in general. The fish community attributes, used here as a proxy for competition, were also related to the consumption of certain food resources as well as to the trophic niche breadth and overlap. Notably, the trophic niche breadth of omnivores and herbivores generally showed opposite relationships with the attributes of macrophytes and fish.

Numerous studies have demonstrated that fish foraging efficiency declines as macrophyte density increases because dense vegetation may restrict the access to prey by decreasing the visibility and swimming speed of fish (Crowder & Cooper, 1982; Savino & Stein, 1982; Sammons & Maceina, 2006; Liversage et al., 2017). However, we found an opposite response as also observed in another field study (Middaugh et al., 2013) and an experiment (Nohner et al., 2018) where higher prey consumption by fish was revealed in habitats with high macrophyte coverage than in low-vegetation habitats. This may reflect the possibility that high density of available prey may overwhelm the negative effects of a structurally dense vegetation (Middaugh et al., 2013). However, some studies also have shown no effect of vegetation density on feeding activity for some fish species (Olson et al., 2003; Pelicice & Agostinho, 2006). The general positive relationship in the fish foraging in our study was especially due to the response of herbivorous fish, since when the guilds were analyzed separately, the herbivores were the only ones that showed a significant relationship between foraging efficiency and macrophyte density. As herbivorous fish can directly graze on the rhizomes, shoots, and stems of submerged plants (Wang et al., 2020), the increase in macrophyte density, even if excessive, favors their foraging because it increases the availability of resources and does not demand efficient maneuverability and performance, attributes required to exploit resources in structurally complex habitats (Prado et al., 2016).

However, the efficiency foraging of omnivorous fish also showed a positive, although not significant, trend with macrophyte density, which is probably also associated to their ability to feed on plants. Although macrophytes provide protection to prey by reducing the visual contact between them and their predators (Dibble, 1996), fish preferences can change, and they can

switch to alternate food sources according to prey availability (Correa & Winemiller, 2014). In general, macrophytes and associated algae may constitute a vital food resource for omnivorous fish (Rao et al., 2015; Gao et al., 2017), and constitute most of their diet in high-macrophyte biomass stands because of the extreme availability of those resources (Aleixo et al., 2022). In fact, in our study, the plant consumption by fish, regardless of trophic guilds, increased significantly along the macrophyte density gradient, suggesting a prevalence of herbivory in the denser macrophyte stands. When there is an energy limitation, omnivores are able to enhance feeding on low trophic position food items (e.g., vegetal material) (Beisner et al., 1997; Arim et al., 2007). Also, according to the Optimal Foraging Theory (MacArthur & Pianka, 1966), predators search for the most profitable prey, considering the balance between energetic gains and metabolic costs. Thus, plant resources may become a more profitable food for fish species in denser macrophyte stands due to their high availability and lack of mobility. However, we might have failed to detect a negative relationship between fish foraging and macrophyte density because we did not sample extremely dense stands as such were not present, likely because floodplain environments are naturally subject to regulation by droughts and floods, which prevents exacerbated proliferation of macrophytes (Pedro et al., 2006).

Despite the higher foraging efficiency, the trophic niche breadth of fish - evaluated by the individual variability - responded negatively to the increase in plant density. Vejříková et al. (2017) and Eloranta et al. (2017) also verified a lower individual variation and narrow trophic niches in the presence of macrophyte and consequently high habitat complexity. This may be associated with the fact that complex habitats can represent a physical barrier to foraging, offering refuge that prevents predator-prey encounters (Priyadarshana et al., 2001; Thomaz & Cunha, 2010), and, thereby, reducing the accessibility to prey. The lower availability of food resources - in this case due to lower accessibility - generally leads fish to consume a smaller variety of food items (Quirino et al., 2017) that are easier to prey on under these conditions (e.g., plants), which does not necessarily imply a smaller amount of food ingested. Then, trophic niche breadth may be higher in habitats with low macrophyte density – not because of food availability (which is reduced), but presumably because they do not protect prey against predation as highly structurally macrophyte stands do (Dibble, 1996; Padial et al., 2009; Choi et al., 2016). The negative response of trophic niche breadth to the increase in macrophyte density was especially clear for omnivorous fish, while the other guilds did not show significant patterns. On the other hand, it has already been verified that the difficulty in accessing specific prey in high biomass stands can lead zooplanktivorous fish to add alternative resources to their

diet, consequently increasing inter-individual variability (Quirino et al., 2021b). Different patterns in niche breadth responses, including null patterns, demonstrated for each trophic guild of fish suggest that they may respond differently to variations in macrophyte density despite the general trend.

Macrophyte diversity, in turn, was positively related to the trophic niche breadth of herbivorous fish. High diversity of plants means different architectures, growth forms, and physiology and, therefore, different types of microhabitats. This leads to various ecological niches, including the liberation of allelochemicals, food sources, and shelter, which supports dense and diverse biotic communities (Habib & Yousuf, 2015; Yofukuji et al., 2021). The diverse structural habitat provided by different architectures can potentially contribute to differences in fish diets (Dibble & Harrel, 1997; Yofukuji et al., 2021). A more diverse macrophyte community, with submerged and floating macrophytes, may facilitate coexistence between plant-associated macroinvertebrates and fish (Clemente et al., 2019). Choi et al. (2016) verified that mixed vegetation zones (containing five macrophyte species) presented higher zooplankton density than zones with less macrophyte species. Yofukuji et al. (2021) observed that an omnivorous fish increased the consumption of invertebrates and decreased the consumption of aquatic plants as macrophyte diversity increased. In our study, there was a significant decline in algae and detritus consumption by fish with the increasing plant diversity, which suggests that in stands with high macrophyte diversity, which offer higher availability of food resources (Choi et al., 2016), fish can replace basal resources by new ones.

Fish diversity was also related to the trophic niche breadth of herbivorous and omnivorous fish with opposite relationships being observed: a positive relationship with herbivores and a negative relationship with omnivores. Moreover, there was an increase in the consumption of higher plants and a decrease in the consumption of invertebrates (except insects) with increasing fish diversity. These observations suggest that fish may consume more low-energy food, such as plants, than invertebrates in a situation of high competition that causes a shortage of animal food (Persson, 1983; Brabrand, 1985). In fact, different fish species prey on different macroinvertebrates, and a high biomass of invertivorous fish can significantly reduce the density of macroinvertebrate prey (Lovell et al., 2017), which may have contributed to the diet shift to plant resources. It is noteworthy that in our study the trophic niche breadth of populations was evaluated according to the variability among individuals, and in situations of greater competition, fish may try to avoid it by reducing among-individual variation and thereby the population niche breadth (Bolnick et al., 2010). However, according to the foraging theory,

organisms can either increase or decrease individual specialization (Araújo et al., 2011). Thus, fish can also increase their diet with alternative items by enhancing diet variation among individuals when competition for resources is high and prey availability is reduced (Svanbäck & Bolnick, 2007; Xia et al., 2020), which may have been the strategy of the herbivores; when facing increased competition for plant resources, they may have added alternative prey, which in turn led to increasing among-individual variation. Thus, trophic niche breadth adjustments become important for the coexistence of different fish species (Corrêa et al., 2011), and each trophic guild can adjust the width by expanding or narrowing it. In fact, trophic niche overlap was also altered according to fish density and diversity in our study, responding positively to these attributes. In this case, when the fish experienced intense competition (high fish density and diversity), regardless of whether they reduced or increased the niche breadth, the general tendency was for the niches to overlap. This result is probably associated with the intense predation by fish that led to limited food resources via top-down regulation (Sinistro, 2010).

Regarding the macrophyte density gradient evaluated in this study, the trophic niche overlap for fish in general increased slightly to an intermediate point of plant density and then declined. This, together with the pattern observed for niche breadth, suggests that while fish reduce niche breadth along the gradient of macrophyte density (except for herbivores), niche overlap among species increases, but this occurs only to a certain extent of plant density when species start to partition the niche more strongly. The greater niche overlap observed in the macrophyte stands with intermediate levels of macrophyte density is justified by the high availability of resources compared to stands with little vegetation and the low restriction of swimming movements compared to denser stands (Thomaz & Cunha, 2010; Kovalenko et al., 2012; Cardozo et al., 2021). When available resources are abundant, trophic niche overlap can be high, but it does not necessarily imply intense competition or it means that the strong competition has not yet led to segregation in resource use (Corrêa et al., 2011). However, as access to prey becomes more restricted in high-density stands, fish guilds can feed on the resources they are most suited to since each species has a foraging ability among macrophyte structures (Prado et al., 2016), leading to reduced niche overlap. A fish that can easily swim through the macrophyte structures is able to forage more efficiently than other fish, thereby segregating and partitioning the habitats (Priyadarshana et al., 2001). In fact, fish may segregate the niche more strongly and, consequently, reduce competition for resources in lakes with abundant macrophytes compared to macrophyte-poor lakes (Eloranta et al., 2017; Vejříková et al., 2017); thus, habitat complexity can fundamentally affect interactions among fish.

Our study gives more insight into how macrophyte density and diversity, as well as fish density and diversity, can strongly influence the use of food resources by fish and hence their trophic niche breadth and segregation. The consumption of higher plants by fish and their foraging efficiency were positively related to macrophyte density, while niche breadth responded negatively. Niche overlap of fish showed an increase at the intermediate levels of macrophyte density. Fish diversity negatively influenced the consumption of higher plants and some invertebrates, while fish diversity and density were positively related to the trophic niche overlap of fish. In addition, our results indicate that the response of the trophic aspects of fish depends on their feeding habits because, despite the general results, we observed different responses (opposite or null) according to the guild, such as, for example, the niche breadth of herbivores that responded oppositely to the breadth of omnivores. Thus, macrophyte vegetation increases the overall physical complexity and improves the availability of resources and, at the same time, reduces the prey encounter rates, favoring herbivory under high plant density conditions. Then, opportunities in specific feeding habitats and the associated vegetation density influence the use of the habitats by fish. However, trophic relationships create complex and unpredictable effects, and despite the general trends observed, other factors such as intra- and interspecific competition and predation risk must be taken into account in studies of the influence of macrophytes on the trophic aspects of the fish communities.

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# 4 SUBMERGED MACROPHYTES AS POTENTIAL DRIVERS OF FISH COMMUNITIES IN 88 TEMPERATE SHALLOW LAKES

## ABSTRACT

1. Fish community structuring is fundamentally affected by a range of lake characteristics, including macrophyte coverage, as these plants provide spawning substrate, nursery area, refuge against predators and food.

2. We assessed the fish-macrophyte relationship at both lake and point scale using an extensive dataset from 88 Danish shallow lakes (maximum depth $\leq 4.5$ m). At the lake scale, we used mean values of fish and macrophyte community samples for each lake, totaling 88 samples. The data from the point scale refers to multiple sampling points in each lake (totaling 595 samples) of both macrophytes and fish communities, which also allowed us to assess the variation within lakes in each lake.

3. We found a general negative relationship between macrophyte coverage and fish abundance and biomass, being stronger at the point scale, whereas the effect was attenuated at the whole lake scale. Contrary to macrophytes, chlorophyll $a$ level showed a positive relationship with fish abundance - except for perch and all fish <10 cm. The deeper and more eutrophic the lakes were, the more fish tended to occupy points covered by macrophytes. Perch generally showed patterns opposed to the other evaluated species such as roach and bream.

4. Our results suggest that fish abundance and biomass respond to a combination of factors that are often intercorrelated and difficult to isolate. Therefore, the response of fish abundance and biomass to macrophyte coverage may be dependent, among other factors, on the fish species, fish size, and study lake.

**Keywords:** fish abundance, fish biomass, habitat complexity, aquatic plants, macrophyte coverage, chlorophyll, water depth

# MACRÓFITAS SUBMERSAS COMO POTENCIAIS PREDITORAS DA COMUNIDADES DE PEIXES EM 88 LAGOS RASOS TEMPERADOS

## RESUMO

1. A estruturação da comunidade de peixes é fundamentalmente afetada por um conjunto de características do lago, incluindo a cobertura de macrófitas, pois essas plantas fornecem substrato para desova, área de berçário, refúgio contra predadores e alimento.

2. Foi avaliada a relação peixe-macrófita nas escalas de lago e de ponto usando um extenso conjunto de dados de 88 lagos rasos dinamarqueses (profundidade máxima ≤ 4,5 m). Na escala de lago, foram utilizados valores médios de amostras de peixes e macrófitas para cada lago, totalizando 88 amostras. Os dados da escala de pontos referem-se aos múltiplos pontos de amostragem em cada lago (totalizando 595 amostras) tanto de macrófitas quanto de comunidades de peixes, o que também permitiu avaliar a variação dentro dos lagos em cada lago.

3. Foi encontrada uma relação negativa geral entre a cobertura de macrófitas e a abundância e biomassa de peixes, sendo mais forte na escala de ponto, enquanto o efeito foi atenuado na escala de lago. Ao contrário das macrófitas, o nível de clorofila *a* mostrou uma relação positiva com a abundância de peixes - exceto para perca e todos os peixes <10 cm. Quanto mais profundos e eutróficos eram os lagos, mais os peixes tenderam a ocupar pontos cobertos por macrófitas. A perca geralmente apresentou padrões opostos às outras espécies avaliadas, como roach e bream.

4. Os resultados sugerem que a abundância e a biomassa de peixes respondem a uma combinação de fatores que muitas vezes são intercorrelacionados e difíceis de isolar. Portanto, a resposta da abundância e biomassa dos peixes à cobertura de macrófitas pode ser dependente, entre outros fatores, da espécie de peixe, tamanho do peixe e lago de estudo.

**Palavras-chave:** abundância de peixes, biomassa de peixes, complexidade de habitat, plantas aquáticas, cobertura de macrófitas, clorofila, profundidade da água

## 4.1 Introduction

Submerged macrophytes are essential structuring elements in aquatic ecosystems and are considered by the European Water Framework Directive (WFD) as one of the four key biological elements used to describe ecological quality in lakes (Søndergaard *et al.*, 2010). They affect the physical and chemical properties of lakes, such as temperature, dissolved oxygen, pH and nutrients (Jeppesen *et al.*, 1998; Song *et al.*, 2019). Particularly in shallow lakes, macrophytes play a critical role by stabilizing the sediment, assimilating nutrients and modifying the trophic structure, all contributing to maintain a clear water state and good water quality (Jeppesen *et al.*, 1998; Thomaz, 2021). Submerged macrophytes may also affect the spatial distribution of organisms as they provide spawning substrate, nursery area, refuge against predators and food (Dibble, Killgore & Harrel, 1996; Slade, Dibble & Smiley, 2005; Quirino *et al.*, 2021b).

Fish may feed on submerged macrophytes (Zapletal *et al.*, 2016; Yu *et al.*, 2021) and on organic detritus, periphyton and aquatic invertebrates attached or associated with the leaves and stems (Pelicice & Agostinho, 2006; Carniatto *et al.*, 2020; Cardozo *et al.*, 2021). Variations in macrophyte structural complexity affect the strength of the fish-prey interactions, including piscivory (Meerhoff *et al.*, 2003, 2007). Consequently, the coverage and density of submerged macrophytes play an essential role for the structure and distribution of fish communities (Dibble & Pelicice, 2010; Carniatto *et al.*, 2013).

Vegetated areas usually harbor higher fish abundance and diversity when compared to environments free of vegetation, and this is particularly true for juveniles and small-sized fish (Agostinho *et al.*, 2007). However, submerged macrophytes create environmental gradients in a range from sparse to dense patches, and fish densities are generally higher in areas with intermediate levels of density (Dibble *et al.* 1996; Cunha *et al.* 2019), while excessive macrophyte density can cause physical and chemical barriers for them reducing the foraging efficiency (Miranda & Hodges, 2000). Therefore, high plant coverage may reduce the abundance and growth of particular fish categories, like larger piscivores and benthic omnivores, because they need free-habitats to move and feed efficiently (Engel, 1987; Persson *et al.*, 1992; Sammons & Maceina, 2006). The different fish species among macrophytes have different plant density preferences, according to their adaptations and tolerances (Lopes *et al.*, 2015). For example, species with elongated body shape can camouflage within plants (Kimura *et al.*, 2021) while other species tolerate low oxygen concentrations (Bulla *et al.*, 2011), making

these two groups more abundant in high dense stands of macrophytes. Thus, the correlations between macrophyte abundance and fish community attributes may be species-dependent and not idiosyncratic.

Fish-macrophyte interactions may also vary with the spatial scale, because plants are considered 'quasi-multifractal objects', encompassing different degrees of complexity at different scales, from patches of plants to single natural macrophyte leaves (Thomaz & Cunha, 2010; Kovalenko, Thomaz & Warfe, 2012). At the lake scale, which considers the whole water body, studies usually approach general patterns on fish-macrophyte relationship, such as fish abundance, composition, distribution and reproduction (Maceina *et al.*, 1991; Cheruvelil *et al.*, 2005). At the point scale, considering the macrophyte patch where the fishes truly exploit (Dibble *et al.*, 1996), studies are able to address the behavioral ecology of fishes and the process by which they interact with the plants, providing insight into how macrophytes affect fish foraging, diets and behavioral responses (Savino & Stein, 1982; Miranda & Hodges, 2000; Padial, Thomaz & Agostinho, 2009; Nohner *et al.*, 2018; Carniatto *et al.*, 2020). In addition, the unimodal relationship between macrophyte coverage and fish attributes, usually observed at the fish microscale (Cunha *et al.*, 2019; Quirino *et al.*, 2021a), may not exist at the whole-lake scale.

Fish diversity and composition also strongly correlate with lake characteristics other than macrophyte coverage, such as area, depth and productivity from phytoplankton (Mehner *et al.*, 2005; Brucet *et al.*, 2013). Larger and deeper lakes usually have higher fish richness and abundance due to their wider range of microhabitats and greater stability than smaller and shallower lakes (Olin *et al.*, 2002; Drakou *et al.*, 2009; Emmrich *et al.*, 2011; Brucet *et al.*, 2013). Regarding productivity from phytoplankton, eutrophication causes an excessive increase in the biomass of these organisms (Wetzel, 2000), which generally lead to higher total fish biomass and density (Jeppesen *et al.*, 2000; Brucet *et al.*, 2013; Yu *et al.*, 2021). However, while planktivorous and omnivorous fish benefit from eutrophication (Chla) (Robertis *et al.*, 2003; Yu *et al.*, 2021), the feeding efficiency of piscivorous fish is often reduced in part due to the high water turbidity and competition with other fish at the juvenile state (Jeppesen *et al.*, 2000). In North European lowland lakes, fish assemblages are dominated by cyprinids, such as roach (*Rutilus rutilus*) and bream (*Abramis bramis*) (Olin *et al*., 2002; Mehner *et al.,* 2005) when eutrophic, while at lower nutrient levels there is a dominance of percids (Persson *et al.*, 1991; Jeppesen *et al.*, 2000; Olin *et al.*, 2002). In addition, due to the weaker predator control

and competition, the contribution of smaller-sized specimens increases with eutrophication (Perrow *et al.*, 1999; Jeppesen *et al.*, 2000).

Submerged macrophytes and phytoplankton biomass (or chlorophyll *a*) are two commonly used water quality indicators (Søndergaard *et al.*, 2016). Macrophytes tend to stabilize shallow lakes in the clear-water state, while environments with high nutrient concentrations negatively influence the development and colonization of aquatic macrophytes (Jeppesen *et al.*, 1997; Song *et al.*, 2019). Shallow lake ecosystems may shift from macrophyte-dominated clear water state to phytoplankton-dominated turbid water state with increasing nutrient loading (Moss, 1990; Scheffer *et al.,* 1993), which affect the food web and fish communities (Mormul *et al.*, 2012).

Most studies of fish-macrophyte interactions cover only a few lakes or a small geographical region (e.g., Maceina *et al.*, 1991; Meerhoff *et al.*, 2003; Hargeby *et al.,* 2005; Middaugh *et al.*, 2013; Lopes *et al.*, 2015). Here we used an extensive dataset from 88 Danish shallow lakes to address the following questions: how are fish community attributes (abundance and biomass), particular fish sizes and particularly fish species related to submerged macrophyte coverage in lakes with different trophic states (chlorophyll *a*), lake area and lake depth? Do these relationships depend on the study scale (lake scale or point scale)? Considering the point scale, will the fish-macrophyte relationship within-lakes vary among lakes? We expected that fish abundances and biomass would increase with lake area, lake depth, chlorophyll *a*, since larger dimensions promote stability and higher habitat availability (Olin *et al.*, 2002; Brucet *et al.*, 2013), while chlorophyll *a* usually means higher productivity (Jeppesen *et al.*, 2000; Yu *et al.*, 2021). Regarding macrophyte coverage, we expected that fish abundances and biomass would respond positively until a certain point where they might start to decrease due to limiting factors of an extremely complex habitat (Cunha et al. 2019). In addition, we predicted that species and size classes would show different responses to macrophyte coverage variation, as species vary in their ecological niches and behaviors to explore the structural complexity provided by macrophytes (Diehl, 1988), and large-bodied species tend to be suppressed at high levels of plant cover, as the foraging time may increase (Dibble *et al.*, 1996). More specifically, we predicted that the fish-macrophyte relationship at the point scale could be different from the lake scale, given that fish can actively select the macrophyte patches according to their offer of refuge and feeding ground (Gotceitas & Colgan,

1989), thereby being supposedly more closely related to near macrophyte patch where they were sampled, which may not be reflected at the whole lake scale.

## 4.2 Methods

### 4.2.1 Study area

The study was carried out in lakes widely distributed across the Danish territory (Figure 1; northernmost lake: 57º 62'21'' N, 10º 34'66'' E; southernmost lake: 54º 71'07'' N, 11º 57'68'' E; westernmost lake: 56º 55'69'' N, 8º 13'82'' E; easternmost lake: 55º 69'02'', N 12º 58'06'' E). However, in this study, the samplings occurred between June and September, covering only Summer and early Autumn. Most Danish lakes are relatively small, shallow and nutrient-rich lakes due to intensive agriculture and urban expansion (Jeppesen *et al.*, 2011; Søndergaard *et al.*, 2020). Also, they can be colonized by several macrophyte taxa, including floating-leaved and submerged species, filamentous algae and emergent plants with submerged forms (Søndergaard *et al.*, 2020).
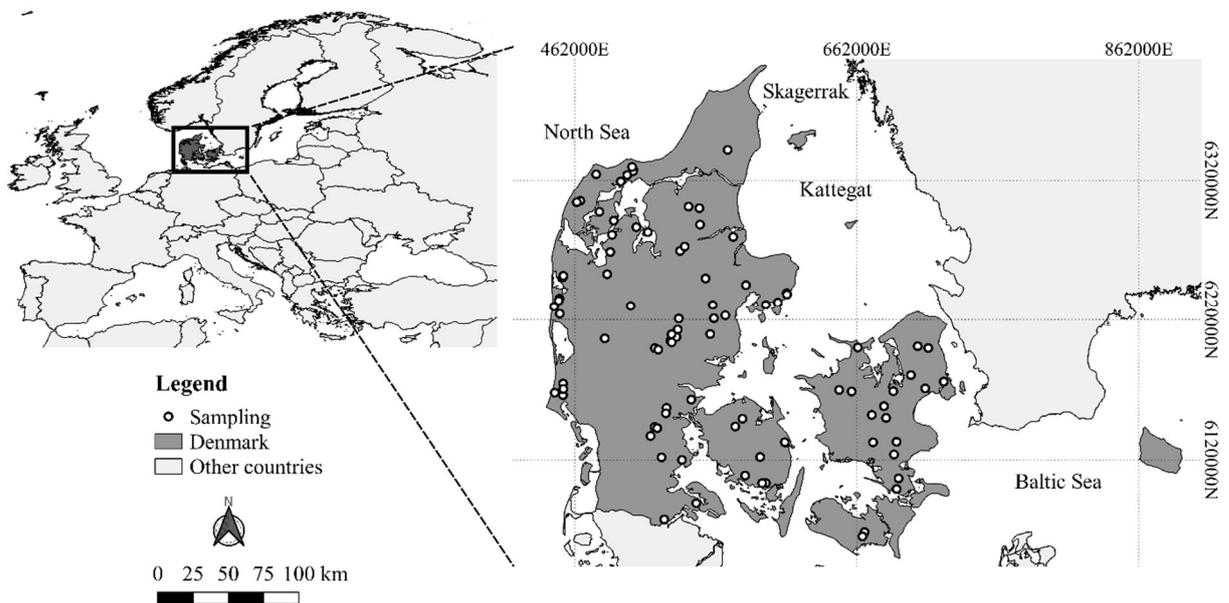


**Fig. 1.** Map of study area showing the lakes used in this study, which were distributed across the Danish territory. Data: DIVA-GIS and GADM (Hijmans *et al.*, 2022). EPSG: 23032.

4.2.2 Sampling

The data used in this study was extracted from an existing database derived from the Danish aquatic monitoring program NOVANA, which has monitored the state of the aquatic environment since 1989. This ongoing program includes data on central physical, chemical, and biological variables, and is based on well-defined and comparable sampling protocols (Svendsen *et al.*, 2005). From the database, we selected only shallow lakes (maximum depth ≤4.5 m) where fish and macrophytes were collected in the same year, in total data from 88 lakes sampled from 2010 to 2020. We focused only on spatial analysis, then when a lake presented more than one year of samplings, we randomly chose one of them, obtaining a unique sampling in time for each lake.

*4.2.2.1 Fish sampling*

Fish were sampled between 14 August and 14 September. They were sampled with benthic multi-mesh gillnets using standardizing sampling designs accredited as the European standard (EN 14757) (CEN (European Committee for Standardisation), 2005). The same type of benthic multi-mesh gillnets (type NORDIC) was used in all lakes. Each net was 1.5 m deep and 42 m long and consisted of 14 units of 3 m length with 14 different mesh sizes placed in random order (6.25, 8, 16.5, 75, 38, 25, 12.5, 33, 50, 22, 43, 30, 60, 10 mm). The total number of nets used per lake varied according to maximum depth and lake size, ranging from 4 to 12 gillnets. Gillnetting was conducted both in the littoral and the pelagic zones and all gillnets were set overnight (about 16 h). A more detailed description of the sampling can be found in Menezes *et al.* (2015).

Fish were counted and their weight (kg) was measured for each species and size class (larger or smaller than 10 cm). In this study, fish abundance and biomass were calculated as catch per unit effort (CPUE, catch per gillnet per night) of fish, using the total number of individuals (CPUEn – considered as abundance) and total weight (CPUEw – considered as biomass) per gillnet and per night. The 40 fish species captured belong to 25 families (Table S1).

*4.2.2.2 Macrophyte sampling*

Submerged macrophytes samplings were conducted between 24 June and 15 August, when the plant community achieves its maximum coverage. They were sampled by measuring cover at 23–743 sampling points (number increasing with lake size) distributed along transects covering the whole lake area and all depth zones, but with the main emphasis on areas with vegetation. To measure the macrophyte cover, a 7-point scale ranging from absent to complete cover (0, 1-5, 5-25, 25-50, 50-75, 75-95 and 95-100%) was used, from which a mean was subsequently calculated (0%, 2.5%, 15%, 37.5%, 62.5%, 85% or 97.5%). At each macrophyte sampling point, water depth (m), species presence and total coverage (in percentage of the total observed area) were estimated using a water glass combined with a rake. We considered only submerged vascular plants and charophytes, excluding filamentous algae, resulting in 101 species or taxa of submerged macrophytes belonging to 32 families (Table S2).

*4.2.2.3 Lake characteristics*

The physical data included lake area (ha) and maximum water depth (m), while chemical data were presented as the mean summer concentrations, including chlorophyll *a* (Chla - µg/L), total phosphorus (TP - mg/L), total nitrogen (TN - mg/L), pH, color (mg Pt/L) and conductivity (mS/m), which were measured at a mid-lake station (integrated sample from top to bottom) from 1 May to 30 September and were used to calculate summer means. TN, TP, and Chla concentrations were analyzed according to Danish standard methods (Jespersen & Christoffersen, 1987; Sondergaard, Kristensen & Jeppesen, 1992) and the other variables were measured in the field using Horiba field sensors.

4.2.3 Data analysis

*4.2.3.1 Spatial scales*

In landscape analysis, the spatial scale consists of two components: grain (the smallest area for which the study has values or characteristics) and extent (the spatial area defined as the landscape) (Mayer & Cameron, 2003; Yamakita & Nakaoka, 2011). In this paper, we analyzed the relationship between fish community and macrophyte coverage using two different size of grain, but keeping the same extent. The data from the first grain size, the lake scale, are similar

(in structure) to those obtained at broad spatial scale, in which each lake is a replicate. Similarly, the data from the second grain size, the point scale, are akin to those obtained in local studies whose sampling designs considered the variation within lakes. In addition, we also analyzed the point scale using a second approach: within-lake.

For the lake scale (88 lakes), fish abundance and biomass were estimated as a mean value per lake, dividing the total CPUEn and CPUEw by the number of gillnets used in each lake, which varied according to the lake dimension. Regarding the explanatory variables, macrophyte coverage was calculated as a mean value based on how large each sampling point represented relatively to the whole lake area, while water depth was calculated as the mean value of the depth measured in each macrophyte point. We also used lake area and summer mean of chlorophyll *a* (Chla) as explanatory variables. The lakes had a wide range of macrophyte coverages (ranging from 0 to 86.9%) and Chla (from 4.4 to 411 µg/L) (Table S3). Most lakes were eutrophic, with a mean TP of 0.18 mg/L and a mean Chla of 75 µg/L (Table S3). In addition, 86.3% of the lakes had a mean depth <2 m, and most of them (73.8%) were >10 ha. All the lakes used were freshwater (conductivity <200 mS/m), non-colored (<100 mg Pt/L) and non-acid (pH >6) (Table S3).

In the analyses considering the point scale, we used each fish gillnets as the samples, totalizing 595 samples. Fish abundance and biomass data were analyzed as the "number of individuals/gillnets/night" and "weight/gillnets/night" caught during one night, respectively, because the sampling method was previously standardized: all gillnets had the same dimension and mesh sizes and was used during the same period. Regarding explanatory variables, since fish and macrophytes were not sampled at the exact same location, we used an interpolation method to predict the macrophyte cover values and water depth around the gillnets areas. For this purpose, we used the Inverse Distance Weighted (IDW) technique in ArcGIS Pro 2.9.0 that estimates cell values by averaging the values of sample data points in the neighborhood of each processing cell, interpolating a raster surface. This method assumes that the influence of an input point on an interpolated value is distance related, thus, the closer a point is to the center of the target cell, the more influence it has on the average (Lam, 1983).

By analyzing each lake separately, the minimum distance between most macrophyte sampling points was used as the cell size, and the minimum distance between most macrophyte transects was used as the maximum distance (Figure 2). The cell size refers to the size of the output raster created, and the maximum distance specifies the limit distance to search for the

nearest input sample points. To calculate the output values, we used a maximum of 6 nearest points from the cell location, since input points far away from where the estimation is being made may have poor or no predictive power (Ploton *et al.*, 2020). After interpolating for any geographic point of the lake, we calculated a mean value for macrophyte coverage and water depth inside a buffer around each gillnet. The buffer diameter had the gillnets length (42 m), since their respective coordinates were taken in the center of the gillnet (Figure 2).
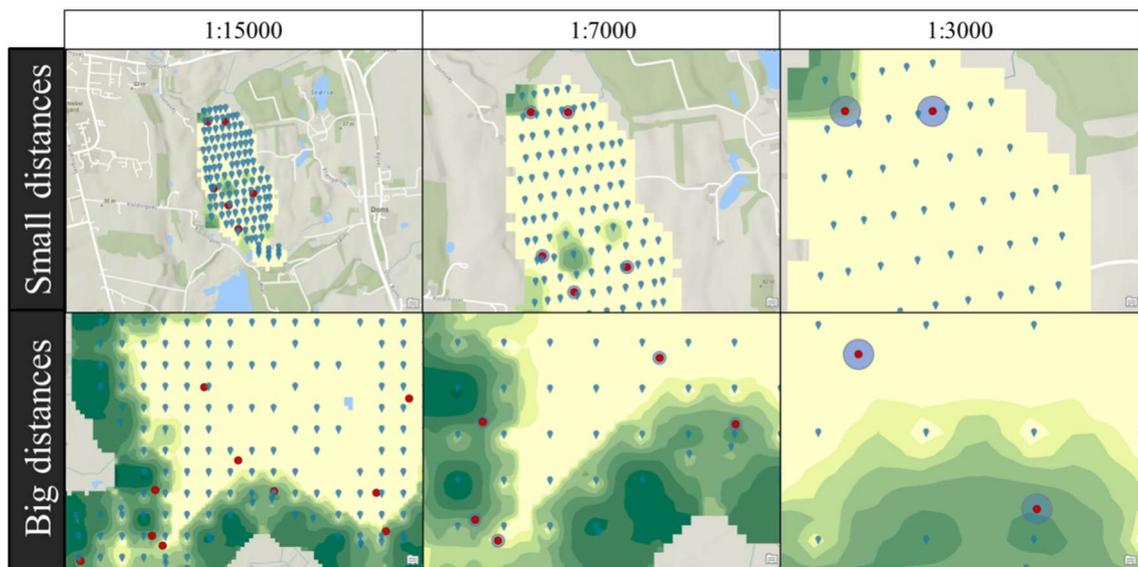


**Figure 2.** Interpolation examples, showing a lake with small or big distances between the macrophyte samplings (blue symbol). The shades of green represent the interpolated values of macrophyte coverage. The red points are the fish sampling stations and the blue buffers around them are the areas from which macrophytes coverage mean were calculated.

Regarding lake area and Chla, we used the same value used for the lake scale, since these lake characteristics also could influence the fish community response at a point scale, and often there is a modest difference on trophic state in surveys within and outside macrophyte stands because of the internal connectivity of lakes given by processes such as water flow, diffusion and wind-driven movement (Song *et al.*, 2019). Moreover, no Chla data were available at the point scale.

*4.2.3.2 Models*

To assess the relationship between fish and the explanatory variables at the point scale, we performed generalized mixed models (GLMMs) using the Gaussian distribution with the identity function. We included the lakes as a random effect because each lake could be

intrinsically subject to unmeasured influences and, therefore, have a different impact on fish community. In addition, we used gillnets coordinates to account for a possible correlation structure, as they could be spatially correlated inside each lake. We fitted generalized linear models (GLMs) using the Gaussian family with the identity function to investigate the relationship between fish and the explanatory variables at the lake scale. We used the "glmmPQL" function from the Mass package (Venables & Ripley, 2002) to perform GLMM and the "glm" function from the R Stats package (R Core Team, 2022) to perform GLM.

To verify how fish attributes respond to macrophyte coverage within the lakes (within-lake approach), we performed Pearson correlation using "cor" function from the R Stats package (R Core Team, 2022) between both communities for each lake separately, using the point scale samples. Subsequently, we used the slopes of each lake as the response variable to run GLMs considering the four explanatory variables of lake scale (mean macrophyte coverage, mean water depth, lake area and mean Chla). To run these models, we obtained the slopes for the lakes with at least 6 points sampled, numbering 78 lakes. When considering lakes with at least 8 points, the total number of lakes was reduced (20), but they provided similar results (Figure S1). Thus, we chose to show the models including the full set of lakes.

For both scales and approaches, we applied the models to five fish response variables in terms of abundance and biomass, numbering 10 models: total fish caught, fish larger than 10 cm, fish smaller than 10 cm, and three species separately: *Rutilus rutilus* (Linnaeus, 1758) (known as roach), *Perca fluviatilis* Linnaeus, 1758 (known as perch) and *Abramis brama* (Linnaeus, 1758) (known as bream), which were the most captured fish species both in terms of number (totaling 77% of the total individuals caught) and biomass (totaling 72% of the total biomass caught – Table S1). We also run models considering the ratio between roach and perch as the response variable, which was calculated by dividing the total number of individuals (or biomass) of roach by the sum of individuals (or biomass) of both roach and perch. This ratio could indicate a succession from the dominance of perch to roach according to the explanatory variables since there is usually a competitive interaction between these species in North European lakes (Persson, 1983).

Visual analysis of the graphs on the relationship between macrophyte coverage and fish variables did not indicate non-linear relationships, and therefore, we ran only first-order models (Figures S2-S3). TN and TP showed multicollinearity (>80%) with Chla and considering that Chla usually has a direct impact on fish whereas the effect of nutrients is indirect via their

relations to several trophic levels (Jeppesen *et al.*, 2000), we run the models using only Chla. However, we also ran models replacing Chla with TN or TP that provided similar results (Table S4 and S5; Figures S4-S8).

Before the models' construction, we scaled the explanatory variables to make their coefficients comparable and we used square root transformation for the response variables (fish abundance and biomass) to approximate them towards a Gaussian distribution. All assumptions (linearity, normality of residuals and homogeneity of variances) were checked and met.

## 4.3 Results

For most models, the macrophyte cover was negatively related to both fish abundance and biomass at the point scale, while Chla was positively related to the fish variables (Figures 3 and 4). Perch abundance was the only fish variable not related to the macrophyte coverage effect, while the abundance of perch and all fish <10 cm did not show a relationship with Chla (Figures 3 and 4). The water depth at the point scale was negatively related to fish abundance for most models, except for roach (Figure 3), while for fish biomass, the water depth was only related (negatively) with fish <10 cm (Figure 4). The lake area was not associated with any of the two response variables at the point scale.

Analysis at the lake scale also revealed general negative relationships (though not significant) of the mean macrophyte coverage with fish abundance and biomass for most models (Figures 3 and 4). The total abundance of fish, the abundance and biomass of fish <10 cm and the abundance of roach were the only fish variables significantly (and negatively) related to macrophyte coverage at the lake scale. Chla was positively and significantly related to the total abundance and biomass of fish, fish >10 cm and bream, the biomass of fish <10 cm, as well as the abundance of roach (Figure 3 and 4). Abundance and biomass of perch reduced with Chla, while deeper waters have higher perch abundance (Figure 3 and 4). The lake area had no effect on the fish variables at the lake scale.

Chla was the only variable significantly (and positively) related to the ratio between roach and perch at both the point and lake scale (Figure 5).
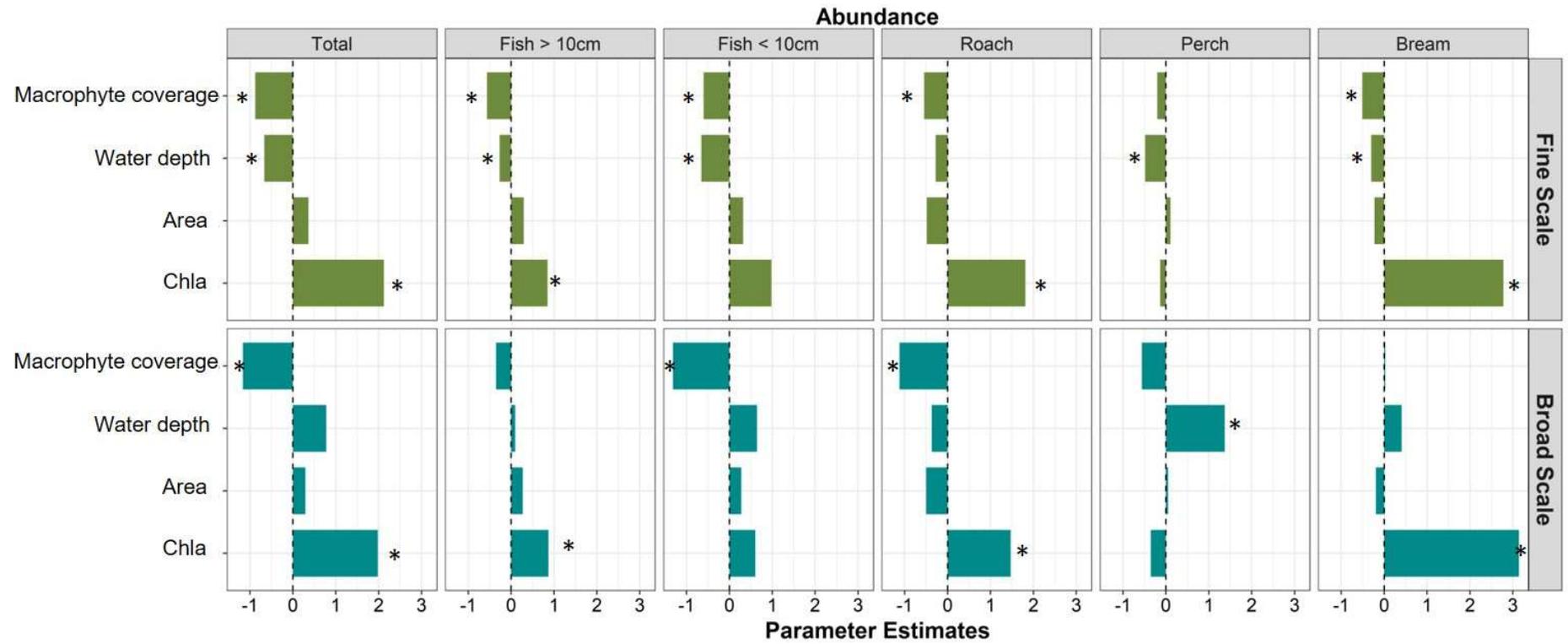
**Figure 3.** Parameters estimates of generalized mixed models (point scale) and generalized linear models (lake scale) for fish abundance in relation to explanatory variables. The dashed line represents the confidence interval. *Statistically significant values.
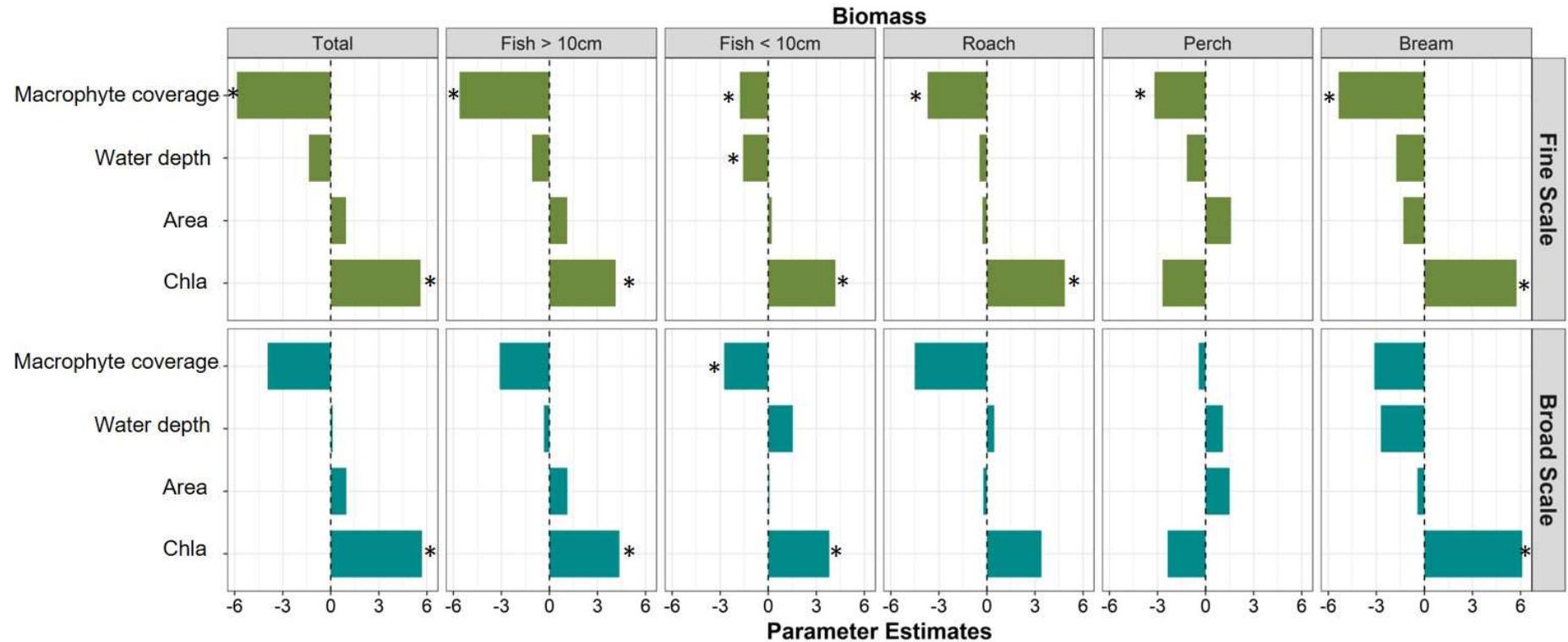
**Figure 4.** Parameters estimates of generalized mixed models (point scale) and generalized linear models (lake scale) for fish biomass in relation to explanatory variables. The dashed line represents the confidence interval. *Statistically significant values.
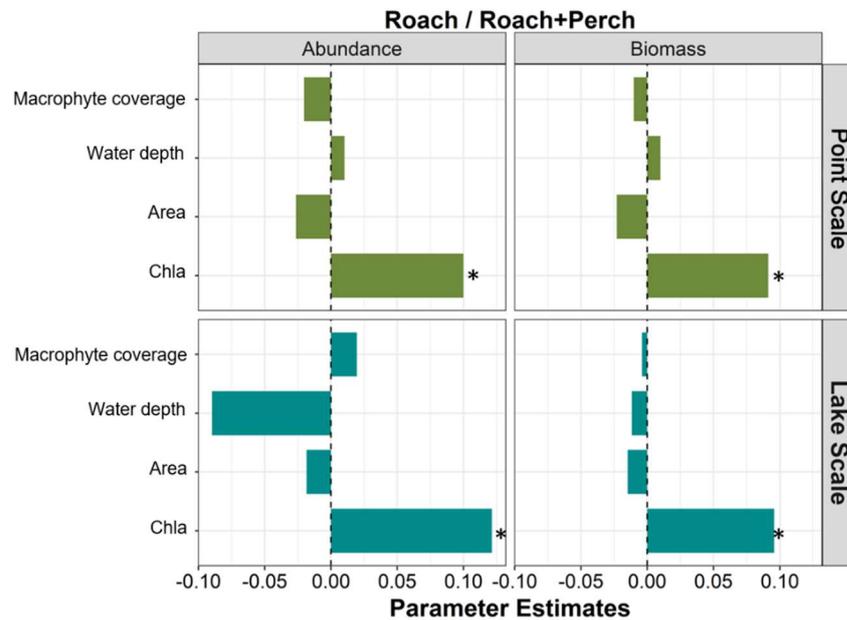
**Figure 5.** Parameters estimates of generalized mixed models (point scale) and generalized linear models (lake scale) for ratio between roach and perch in relation to the explanatory variables. The dashed line represents the confidence interval. *Statistically significant values.

Considering the within-lake approach, for most lakes, the relationship between fish and macrophyte coverage around the gillnet was negative, resulting in negative mean slopes (Table 1). However, some lakes showed positive relationships, and for fish <10 cm the proportion of lakes with a positive and negative association was similar (Table 1). Regarding the models, there was a negative relationship between the lake macrophyte coverage and the slope between fish and macrophyte coverage around the gillnet (Figure 6), especially for the total abundance of fish and the abundance and biomass of fish <10 cm. Furthermore, a positive relationship between macrophyte coverage and fish mainly counts for deeper lakes, as there was a positive effect of mean water depth of the lake and the slope, which was mainly observed for fish biomass (total, >10 cm, roach and perch). Chla also had a positive influence on the fish-macrophyte relationship within the lake only for perch biomass (Figure 6).

**Table 1.** Mean values and proportion of positive and negative values of slopes for the relationship between fish and macrophyte coverage around the gillnet. N= number of individuals; w= weight.

| | Total (n) | Total (w) | >10 cm (n) | >10 cm (w) | <10 cm (n) | <10 cm (w) | Roach (n) | Roach (w) | Perch (n) | Perch (w) | Bream (n) | Bream (w) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean values | -0.06 | -0.13 | -0.09 | -0.14 | 0.00 | -0.01 | -0.04 | -0.15 | 0.03 | -0.11 | -0.15 | -0.18 |
| Positive values (%) | 43.3 | 31.3 | 38.8 | 31.3 | 49.2 | 46.3 | 44.6 | 36.9 | 53.0 | 39.4 | 26.3 | 26.3 |
| Negative values (%) | 56.7 | 67.2 | 61.2 | 67.2 | 49.2 | 52.2 | 55.4 | 63.1 | 45.4 | 60.6 | 71.0 | 73.7 |
| Neutral values (%) | 0 | 1.49 | 0 | 1.49 | 1.49 | 1.49 | 0 | 0 | 1.52 | 0 | 2.63 | 0 |
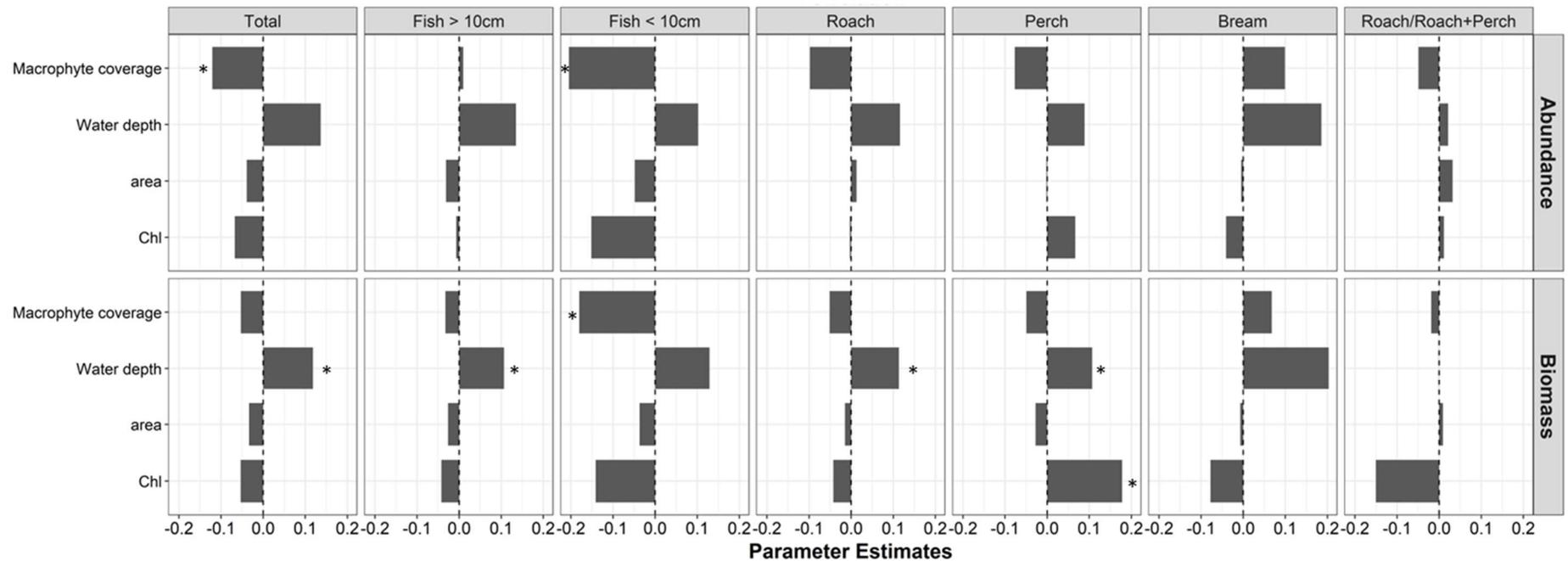


**Figure 6.** Parameters estimates of generalized linear models for the slopes from correlation between fish and macrophyte coverage within the lakes.

*Statistically significant values

## 4.4 Discussion

Our findings revealed that, with the exception of the lake area, the considered predictors - macrophyte coverage, Chlorophyll *a* (Chla) and water depth – had, generally, significant relationships with the fish abundance and biomass at both scales considered: lake scale and point scale. In general, the evaluated attributes of fish community (abundance and biomass) tended to respond negatively to the increase in macrophyte coverage, which was mainly verified at the point scale, not the lake scale. On the other hand, Chla tended to generate positive relationships on both scales, except for perch and fish >10 cm. When evaluating the relationship between fish and macrophytes within-lakes, with the exception of abundance and biomass of fish <10 cm, there was for most lakes a negative relationship, especially for lakes with the higher macrophyte coverage and also shallower. Regarding water depth, it also tends to be more important for the community at the point scale than at lake scale, with negative relationships with fish abundance.

### 4.4.2 Relationship between fish attributes and macrophyte coverage

Although macrophytes are recognized for improving structural heterogeneity of microhabitats and for increasing habitat complexity in aquatic ecosystems (Thomaz & Cunha, 2010), and studies indicate that intermediate densities of macrophytes tend to support the greatest abundance of fish (Ferrer-Montaño & Dibble, 2002; Cunha *et al.*, 2019), our study showed a negative relationship between macrophyte coverage and fish abundance and biomass for most of the lakes when analyzed the within-lake approach.

The fish–habitat complexity relationship also considers changes in foraging efficiency and predator encounter rates (Miranda & Hodges, 2000), and the visual predator encounters are important to prey habitat selection (Savino & Stein, 1982). Despite the structural complexity of macrophyte is clearly important determining the predation risk, the direction of this mediation is strongly influenced by regional predator pools (Farina *et al.*, 2014). In predator-less environments, for example, the hump-shaped relationship with macrophyte complexity may not persist and fish counts can peak at low macrophyte complexity (Wilson *et al.*, 2015), while in dense stands of submerged macrophytes, the visual and movements barriers created may restrict the efficient forage of predatory fish, reducing their prey capture rates and narrowing their diets in these environments (Crowder & Cooper, 1982; Savino & Stein, 1982; Diehl, 1988). This can

lead fish to avoid staying in points in the lake with high plant coverage and actively seek points with sparser macrophytes, which provide food and greater prevention against predation than areas without macrophytes.

However, some lakes showed positive correlations between fish abundance and biomass and macrophyte coverage at the point scale, especially for fish <10 cm, which suggests that despite the general trend, macrophytes may also have positive effects on fish populations. In fact, the macrophyte community may partition available space according to fish size. Thus, while larger fish rarely penetrate foliage and are confined in the pelagic region than within plant stands (Engel, 1987), small fishes usually inhabit macrophytes where they seek refuge and food (Dibble & Pelicice, 2010). Here, we analyzed fish smaller and larger than 10 cm, but usually in studies that show the hump-shaped response to the macrophyte density, fish have a mean body length of around 3.2 cm (Cunha *et al.*, 2019) or 2.4 cm (Lopes *et al.*, 2015). Thus, smallest and largest fish could not be properly analyzed in our study (only having data pooled in two size categories). However, our results still demonstrated general trends, as there was a high proportion of lakes with positive relationships between macrophyte coverage and fish <10 cm.

Specifically, perch abundance was the only fish variable that was not affected by the macrophyte coverage at both scales. As fish present variable size, feeding habits, behavior, and abilities to use the levels of macrophyte density, the response to vegetated habits is species-dependent (Meerhoff *et al.*, 2003; Lopes *et al.*, 2015). Certain fishes prefer areas of fairly dense macrophytes and others prefer areas with sparser vegetation or the borders of a macrophyte stand (Lopes *et al*., 2015). Perch usually dominates lakes with dense submerged macrophytes, and their growth rate and recruitment can be positively related to a period of expanding submerged vegetation (Hargeby *et al.*, 2005; Mehner *et al.*, 2005). These results could be explained by the fact that perch, during the juvenile and benthivorous stage, is considered an efficient visual predator on benthic invertebrates, especially in good light conditions, being capable of feeding on chironomid larvae even in a complex vegetation stand (Diehl, 1988). Such structured habitats offer high biomasses of macroinvertebrates which makes benthivorous perch a superior competitor in dense vegetation, for example, relative to roach and bream, which are planktivorous and most efficient foragers in sparse vegetation (Diehl, 1988; Persson & Eklov, 1995). Interestingly, as opposed to perch abundance, perch biomass was still negatively related to macrophyte coverage at the point scale, which reflected the importance of

large and consequently piscivorous perch that generally occupies the pelagic region in many North European lakes (Persson *et al.*, 1992).

At the whole lake scale, the patterns were not so evident, and despite the same negative trend with macrophyte coverage, few fish groups demonstrated significant relationships, which means that even though fish can avoid dense areas of submerged macrophytes, as they are able to select favorite locations along the range between dense macrophytes and open area (Lopes *et al.*, 2015), it does not necessarily mean lower fish abundance and biomass at the lake scale. However, some of our models did respond significantly (and negatively) to macrophyte coverage at the lake scale: total fish abundance, roach abundance, as well as abundance and biomass of fish <10 cm. Instead of supporting the hypothesis of optimal intermediate macrophyte coverage, studies conducted in multiple North temperate lakes found that excessive plant growth in a lake can reduce fish growth (Cheruvelil *et al.*, 2005). Maceina *et al.* (1991), Olson *et al.* 1998 and Unmuth *et al.* (2001) showed that total removal of aquatic plants in lakes increased the growth, survival and abundance of some species, justifying why macrophyte harvesting is suggested as a way to improve fish growth in lakes with high densities of submerged macrophytes (Olson *et al.*, 1998). One of the reasons may be the increased predation efficiency after plant removal (Sammons & Maceina, 2006). In addition, our results showed that the negative relationship between macrophytes and total fish abundance, as well as abundance and biomass of fish <10 cm was strongly associated with those lakes with high plant coverage, as evidenced at within-lakes approach. The observed pattern could not only reflect the macrophyte coverage per se, as the lakes with a high density of submerged macrophytes have a low amount of nutrients in the water and greater water transparency, which may favor predation on fish (Bunnell *et al.*, 2021). High predation rates from piscivorous fish in macrophyte patches may lead fish to avoid these places because of their inability to keep a sufficient distance to predators in a limited space, thereby adopting an open-water antipredator strategy (Jacobsen & Perrow, 1998). These studies showing negative responses of fish abundance to macrophyte coverage are often considering open water predators, which is contrary to most studies showing macrophyte as great refuges for fish (Dibble *et al.*, 1996; Agostinho *et al.*, 2007).

### 4.4.3 Relationship between fish attributes and Chlorophyll *a*

As expected, most models of fish abundance and biomass showed a positive relationship with Chla, which is in accordance with the relationship observed in several studies in temperate lakes (Jeppesen *et al.*, 2000; Olin *et al.*, 2002; Brucet *et al.*, 2013; Menezes *et al.*, 2015). In eutrophic lakes, there is a higher food availability from the zooplankton and macroinvertebrates communities, which generates a higher fish production/weight (Jeppesen *et al.*, 2000; Olin *et al.*, 2002). Also, eutrophication of lakes can generate changes in the physical environment, for example, increasing water turbidity, which leads to nearly complete elimination of submerged macrophytes as they are often light-limited (Scheffer *et al.*, 1993). As many fish species are adapted to a particular light range, a change in water transparency may affect the competitive interactions between fishes (Trochine *et al.*, 2022). In fact, perch abundance and biomass showed opposite (negative) trends at both scales and when analyzed the ratio between roach and perch, there was always a significant and positive relationship, indicating a shift from a dominance of perch to a dominance of roach along the gradient of productivity (here measured as Chla), which is congruent with earlier results in a large number of lakes (Persson *et al.*, 1991; Jeppesen *et al.*, 2000; Olin *et al.*, 2002).

Cyprinids and percids typically show different responses to the trophic status of a lake (Diehl, 1988). In fact, at lower nutrient levels, it is common to find a dominance of perch, and the large adult piscivorous perch can potentially regulate the roach population by predation (Mehner *et al.*, 2005). Its marked decline with nutrient enrichment may be related to the visual foraging of perch in good light conditions (Diehl, 1988). Being more dependent on visual cues, the decrease in water transparency leads to lower visibility and lower feeding efficiency (Menezes *et al.*, 2013). This may release cyprinids from the predation (Persson *et al.*, 1991), and facilitate a succession from perch to roach (Jeppesen *et al.*, 2000; Olin *et al.*, 2002). Besides, food consumption by roach is less affected by turbidity and absence of light (Diehl, 1988), as it presents higher feeding efficiency at low light intensities, feeding on small zooplankton and primary producers (such as bluegreen algae) (Peterka & Matěna, 2009), thus favoring them in the competition with perch (Persson, 1983).

Moreover, Chla positively influenced the perch biomass-macrophyte coverage relationship within lakes, indicating that in more eutrophic lakes perch tend to occupy macrophyte structures. Both macrophyte cover and high turbidity (which may be reflected by Chla concentration), can affect predator–prey interactions, but these variables add different

difficulties to fish foraging (Figueiredo, Mormul & Benedito, 2013). Macrophyte coverage can reduce the fish prey encounter-rates by adding physical barriers (Meerhoff *et al.*, 2007; Clemente *et al.*, 2019), whereas turbidity influence the reaction distance between fish and their prey by negatively affecting visibility (Snickars, Sandström & Mattila, 2004). However, the effect of increased turbidity is supposed to be stronger on predation than that of macrophyte coverage (Carter *et al.*, 2010; Pekcan-Hekim *et al.*, 2010), thus when visibility is so reduced, predators may switch to other means of finding prey items or may forage elsewhere (Figueiredo *et al.*, 2013). This finding suggests that the effect of macrophyte cover on fish abundance also depends on lake productivity (here expressed as Chla).

4.4.4 Relationship between fish attributes and water depth and lake area

The water depth at the point scale was negatively related to abundance of most fish in addition to the biomass of fish <10 cm. Water depth affects water characteristics, contributing to variations in fish assemblage attributes, especially at the local lake scale (Menezes *et al.*, 2015). Although deeper lakes tend to be most species-rich and diverse (Brucet *et al.*, 2013), less fish per unit of volume, especially for cyprinids, can be found in deeper lakes (Mehner *et al.*, 2005, 2007), where is expected a lower fish predation pressure on their prey (Jeppesen *et al.*, 1997, 2000). However, despite that the water depth negatively affected the perch abundance at the point scale, the mean lake depth had a positive effect for this species, which may be associated with the fact that in deep lakes, the influence of wind-driven waves that agitate the lake substrate and resuspend nutrients and sediments is lower, promoting clearer water (Arfi, Guiral & Bouvy, 1993; Roozen *et al.*, 2003), advantageous condition to visual predators, such as perch. In fact, the greater stability of deep lakes can result in assemblages dominated by large-body species, species less tolerant of extreme water quality conditions, and predators (Miranda, 2011). When analyzing the relationship between fish and macrophytes at the within-lake approach, it tended to be positive in deeper lakes. Thus, in deeper lakes, where supposedly there are more habitats to be occupied (Jeppesen *et al.*, 2000), fish may occupy areas with submerged macrophytes.

It is widely accepted that lake area plays a positive effect on fish richness, abundance and biomass as, in theory, larger lakes have more habitats and thus allow less competition (Horppila *et al.*, 2000; Olin *et al.*, 2002; Drakou *et al.*, 2009; Emmrich *et al.*, 2011; Brucet *et al.*, 2013). However, we found no such relationship, concurring with studies of lakes in China (Zhao *et al.*,

2006; Yu *et al.*, 2021), which suggest that other lake characteristics overshadow the area effect. Also, most fish species are usually found in the littoral zone, and the positive relationship between fish abundance and lake area could be clearer when considering only the littoral than the offshore zone of lakes, because the latter presents low habitat heterogeneity (Menezes *et al.*, 2013). Here, we placed gillnets randomly, both littoral and pelagic zones, which may have masked such a relationship. Moreover, we restricted the analysis to lakes with a max depth below <4.5m and therefore did not include large and deep lakes. Also, the lakes were mostly eutrophic, both factors that may have reduced habitat variability with impact on the effect of the lake area on fish abundance, as also seen for fish richness in a study encompassing 36 lakes in China (Yu *et al.*, 2021).

## 4.5 Conclusion

In summary, the fish abundance and biomass responded to a combination of factors that are often intercorrelated and difficult to isolate, which affect swimming, food availability, predator-prey interactions and competition. Many of the field studies regarding the relationship between fish and macrophyte have sampled a few lakes or one lake only. Using 88 Danish lakes, our findings showed there typically was a negative relationship between fish and macrophyte coverage, and that this relationship was more evident at the point scale than at the whole lake scale. In addition, we found that fish catches increased with lake productivity and decreased with water depth, and the deeper and more eutrophic the lakes are, the more fish tend to occupy points covered by macrophytes. Despite the point water depth showing a negative relationship with fish attributes, perch abundance showed a positive relationship at the lake scale. In fact, perch generally showed patterns contrary to the total community and the other evaluated species. Thus, the relationship between fish and macrophytes, as well as other lake characteristics, differ depending on the fish species, size, scale and the lake studied. Also, it is plausible that other plant characteristics not measured might be additionally important and may also help account for variability in fish attributes over a range of scales, such as macrophyte growth forms and species, each with different implications for fish.

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## 5 CONCLUDING REMARKS

We aimed to assess the fish-macrophyte relationship, specifically to understand how the configuration (density, coverage and diversity) of macrophyte in a point/stand or at the whole lake can drive fish community in multiple aspects (taxonomic, functional and trophic). Thirty macrophyte stands were sampled along a river with low current velocity in Brazil and 88 lakes distributed across Denmark territory, this last one considering two scales (point scale and lake scale).

The results revealed that the highest functional richness occurred in stands with intermediate macrophyte density and high macrophyte diversity. The variation in macrophyte density among stands was the main factor responsible for the variations in functional beta diversity. The turnover component increased more rapidly after a certain point of the macrophyte density gradient. The macrophyte stands that contributed most to overall functional beta diversity had low and high functional richness and, consequently, the lowest and highest plant densities, as well as low plant diversity. These results suggested that habitat complexity assessed through macrophyte density and diversity influenced the filtering of fish functional traits of fish communities (functional alpha diversity), as well as their functional beta diversity.

Based on analyzes of fish stomachs we investigated if the variations in macrophyte density and diversity in the stands where fish were caught could influence the consumption of particular food categories, foraging efficiency, trophic niche breadth and overlap of fish, both in general and according to trophic guilds. We showed that increasing macrophyte density favored herbivory, probably because high macrophyte density levels may reduce the prey encounter rates. Also, macrophyte density was positively related to fish foraging efficiency. In general, fish reduced their trophic niche breadth along the gradient of macrophyte density, while niche overlap among species is high in intermediate plant density. Macrophyte diversity was negatively related to the consumption of higher plants by fish, but positively related to insects' consumption. Despite the general patterns, our findings showed that trophic relationships of fish among macrophyte stands are dependent on trophic guilds and interspecific competition.

Most studies of fish-macrophyte interactions cover only a few lakes or a small geographical region, but here we used an extensive field dataset from 88 lakes encompassing the Danish territory extent. Interestingly, contrary to most tropical studies, we found that the evaluated attributes of fish community tended to respond negatively to the increase in

macrophyte coverage, which was mainly verified at the point scale, whereas this relationship was attenuated at the whole lake scale. On the other hand, Chla tended to generate positive relationships on both scales. In addition, we showed that fish catches increased with lake productivity and decreased with water depth, and the deeper and more eutrophic the lakes are, the more fish tend to occupy points covered by macrophytes. In summary, these results suggest that fish abundance and biomass respond to a combination of often intercorrelated and difficult to isolate, which affect swimming, food availability, predator-prey interactions and competition, affecting species and fish size classes differently.

In conclusion, our findings emphasize the role of macrophytes as environmental filters selecting functional traits in fish communities and strongly influencing the use of food resources by fish, their trophic niche breadth and overlap, as well as their abundances. Interestingly, the temperate environments studied here generally presented a lower fish richness than the tropical environment, while the opposite was verified for the macrophyte richness, with more submerged species sampled in the Danish lakes. In general, we demonstrated that the response of trophic niche breadth along the macrophyte gradient depends on their feeding habits, with herbivorous responding oppositely to omnivores, while some trophic guilds presented null responses to the gradient of macrophyte density and diversity. In the same sense, the implications of macrophyte coverage to fish abundance and biomass are dependent on the species and fish size evaluated, and some species can show responses contrary to the general community response. Moreover, the relationships observed at the point scale may not apply to the whole-lake scale, reflecting the importance of the scale considered, and the response may be dependent, among other factors, on the studied lake. Also, the fish community response to macrophyte may be dependent, among other factors, on the studied lake. We showed that fish respond to different macrophyte measurements, such as coverage percentage, density and diversity. Thus it is plausible that other plant characteristics (e.g., growth forms and species) might be additionally important to fish community variation. In addition, other factors such as intra and interspecific competition and predation risk must be taken into account in studies of the influence of macrophytes on trophic aspects of the fish community. In fact, the variation in abundance, trophic niche and functional traits of fish observed in this study is probably a result of a complex combination of factors such as food availability, shelter from predators, physical space for locomotion and trophic state of water, all influenced directly or indirectly by aquatic macrophytes.

**APPENDIX A -** Upper Paraná River water level
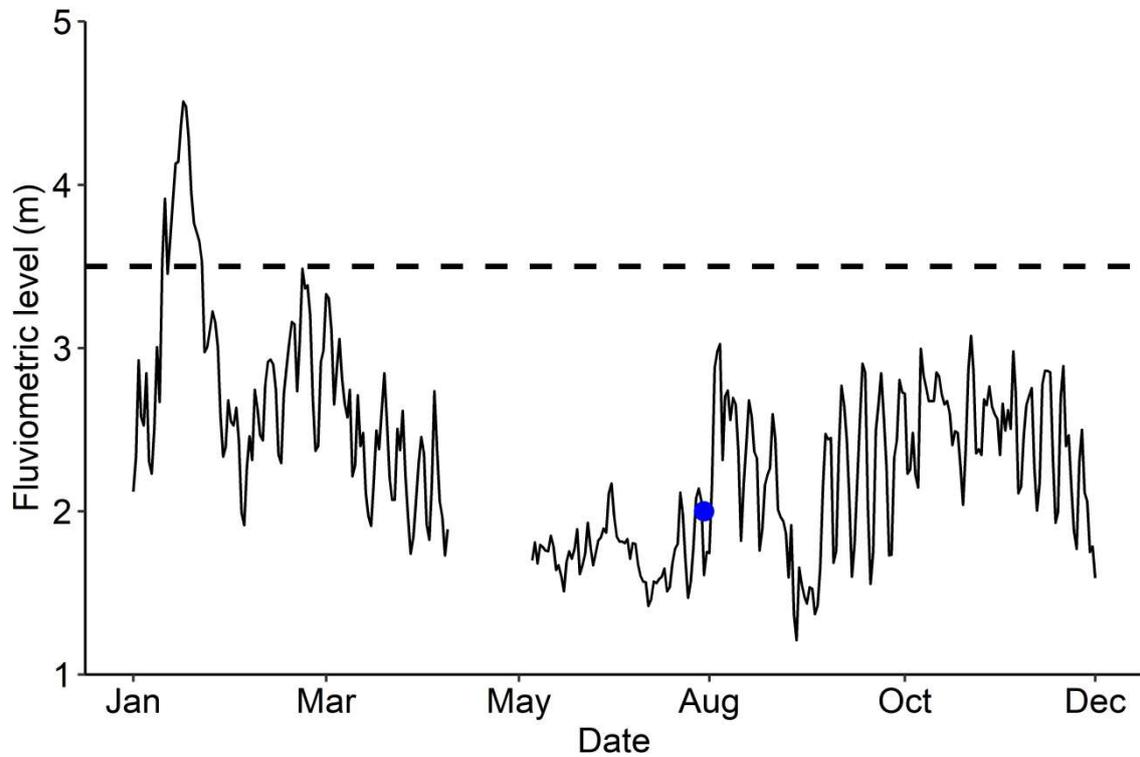


**Fig. S1.** Upper Paraná River water level recorded between Jan 2018 and Dez 2018. The blue circle represent the sampling time. The dashed line correspond to the threshold of the water level for riverbank overflow in the Upper Paraná River floodplain (above 3.5 m – Comumello et al., 2003).

**APPENDIX B -** Photos of different levels of macrophyte density and diversity
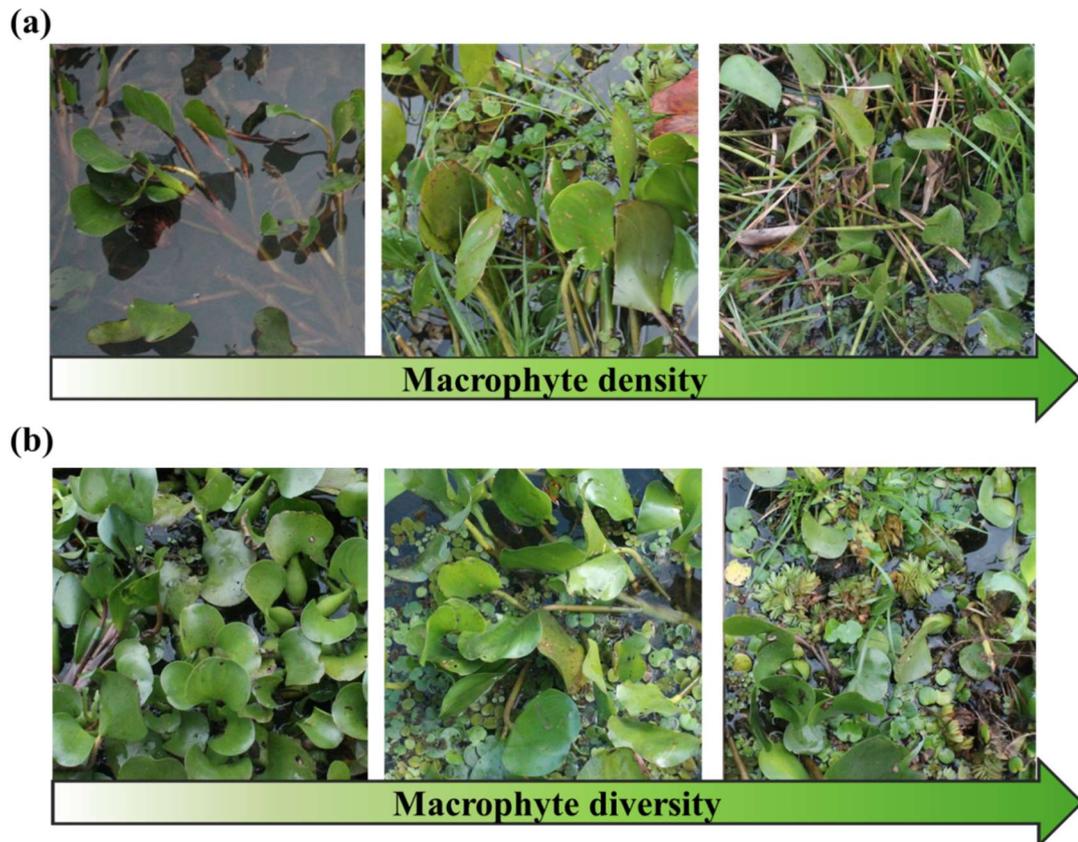


**Fig. S2** Photos of different levels of density (A) and diversity (B) of macrophytes sampled in the Baía River, Brazil

**APPENDIX C -** Relationship between Gower and Euclidean distances
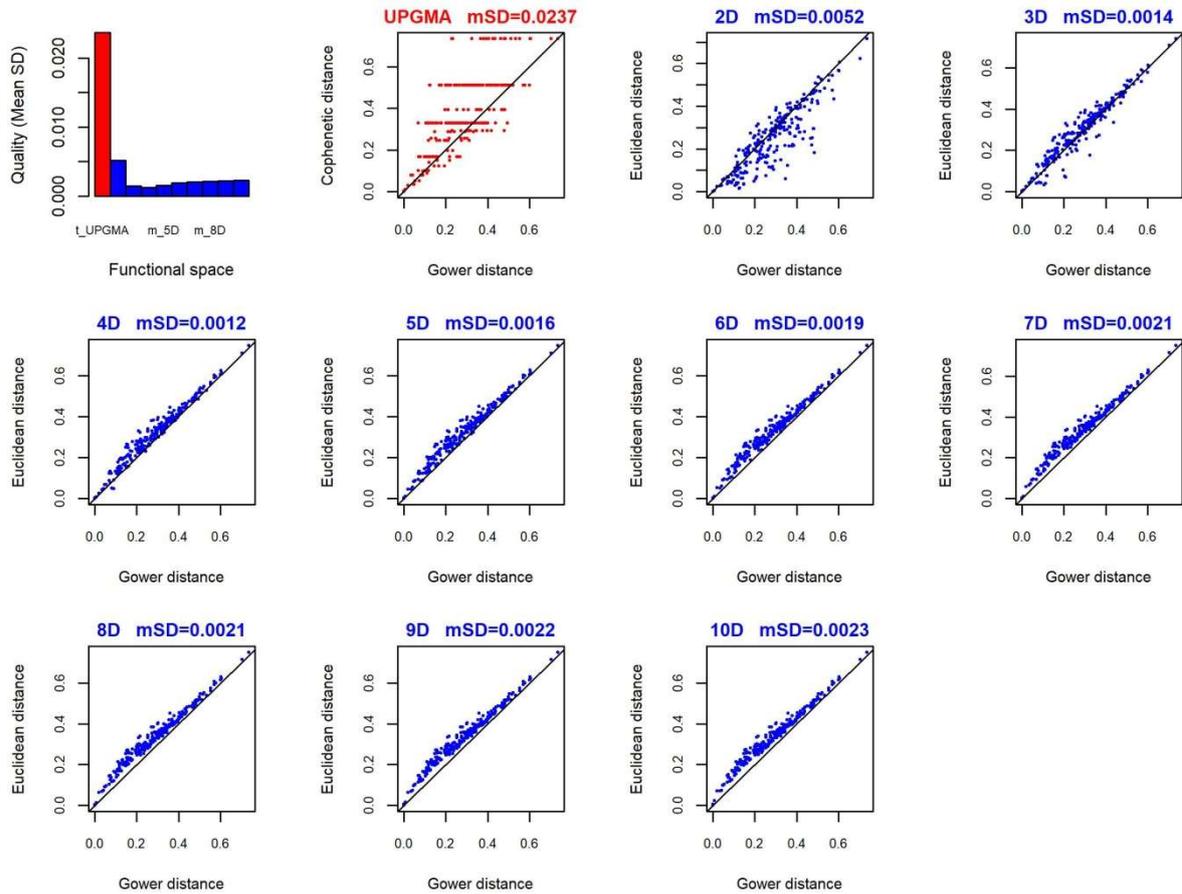


**Fig. S3** Relationship between the initial functional distance (Gower distance) and the standardized distances in the new space generated by PCoA (Euclidean distance). The lower the mean squared deviation (mSD), the higher the quality of the functional space

**APPENDIX D -** Boxplots of pairwise dissimilarities
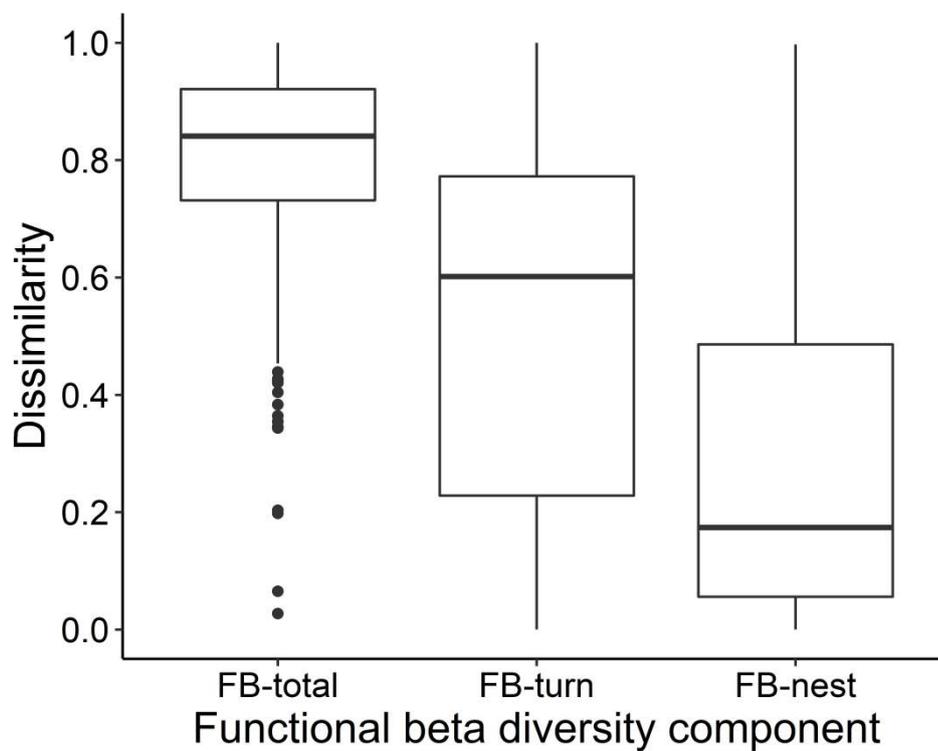


**Fig. S4** Boxplots of pairwise dissimilarities for the total (FBD-total), turnover (FBD-turn) and nestedness (FBD-nest) of fish caught in a gradient of aquatic macrophytes. The horizontal lines represent the median value, box represents 25th and 75th percentiles, whiskers represent respectively the smallest and largest value within 1.5 times interquartile range below and above percentiles, and dots represent outliers

**APPENDIX E -** Plots of I-splines
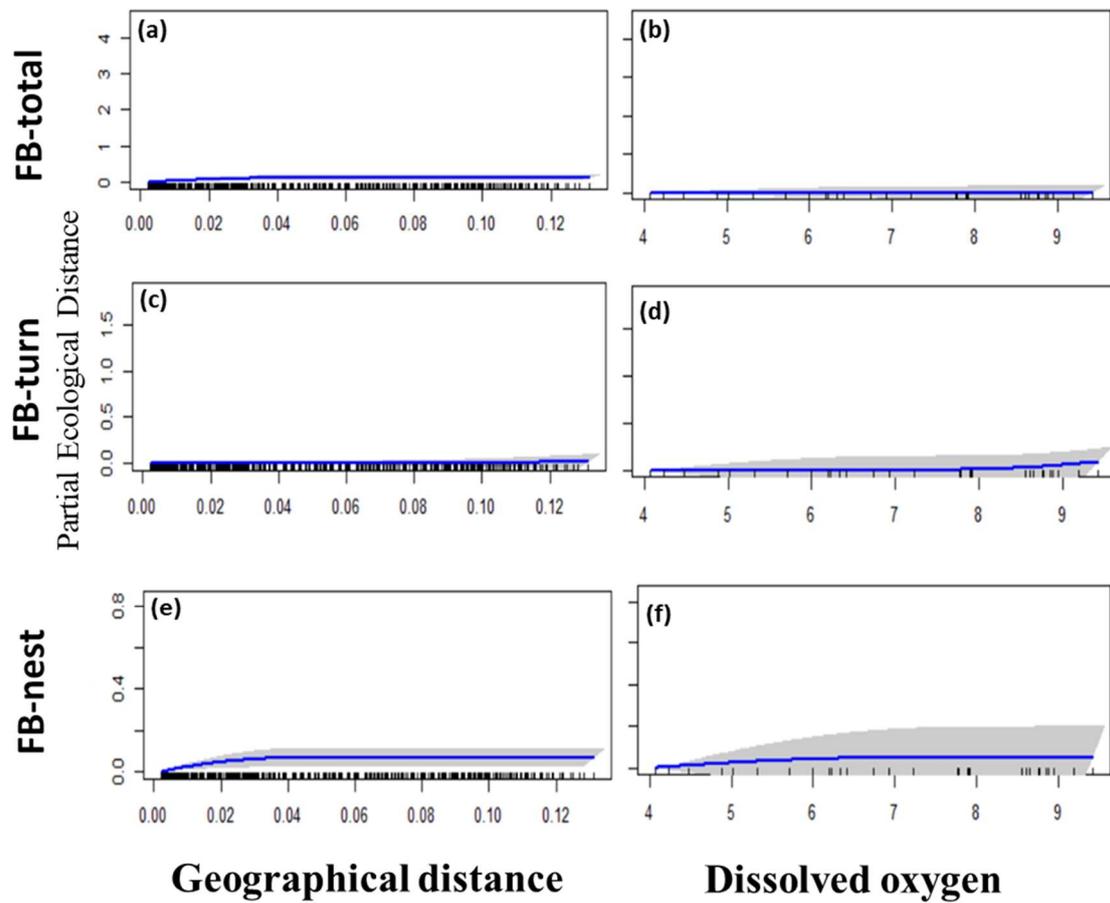


**Fig. S5** Plots of I-splines of the predictor variables (blue) and confidence intervals from bootstrapping (grey) for each component of beta diversity of fish along geographic distance (A, C, and E) and dissolved oxygen (B, D and F) gradients

**APPENDIX F -** Limnological characterization of macrophyte stands

**Table S1** Mean values ± standard deviation of temperature, pH, conductivity, and dissolved oxygen (DO) estimated in the multispecific stands of aquatic macrophytes, in the Baía River, Brazil

| Marcrophyte stand | Temperature (ºC) | pH | Conductivity (µS/cm) | DO (mg/L) |
|---|---|---|---|---|
| 1 | 19.26 ± 0.36 | 6.75 ± 0.55 | 0.03 ± 0.009 | 4.89 ±1.23 |
| 2 | 19.25 ± 0.36 | 6.61 ± 0.64 | 0.02 ± 0 | 4.07 ± 0.24 |
| 3 | 19.08 ± 0.73 | 6.61 ± 0.33 | 0.02 ± 0.001 | 6 ± 2.05 |
| 4 | 19.29 ±0.49 | 6.74 ± 0.35 | 0.02 ± 0.001 | 4.23 ± 1.25 |
| 5 | 19.25 ± 0.45 | 6.93 ± 0.14 | 0.02 ± 0.001 | 5.32 ± 1.89 |
| 6 | 19.35 ± 0.35 | 7.07 ± 0.06 | 0.02 ± 0.001 | 5.02 ± 1.19 |
| 7 | 19.72 ± 1.07 | 6.63 ± 0.66 | 0.03 ± 0.01 | 6.42 ± 1.39 |
| 8 | 19.8 ± 1.02 | 7.04 ± 0.6 | 0.02 ± 0.002 | 6.74 ± 0.85 |
| 9 | 19.83 ± 1.12 | 6.98 ± 0.53 | 0.02 ± 0.003 | 6.34 ± 1.34 |
| 10 | 19.92 ± 1.1 | 6.42 ± 0.45 | 0.02 ± 0.003 | 4.47 ± 2.56 |
| 11 | 19.77 ± 0.81 | 6.71± 0.8 | 0.02 ± 0.001 | 6.93 ± 2.28 |
| 12 | 19.95 ± 1 | 6.69 ± 0.48 | 0.02 ± 0.001 | 5.71 ± 1.48 |
| 13 | 19.34 ± 0.79 | 6.41 ± 0.37 | 0.02 ± 0.001 | 6.23 ± 3.36 |
| 14 | 19.22 ± 0.66 | 6.57 ± 0.17 | 0.02 ± 0.001 | 7.9 ± 1.03 |
| 15 | 19.24 ± 0.85 | 6.53 ± 0.29 | 0.02 ± 0.001 | 7.24 ± 1.42 |
| 16 | 19.24 ± 0.7 | 6.33 ± 0.43 | 0.02 ± 0 | 8.66 ± 1.49 |
| 17 | 19.03 ± 0.89 | 6.69 ± 0.32 | 0.02 ± 0.001 | 8.89 ± 1.38 |
| 18 | 19.25 ± 0.86 | 6.58 ± 0.37 | 0.02 ± 0.001 | 9.19 ± 1.08 |
| 19 | 18.47 ± 0.66 | 6.42 ± 0.18 | 0.02 ± 0 | 7.77 ± 0.29 |
| 20 | 18.31 ± 0.34 | 6.33 ± 0.2 | 0.02 ± 0.004 | 8.96 ± 2.89 |
| 21 | 18.36 ± 0.47 | 6.22 ± 0.35 | 0.02 ± 0.012 | 7.78 ± 1.93 |
| 22 | 18.75 ± 0.38 | 6.45 ± 0.11 | 0.02 ± 0.001 | 7.92 ± 1.48 |
| 23 | 18.73 ± 0.25 | 6.48 ± 0.2 | 0.03 ± 0.007 | 7.9 ± 1.47 |
| 24 | 18.9 ± 0.19 | 6.21 ± 0.26 | 0.03 ± 0.012 | 6.2 ± 3.57 |
| 25 | 19.59 ± 1.34 | 6.93 ± 0.13 | 0.05 ± 0.008 | 8.78 ± 1.82 |
| 26 | 19.56 ± 0.91 | 7.07 ± 0.16 | 0.06 ± 0.003 | 9.43 ± 1.63 |
| 27 | 19.54 ± 0.88 | 7.09 ± 0.16 | 0.05 ± 0.005 | 8.86 ± 1.21 |
| 28 | 19.65 ± 0.64 | 7.28 ± 0.07 | 0.06 ± 0.001 | 8.62 ± 1.04 |
| 29 | 19.75 ± 0.58 | 7.51 ± 0.11 | 0.06 ± 0.001 | 8.76 ± 0.88 |
| 30 | 19.73 ± 0.52 | 7.43 ± 0.13 | 0.06 ± 0.002 | 8.56 ± 1.21 |

**APPENDIX G -** Description of the functional traits of fish

**Table S1:** Description of traits used in the analysis.

| Niche dimension | Functional Trait | Levels | Description | Reference |
|---|---|---|---|---|
| Life History | Fertilization | Internal | The deposition of sperm within the female by the male. | Froese & Pauly, 2017 |
| | | External | Meeting of male and female gametes occurs outside the female's body, after the male and female expelled their gametes in water. | |
| | Parental Care | Yes or No | Any investment by parents in offspring that increases the offsprings' chances of surviving, including guarding, nest building, fanning, splashing, external egg carrying, egg burying, moving eggs or young, ectodermal feeding, oral brooding, etc. | Froese & Pauly, 2017 |
| | Spawning | Partial | Lots of oocytes at different stages of development. The oocytes of each lot develop synchronously and when the most developed lot reach full maturation, they are eliminated. | Vazzoler, 1996 |
| | | Total | At each breeding period two lots of oocytes are evidenced within the ovaries: reserve stock oocytes and oocytes that will synchronously mature and be eliminated during the spawning period. | |
| | | Intermittent | No lots are evident inside the ovaries. Oocytes are present at all stages of development, and their elimination occurs as they reach full maturation. | |
| Trophic Niche | Trophic Guild | Detritivorous | Species that feed predominantly on detritus and sediment | Nowakowski et al., 2008; Delariva et al., 2013 |
| | | Aquatic insectivorous | Species that feed predominantly on aquatic insects | |
| | | Insectivorous | Species that feed predominantly on insects (aquatic and terrestrial in similar proportion) | |
| | | Planktivorous | Species that feed predominantly on zooplankton or phytoplankton | |
| | | Piscivorous | Species that feed predominantly on fish | |
| | | Invertivorous | Species that feed predominantly on aquatic invertebrates | |
| | | Herbivorous | Species that feed predominantly on vascular plants parts, as leaves, seeds, and fruits | |
| | | Algivorous | Species that feed predominantly on algae | |
| | | Omnivorous | Species that feed on animal food items (insect or zooplankton) and vegetable food items (leaves, fruits, seeds, and algae) in similar proportion | |
| Ecomorphology | Body shape | Fusiform | Body shape cylindrical or nearly so and tapers toward the ends. | Froese & Pauly, 2017 |
| | | Deep | Fishes are laterally compressed (very thin when viewed from the front). | |

| Niche dimension | Functional Trait | Levels | Description | Reference |
|---|---|---|---|---|
| | | Compressed | Body flattened laterally. | |
| | Maximum Total Length (mm) | Continuous | The greatest length between the most anterior point of the body (snout) and the most posterior point (tail), in a straight line. | Graça & Pavanelli, 2007 |

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**APPENDIX H -** Functional traits of the fish species sampled

**Table S3** Traits of the fish species sampled in aquatic macrophyte stands in the Baía River, Brazil. PC= Parental care; TL= total length

| | Trophic Niche | Life History | | | Ecomorphology | |
| --- | --- | --- | --- | --- | --- | --- |
| Taxa | Trophic Guild | PC | Spawning | Fertilization | Body Format | TL Max |
| **CHARACIFORMES** | | | | | | |
| **Anostomidae** | | | | | | |
| *Leporinus lacustris* Campos, 1945 | omnivorous[2] | no[3] | partial[2] | external[2,4] | Deep[5] | 57.37 |
| **Characidae** | | | | | | |
| *Astyanax lacustris* (Lütken, 1875) | omnivorous | no[3] | partial[2] | external[2,4] | Deep[5] | 48.94 |
| *Aphyocharax anisitsi* Eigenmann, Kennedy, 1903 | insectivorous (aq) | no[2] | partial[2] | external[2] | Fusiform[5] | 39.44 |
| *Aphyocharax dentatus* Eigenmann, Kennedy, 1903 | insectivorous (aq)[*] | no[2] | partial[2] | external[2] | Fusiform[5] | 39.95 |
| *Hemigrammus ora* Zarske, Le Bail, Géry, 2006 | omnivorous | no[2] | total[2] | external[2] | Fusiform[5] | 46.08 |
| *Hyphessobrycon eques* (Steindachner, 1882) | invertivorous | no[2] | total[2] | external[2] | Moderately deep[5] | 36.73 |
| *Hyphessobrycon moniliger* Moreira, Lima, Costa, 2002 | insectivorous | no[2*] | total[1,2*] | external[2*] | Moderately deep[5] | 42.47 |
| *Moenkhausia bonita* Benine, Castro, Sabino, 2004 | invertivorous | no[2,4] | partial[2] | external[2,4] | Fusiform[5] | 48.31 |
| *Moenkhausia forestii* Benine, Mariguela, Oliveira, 2004 | insectivorous | no[2,4] | partial[2] | external[2,4] | Deep[2] | 48.05 |
| *Moenkhausia sanctaefilomenae* (Steindachner, 1907) | omnivorous | no[2,4] | partial[2] | external[2,4] | Deep[2] | 48.94 |
| *Psellogrammus kennedyi* (Eigenmann, 1903) | herbivorous | no[2,4] | partial[2] | external[4] | Deep and compressed[5] | 50.22 |
| *Roeboides descalvadensis* Fowler, 1932 | insectivorous (aq) | no[2,4] | partial[2] | external[2,4] | Deep[2] | 46.57 |
| *Serrapinnus calliurus* (Boulenger, 1900) | algivorous | no[2] | partial[2] | external[2] | Fusiform[5] | 35.10 |
| *Serrapinnus notomelas* (Eigenmann, 1915) | omnivorous | no[2] | partial[2] | external[2] | Fusiform[5] | 40.13 |
| *Serrapinnus* sp. 1 | omnivorous[*] | no[3] | partial[2] | external[2] | Fusiform[5] | 22.96 |
| *Serrapinnus* sp. 2 | omnivorous[*] | no[3] | partial[2] | external[2] | Fusiform[5] | 26.05 |
| **Crenuchidae** | | | | | | |
| *Characidium* sp. | insectivorous (aq)[6*] | no[2] | partial[2] | external[2] | Moderately deep[5] | 32.60 |
| **Curimatidae** | | | | | | |
| *Steindachnerina brevipinna* (Eigenmann, Eigenmann, 1889) | detritivorous[2] | no[3] | total[2] | external[2,4] | Fusiform[5] | 56.73 |
| **Erythrinidae** | | | | | | |
| *Hoplias mbigua* Azpelicueta, Benítez, Aichino, Mendez, 2015 | piscivorous[2,4] | yes[3] | partial[2] | external[2,4] | Fusiform[5] | 81.39 |
| **Lebiasinidae** | | | | | | |
| *Pyrrhulina australis* Eigenmann, Kennedy, 1903 | insectivorous | no[2] | total[2] | external[2] | Fusiform[5] | 39.01 |

| | Trophic Niche | Life History | | | Ecomorphology | |
|---|---|---|---|---|---|---|
| **GYMNOTIFORMES** | | | | | | |
| **Hypopomidae** | | | | | | |
| *Brachyhypopomus gauderio* Giora, Malabarba, 2009 | invertivorous[1]* | yes[2,4] | partial[2] | external[2,4] | Fusiform and compressed[5] | 89.45 |
| **CICHLIFORMES** | | | | | | |
| **Cichlidae** | | | | | | |
| *Apistogramma combrae* (Regan, 1906) | insectivorous (aq) | yes[2] | partial[2] | external[2] | Fusiform[5] | 45.23 |
| *Crenicichla britskii* Kullander, 1982 | insectivorous (aq) | yes[3] | partial[2] | external[2,4] | Deep[5] | 74.53 |
| *Laetacara araguaiae* Ottoni, Costa, 2009 | insectivorous | yes[2,4] | partial[2] | external[2,4] | Fusiform[5] | 52.07 |
| **CYPRINODONTIFORMES** | | | | | | |
| **Poecilidae** | | | | | | |
| *Pamphorhychties hollandi* (Henn, 1916) | detritivorous | no[2]* | intermittent | internal[2] | Fusiform[5] | 31.24 |

\* Classification based on genus

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**APPENDIX I -** Diet composition of fish sampled

**Table S4** Diet composition[*] (volume percentage) of fish species inhabiting aquatic macrophytes of Baía River, Brazil. N= number of stomachs analyzed; aq= aquatic; te= terrestrial

| Species | N | High plants | algae | insect (aq) | insect (te) | zooplankton | detritus/sediment | others | GUILD |
|---|---|---|---|---|---|---|---|---|---|
| *A. anisitsi* | 20 | 1.54 | 0.58 | 60.31 | 28.71 | 5.20 | 0.19 | 3.47 | insectivorous (aq) |
| *A. combrae* | 19 | 4.78 | 1.12 | 64.89 | 0.84 | 22.75 | 0.56 | 5.06 | insectivorous (aq) |
| *A. lacustris* | 2 | 40 | | 4.67 | 55.33 | | | | omnivorous |
| *C. britski* | 4 | 0.36 | | 84.00 | 13.82 | 1.09 | | 0.73 | insectivorous (aq) |
| *H. ora* | 20 | 49.58 | 6.37 | 24.11 | 16.62 | 2.77 | | 0.55 | omnivorous |
| *H. eques* | 20 | 1.02 | 0.34 | 23.72 | 14.58 | 54.24 | 1.35 | 4.75 | invertivorous |
| *H. moniliger* | 13 | 31.88 | 1.88 | 45.93 | 17.18 | 2.5 | | 0.63 | insectivorous |
| *L. araguaiae* | 20 | 23.75 | 0.71 | 44.66 | 23.89 | 3.04 | | 3.95 | insectivorous |
| *M. bonita* | 19 | 2.39 | | 56.00 | 5.97 | 34.09 | | 1.55 | invertivorous |
| *M. forestii* | 20 | 12.74 | 6.85 | 56.53 | 20.92 | 2.13 | | 0.83 | insectivorous |
| *M. sanctafilomenae* | 20 | 53.27 | 0.97 | 27.72 | 12.23 | 0.97 | | 4.84 | omnivorous |
| *P. hollandi* | 20 | 2.12 | 12.37 | 17.31 | | 4.95 | 62.54 | 0.71 | detritivorous |
| *P. kennedyi* | 20 | 69.43 | 11.13 | 12.35 | | 5.47 | | 1.62 | herbivorous |
| *P. australis* | 20 | 4.58 | | 59.15 | 29.32 | 2.37 | | 4.58 | insectivorous |
| *R. descalvadensis* | 5 | | | 75.49 | | 10.78 | 2.95 | 10.78 | insectivorous (aq) |
| *S. calliurus* | 20 | 6.82 | 70.45 | 6.06 | | 13.64 | | 3.03 | algivorous |
| *S. notomelas* | 20 | 14.20 | 52.47 | 6.18 | | 23.15 | 3.00 | 1.00 | omnivorous |

[*] Diet composition was used to classified species into guilds. For this, we analyzed up to 20 stomachs per species under a stereoscopic and optical microscope and the food contents identified up to the lowest possible taxonomic level. Food items were identified and quantified by the volumetric method using a gridded plate, where the volume was obtained in mm³ and later transformed into ml (Hellawell and Abel 1971). The classification of species in guilds considered the predominance of a given resource in the diet (volume> 60%). For species that did not have a dominant resource, we consider the sum of the two main items. Thus, the species were classified into: detritivorous (preference for decomposing particulate organic matter and mineral particles), herbivorous (preference for higher plant, such as leaves, fruits and seeds), algivorous (preference for algae), aquatic insectivores (preference for aquatic insects), insectivorous (diet composed of aquatic and terrestrial insects), invertivorous (diet composed of aquatic insects and zooplankton) and omnivorous (diet composed of insects and higher plants). Species that did not have a sufficient number of stomachs with food content (N≥2) were classified according to the available literature.

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**APPENDIX J -** Macrophyte species sampled

**Table S5.** Macrophyte species sampled in the Baía River, Brazil

| Taxa | Growth form |
|---|---|
| **ARACEAE** | |
| *Pistia stratiotes* L. | free floating |
| **ARALIACEAE** | |
| *Hydrocotile ranunculoides* L. | free floating |
| **CYPERACEAE** | |
| *Eleocharis* sp. | emergent |
| *Oxycaryum cubense* (Poepp. & Kunth) Lye | epiphyte |
| **HYDROCHARITACEAE** | |
| *Limnobium laevigatum* (H. B. K. ex Willd.) Heine | free floating |
| **MENYANTHACEAE** | |
| *Nymphoides indica* (L.) Kuntze | rooted floating |
| **POACEAE** | |
| *Paspalum repens* Berg. | rooted floating |
| **POLYGONACEAE** | |
| *Polygonum acuminatum* Kunth. | emergent |
| *Polygonum ferrugineum* Weed. | emergent |
| *Polygonum stelligerum* Cham. | emergent |
| **PONTEDERIACEAE** | |
| *Eichhornia crassipes* (Mar.) Salsus. | free floating |
| *Eichhornia azurea* Kunth. | rooted floating |
| **RICCIACEAE** | |
| *Ricciocarpos natans* (L.) | free floating |
| **SALVINIACEAE** | |
| *Azolla filiculoides* Lam. | free floating |
| *Salvinia auriculata* Auble. | free floating |
| *Salvinia biloba* Raddi. | free floating |
| *Salvinia minima* Bak | free floating |

**APPENDIX K -** Likelihood ratio tests

**Table S6** Results of likelihood ratio test for models regressing functional richness (FRic) on density and diversity macrophytes

| Models | $\chi^2$ | P |
|---|---|---|
| First order vs. Second order model | 0.514 | 0.473 |
| First order vs. Third order model | 10.241 | 0.006 |
| Second order vs. Third order model | 9.727 | 0.002 |

**Table S7** Results of likelihood ratio test for models regressing LCBD (FB-total, FB-turn and FB-nest) on functional richness (FRic)

| Models | $\chi^2$ | p |
|---|---|---|
| **LCBD FB-total** | | |
| First order vs. Second order model | 39.474 | < 0.0001 |
| First order vs. Third order model | 40.861 | < 0.0001 |
| Second order vs. Third order model | 1.387 | 0.239 |
| **LCBD FB-turn** | | |
| First order vs. Second order model | 3.6 | 0.058 |
| First order vs. Third order model | 13.266 | 0.001 |
| Second order vs. Third order model | 9.667 | 0.002 |
| **LCBD FB-nest** | | |
| First order vs. Second order model | 4.478 | 0.034 |
| First order vs. Third order model | 24.27 | < 0.0001 |
| Second order vs. Third order model | 19.791 | < 0.0001 |

**Table S8** Results of likelihood ratio test for models regressing LCBD (FB-total, FB-turn and FB-nest) on density and diversity macrophytes

| Models | $\chi^2$ | p |
|---|---|---|
| **LCBD-total** | | |
| First order vs. Second order model | 0.794 | 0.373 |
| First order vs. Third order model | 8.006 | 0.018 |
| Second order vs. Third order model | 7.213 | 0.007 |
| **LCBD-turn** | | |
| First order vs. Second order model | 2.199 | 0.138 |
| First order vs. Third order model | 2.629 | 0.268 |
| Second order vs. Third order model | 0.429 | 0.512 |
| **LCBD-nest** | | |
| First order vs. Second order model | 8.536 | 0.003 |
| First order vs. Third order model | 9.222 | 0.009 |
| Second order vs. Third order model | 0.686 | 0.407 |

**APPENDIX L –** Macrophyte species

**Table S1** Macrophyte species sampled in the Baía River, Brazil. FO%= Frequence of occurrence of each macrophyte species along the 30 sampled macrophyte stands.

| Taxa | FO% |
|------|-----|
| **Araceae** | |
| *Pistia stratiotes* L. | 6.67 |
| **Araliaceae** | 66.67 |
| *Hydrocotile ranunculoides* L. | |
| **Cyperaceae** | |
| *Eleocharis* sp. | |
| *Oxycaryum cubense* (Poepp. & Kunth) Lye | 3.33 |
| **Hydrocharitaceae** | 33.3 |
| *Limnobium laevigatum* (H. B. K. ex Willd.) Heine | |
| **Menyanthaceae** | 56.67 |
| *Nymphoides indica* (L.) Kuntze | |
| **Poaceae** | 3.33 |
| *Paspalum repens* Berg. | |
| **Polygonaceae** | 26.67 |
| *Polygonum acuminatum* Kunth. | |
| *Polygonum ferrugineum* Weed. | 6.67 |
| *Polygonum stelligerum* Cham. | 10.0 |
| **Pontederiaceae** | 20.0 |
| *Eichhornia crassipes* (Mar.) Salsus. | |
| *Eichhornia azurea* Kunth. | 33.33 |
| **Ricciaceae** | 88.67 |
| *Ricciocarpos natans* (L.) | |
| **Salviniaceae** | 40.0 |
| *Azolla filiculoides* Lam. | |
| *Salvinia auriculata* Auble. | 36.67 |
| *Salvinia biloba* Raddi. | 76.67 |
| *Salvinia minima* Bak | 73.33 |

**APPENDIX M –** Fish species

**Table S2** Fish species sampled in aquatic macrophyte stands in the Baía River, Brazil. FO%= Frequence of occurrence of each fish species along the 30 sampled macrophyte stands.

| Taxa | FO% |
|---|---|
| **CHARACIFORMES** | |
| **Anostomidae** | |
| *Leporinus lacustris* Campos, 1945 | 3.33 |
| **Characidae** | |
| *Astyanax lacustris* (Lütken, 1875) | 6.67 |
| *Aphyocharax anisitsi* Eigenmann, Kennedy, 1903* | 23.33 |
| *Aphyocharax dentatus* Eigenmann, Kennedy, 1903 | 3.33 |
| *Hemigrammus ora* Zarske, Le Bail, Géry, 2006* | 36.67 |
| *Hyphessobrycon eques* (Steindachner, 1882)* | 86.67 |
| *Hyphessobrycon moniliger* Moreira, Lima, Costa, 2002* | 30.0 |
| *Moenkhausia bonita* Benine, Castro, Sabino, 2004* | 66.67 |
| *Moenkhausia forestii* Benine, Mariguela, Oliveira, 2004* | 100 |
| *Moenkhausia sanctaefilomenae* (Steindachner, 1907)* | 66.67 |
| *Psellogrammus kennedyi* (Eigenmann, 1903)* | 66.67 |
| *Roeboides descalvadensis* Fowler, 1932* | 13.33 |
| *Serrapinnus calliurus* (Boulenger, 1900) | 56.67 |
| *Serrapinnus notomelas* (Eigenmann, 1915) | 70.0 |
| *Serrapinnus* sp. 1 | 3.33 |
| *Serrapinnus* sp. 2 | 3.33 |
| **Crenuchidae** | |
| *Characidium* sp. | 3.33 |
| **Curimatidae** | |
| *Steindachnerina brevipinna* (Eigenmann, Eigenmann, 1889) | 3.33 |
| **Erythrinidae** | |
| *Hoplias mbigua* Azpelicueta, Benítez, Aichino, Mendez, 2015 | 3.33 |
| **Lebiasinidae** | 66.67 |
| *Pyrrhulina australis* Eigenmann, Kennedy, 1903* | |
| **GYMNOTIFORMES** | |
| **Hypopomidae** | |
| *Brachyhypopomus gauderio* Giora, Malabarba, 2009 | 3.33 |
| **CICHLIFORMES** | |
| **Cichlidae** | |
| *Apistogramma combrae* (Regan, 1906)* | 76.67 |
| *Crenicichla britskii* Kullander, 1982 | 16.67 |
| *Laetacara araguaiae* Ottoni, Costa, 2009* | 43.33 |
| **CYPRINODONTIFORMES** | |
| **Poecilidae** | |
| *Pamphorhychties hollandi* (Henn, 1916) | 50.0 |

*Species used in trophic niche analyses.

**APPENDIX N –** Environmental characteristcs of macrophyte stands

**Table S3** Environmental characteristics in each macrophyte stand sampled in the Baía River. NA= No available data.

| Stand | Water temperature (ºC) | pH | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Water depth (cm) | Macrophyte diversity (Shannon Index) | Macrophyte biomass (g/m$^2$) | Dominating macrophyte species (>70% of biomass) |
|---|---|---|---|---|---|---|---|---|
| 1 | 19.3 ± 0.4 | 6.75 ± 0.55 | 0.03 ± 0.009 | 4.89 ±1.23 | 125 | 2.03 | 1140.5 | *E. azurea* (24.1%) *E. crassipes* (48.8%) |
| 2 | 19.2 ± 0.4 | 6.61 ± 0.64 | 0.02 ± 0 | 4.07 ± 0.24 | 135 | 2.22 | 1404.9 | *E. azurea* (45.3%) *O. cubense* (14.3%) *P. stelligerum* (15.7%) |
| 3 | 19.1 ± 0.7 | 6.61 ± 0.33 | 0.02 ± 0.001 | 6.00 ± 2.05 | 65 | 1.95 | 1752.1 | *E. azurea* (29.1%) *O. cubense* (34%) *S. biloba* (21%) |
| 4 | 19.3 ±0.5 | 6.74 ± 0.35 | 0.02 ± 0.001 | 4.23 ± 1.25 | 60 | 0.68 | 1370.1 | *E. azurea* (87.9%) |
| 5 | 19.2 ± 0.4 | 6.93 ± 0.14 | 0.02 ± 0.001 | 5.32 ± 1.89 | 70 | 0.49 | 1886.8 | *E. azurea* (92.5%) |
| 6 | 19.3 ± 0.3 | 7.07 ± 0.06 | 0.02 ± 0.001 | 5.02 ± 1.19 | 130 | 0.73 | 1508.5 | *E. azurea* (18.9%) *E. crassipes* (80.7%) |
| 7 | 19.7 ± 1.1 | 6.63 ± 0.66 | 0.03 ± 0.01 | 6.42 ± 1.39 | 55 | 0.58 | 1659.7 | *E. azurea* (92%) |
| 8 | 19.8 ± 1.0 | 7.04 ± 0.6 | 0.02 ± 0.002 | 6.74 ± 0.85 | 75 | 0.34 | 1503.6 | *E. azurea* (94.8%) |
| 9 | 19.8 ± 1.1 | 6.98 ± 0.53 | 0.02 ± 0.003 | 6.34 ± 1.34 | 45 | 0.71 | 2210.2 | *E. azurea* (84.2%) *E. crassipes* (17.7%) |
| 10 | 19.9 ± 1.1 | 6.42 ± 0.45 | 0.02 ± 0.003 | 4.47 ± 2.56 | 53 | 0.85 | 7066.3 | *E. crassipes* (75.5%) *O. cubense* (23.7%) |

| 11 | 19.8 ± 0.8 | 6.71± 0.80 | 0.02 ± 0.001 | 6.93 ± 2.28 | 60 | 0.89 | 1288.1 | *E. azurea* (83.2%) |
|----|-----------|------------|--------------|-------------|-----|-------|--------|---------------------|
| 12 | 19.9 ± 1.0 | 6.69 ± 0.48 | 0.02 ± 0.001 | 5.71 ± 1.48 | 50 | 1.67 | 2106.6 | *E. azurea* (68.3%) *P. repens* (10.6%) |
| 13 | 19.3 ± 0.8 | 6.41 ± 0.37 | 0.02 ± 0.001 | 6.23 ± 3.36 | 55 | 1.75 | 3062.9 | *E. azurea* (53.3%) *E. crassipes* (31.2%) |
| 14 | 19.2 ± 0.7 | 6.57 ± 0.17 | 0.02 ± 0.001 | 7.9 ± 1.03 | NA | 0.25 | 366.2 | *E. azurea* (96.1%) |
| 15 | 19.2 ± 0.8 | 6.53 ± 0.29 | 0.02 ± 0.001 | 7.24 ± 1.42 | 75 | 0.79 | 1909.6 | *E. azurea* (83.9%) |
| 16 | 19.2 ± 0.7 | 6.33 ± 0.43 | 0.02 ± 0 | 8.66 ± 1.49 | 60 | 0.005 | 940.3 | *E. azurea* (99.9%) |
| 17 | 19.0 ± 0.9 | 6.69 ± 0.32 | 0.02 ± 0.001 | 8.89 ± 1.38 | 50 | 0 | 1066.7 | *E. azurea* (100%) |
| 18 | 19.2 ± 0.9 | 6.58 ± 0.37 | 0.02 ± 0.001 | 9.19 ± 1.08 | 60 | 0 | 900.3 | *E. azurea* (100%) |
| 19 | 18.5 ± 0.7 | 6.42 ± 0.18 | 0.02 ± 0 | 7.77 ± 0.29 | 135 | 0.23 | 2299.8 | *E. azurea* (96.3%) |
| 20 | 18.3 ± 0.3 | 6.33 ± 0.20 | 0.02 ± 0.004 | 8.96 ± 2.89 | 95 | 0.62 | 3551.5 | *E. crassipes* (87.2%) |
| 21 | 18.4 ± 0.5 | 6.22 ± 0.35 | 0.02 ± 0.012 | 7.78 ± 1.93 | 110 | 0.76 | 1310.5 | *E. azurea* (79.1%) |
| 22 | 18.7 ± 0.4 | 6.45 ± 0.11 | 0.02 ± 0.001 | 7.92 ± 1.48 | 180 | 0.54 | 1683.5 | *E. azurea* (87.5%) |
| 23 | 18.7 ± 0.2 | 6.48 ± 0.20 | 0.03 ± 0.007 | 7.90 ± 1.47 | 75 | 0.10 | 650.9 | *E. azurea* (98.6%) |
| 24 | 18.9 ± 0.2 | 6.21 ± 0.26 | 0.03 ± 0.012 | 6.20 ± 3.57 | 220 | 1.88 | 1369.5 | *E. azurea* (37.9%) |
| 25 | 19.6 ± 1.3 | 6.93 ± 0.13 | 0.05 ± 0.008 | 8.78 ± 1.82 | 190 | 1.01 | 1787.9 | *L. laevigatum* (74.5%) *S. biloba* (33.1%) |
| 26 | 19.6 ± 0.9 | 7.07 ± 0.16 | 0.06 ± 0.003 | 9.43 ± 1.63 | 260 | 1.09 | 1562.3 | *E. azurea* (79.8%) |
| 27 | 19.5 ± 0.9 | 7.09 ± 0.16 | 0.05 ± 0.005 | 8.86 ± 1.21 | 300 | 1.56 | 1705.7 | *E. azurea* (66.3%) *H. ranunculoides* (13.9%) |
| 28 | 19.6 ± 0.6 | 7.28 ± 0.07 | 0.06 ± 0.001 | 8.62 ± 1.04 | 135 | 2.03 | 1056.5 | *E. crassipes* (17.9%) *P. ferrugineum* (40.1%) *P. stelligerum* (27%) |
| 29 | 19.7 ± 0.6 | 7.51 ± 0.11 | 0.06 ± 0.001 | 8.76 ± 0.88 | 270 | 1.27 | 933.2 | *E. azurea* (65.9%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *S. auriculata* (18.2%) |
| 30 | 19.7 ± 0.5 | 7.43 ± 0.13 | 0.06 ± 0.002 | 8.56 ± 1.21 | 290 | 1.10 | 1429.1 | *E. azurea* (47.5%) |
| | | | | | | | | *H. ranunculoides* (50.9%) |

**APPENDIX O –** Diet composition of fish species

**Table S4.** Diet composition (volume percentage) and trophic guild of fish species inhabiting aquatic macrophytes of Baía River, Brazil. N= number of stomachs analyzed.

| Species | N | Higher plant | Algae | Insects | Other invertebrates | Fish | Detritus | Trophic guild |
|---|---|---|---|---|---|---|---|---|
| *Aphyocharax anisitsi* | 33 | 1.19 | 0.89 | 85.91 | 11.13 | 0.73 | 0.15 | Insetivore |
| *Apistogramma combrae* | 51 | 2.77 | 1.08 | 54.39 | 31.77 | - | 9.99 | Invertivore |
| *Astyanax lacustris* | 2 | 40 | - | 60 | - | - | - | Omnivore |
| *Crenicichla britskii* | 4 | 0.36 | - | 97.82 | 1.82 | - | - | Insetivore |
| *Hemigrammus ora* | 77 | 55.05 | 3.82 | 33.79 | 6.65 | 0.69 | - | Omnivore |
| *Hyphessobrycon eques* | 267 | 2.58 | 0.49 | 25.97 | 70.7 | 0.11 | 0.15 | Invertivore |
| *Hyphessobrycon moniliger* | 13 | 31.88 | 1.87 | 63.13 | 3.12 | - | - | Insetivore |
| *Laetacara araguaiae* | 46 | 26.3 | 0.78 | 64.95 | 7.8 | - | 0.17 | Insetivore |
| *Moenkhausia bonita* | 278 | 1.04 | 0.55 | 73.19 | 25.21 | - | 0.01 | Insetivore |
| *Moenkhausia forestii* | 612 | 35.98 | 3.2 | 51.66 | 8.22 | - | 0.94 | Omnivore |
| *Moenkhausia sanctaefilomenae* | 61 | 49.93 | 3.93 | 38.89 | 7.07 | 0.18 | - | Omnivore |
| *Psellogrammus kennedyi* | 50 | 66.27 | 12.8 | 11.53 | 9.12 | 0.07 | 0.21 | Herbivore |
| *Pyrrhulina australis* | 92 | 1.31 | 0.12 | 85.53 | 12.96 | 0.04 | 0.04 | Insetivore |
| *Roeboides descalvadensis* | 5 | - | - | 75.49 | 10.78 | 10.78 | 2.95 | Insetivore |
| *Serrapinnus calliurus* | 119 | 10.47 | 49.94 | 4.66 | 27.53 | 0.46 | 6.94 | Herbivore |
| *Serrapinnus notomelas* | 142 | 15.61 | 45.98 | 14.15 | 19.94 | 0.24 | 4.08 | Herbivore |

**APPENDIX P –** The number of stomachs analyzed

**Table S5.** The number of stomachs of each fish species analyzed in each macrophyte stand to calculate trophic niche breadth and overlap.

| Macrophyte stand | *A. anisitsi* | *A. combrae* | *H. ora* | *H. eques* | *H. moniliger* | *L. araguaiae* | *M. bonita* | *M. forestii* | *M. sanctaefilomenae* | *P. kennedyi* | *P. australis* | *R. descalvadensis* | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | 3 | | 18 | | | 2 | | **23** |
| 2 | | 2 | | 10 | | 3 | | 25 | 2 | | 3 | | **45** |
| 3 | 6 | 2 | 4 | 19 | 3 | | | 20 | | 2 | 4 | | **60** |
| 4 | 17 | 3 | 8 | 31 | | | 30 | 30 | 6 | 5 | | | **130** |
| 5 | | 4 | | 3 | | | | 4 | | | 2 | | **13** |
| 6 | | | | | | | 14 | 4 | | | | | **18** |
| 7 | | 9 | 6 | 7 | | 9 | | 29 | 3 | | 11 | | **74** |
| 8 | 6 | | 26 | 17 | | | 30 | 30 | 5 | | | | **114** |
| 9 | | 3 | 15 | 29 | | | 31 | 30 | 7 | 2 | 3 | | **120** |
| 10 | | | | | | 2 | | 9 | 2 | | 4 | | **17** |
| 11 | | | 2 | 22 | | 2 | | 3 | | | | | **29** |
| 12 | | | 8 | 18 | 5 | 6 | 4 | 30 | 2 | 8 | 5 | | **86** |
| 13 | | | | | | | 2 | 12 | 2 | | | | **16** |
| 14 | | | | 14 | | | 8 | 9 | | | | | **31** |
| 15 | | | | | | | 3 | 3 | | | | | **6** |
| 16 | | | 2 | 11 | 2 | | | 20 | | | | | **35** |
| 17 | | | 5 | 3 | | | 19 | 17 | | | | | **44** |
| 18 | | | | | | | 7 | 6 | | | | 2 | **15** |
| 19 | | | | 7 | | | | 30 | | | 2 | | **39** |
| 20 | | 3 | | | | | | 30 | 2 | 5 | | | **40** |
| 21 | | | | 2 | | | 23 | 30 | 4 | | 3 | | **62** |
| 22 | | | | 10 | | | | 29 | 2 | | 9 | | **50** |
| 23 | | 2 | | | | | 9 | 6 | 2 | | | | **19** |
| 24 | | 3 | | 12 | | 3 | | 21 | | | 5 | | **44** |
| 25 | | 2 | | 31 | | 9 | | 30 | | | 8 | | **80** |
| 26 | | | | 15 | | | | 24 | 4 | | 7 | | **50** |
| 27 | | | | 2 | | 6 | | 30 | 2 | | 9 | | **49** |
| 28 | 3 | | | 2 | | | 30 | 25 | | 12 | 5 | | **77** |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | | 10 | | | | | 4 | 17 | 5 | 2 | 3 | | 41 |
| 30 | | | | | | | 30 | 30 | 7 | 6 | 6 | | 79 |
| **Total** | **32** | **43** | **76** | **265** | **10** | **43** | **244** | **601** | **57** | **42** | **91** | **2** | **1506** |

**APPENDIX Q -** Likelihood ratio tests

**Table S6** Results of likelihood ratio test for models regressing fish foraging efficiency assessed by the mean of the degree of stomach fullness (mDF) for macrophyte and fish attributes.

| Models | $\chi^2$ | P |
|---|---|---|
| First order vs. Second order model | 0.001 | 0.98 |
| First order vs. Third order model | 0.211 | 0.89 |
| Second order vs. Third order model | 0.211 | 0.64 |

**Table S7** Results of likelihood ratio test for models regressing trophic niche breadth assessed by the mean of the distance from the centroid (mDC) for macrophyte and fish attributes.

| Models | $\chi^2$ | P |
|---|---|---|
| First order vs. Second order model | 2.754 | 0.10 |
| First order vs. Third order model | 4.541 | 0.10 |
| Second order vs. Third order model | 1.787 | 0.18 |

**Table S8** Results of likelihood ratio test for models regressing trophic niche overlap assessed by the mean of the distance from the centroid (mDC) for macrophyte and fish attributes. * = significant value.

| Models | $\chi^2$ | P |
|---|---|---|
| First order vs. Second order model | 4.333 | 0.04* |
| First order vs. Third order model | 4.391 | 0.11 |
| Second order vs. Third order model | 0.058 | 0.81 |

**APPENDIX R –** Fish species sampled in Denmark

**Table S1.** Fish species sampled in Danish lakes

| Taxon | abundance (%) | biomass (%) |
|---|---|---|
| **ANGUILLIFORMES** | | |
| **Anguillidae** | | |
| *Anguilla anguilla* (Linnaeus, 1758) | 0.01 | 0.01 |
| **BELONIFORMES** | | |
| **Belonidae** | | |
| *Belone belone* (Linnaeus, 1760) | 0.01 | 0.1 |
| **CARANGIFORMES** | | |
| **Carangidae** | | |
| *Trachurus trachurus* (Linnaeus, 1758) | <0.01 | 0.01 |
| **CLUPEIFORMES** | | |
| **Clupeidae** | | |
| *Clupea harengus* Linnaeus, 1758 | 0.46 | 0.31 |
| **Clupeidae** | | |
| *Sprattus sprattus* (Linnaeus, 1758) | 0.52 | 0.03 |
| **Engraulidae** | | |
| *Engraulis encrasicolus* (Linnaeus, 1758) | <0.01 | 0.01 |
| **Cobitidae** | | |
| **CYPRINIFORMES** | | |
| *Cobitis taenia* Linnaeus, 1758 | <0.01 | <0.01 |
| **Cyprinidae** | | |
| *Carassius auratus* (Linnaeus, 1758) | 0.01 | 0.35 |
| *Carassius carassius* (Linnaeus, 1758) | 1.47 | 4.68 |
| *Cyprinus carpio* Linnaeus, 1758 | 0.77 | 3.83 |
| **Gobionidae** | | |
| *Gobio gobio* (Linnaeus, 1758) | 0.05 | 0.01 |
| **Leuciscidae** | | |
| *Abramis brama* (Linnaeus, 1758) | 9.23 | 11.62 |
| *Alburnus alburnus* (Linnaeus, 1758) | 0.83 | 0.2 |
| *Blicca bjorkna* (Linnaeus, 1758) | 0.78 | 1.03 |
| *Leucaspius delineatus* (Heckel, 1843) | 2.73 | 0.2 |
| *Leuciscus idus* (Linnaeus, 1758) | 0.06 | 0.18 |
| *Leuciscus leuciscus* (Linnaeus, 1758) | <0.01 | <0.01 |
| *Rutilus rutilus* (Linnaeus, 1758) | 41.26 | 33.85 |
| *Scardinius erythropthalmus* (Linnaeus, 1758) | 2.1 | 2.99 |
| **Tincidae** | | |
| *Tinca tinca* (Linnaeus, 1758) | 0.16 | 2.46 |
| **ESOCIFORMES** | | |
| **Esocidae** | | |
| *Esox lucius* Linnaeus, 1758 | 0.17 | 7.51 |
| **GADIFORMES** | | |

**Gadidae**
| | | |
|---|---|---|
| *Melangius merlangius* (Linnaeus, 1758) | <0.01 | <0.01 |

**GOBIIFORMES**

**Gobiidae**
| | | |
|---|---|---|
| *Gobius niger* Linnaeus, 1758 | 0.16 | 0.04 |

**Gobiidae**
| | | |
|---|---|---|
| *Gobiusculus flavenscens* (Fabricius, 1779) | <0.01 | <0.01 |

**MUGILIFORMES**

**Mugilidae**
| | | |
|---|---|---|
| *Mugil chelo* | 0.11 | 0.07 |

**OSMERIFORMES**

**Osmeridae**
| | | |
|---|---|---|
| *Osmerus eperlanus* (Linnaeus, 1758) | 4.04 | 0.57 |

**PERCIFORMES**

**Ammodytidae**
| | | |
|---|---|---|
| *Ammodytes tobianus* Linnaeus, 1758 | 0.01 | <0.01 |
| *Hyperoplus lanceolatus* (Le Sauvage, 1824) | <0.01 | <0.01 |

**PERCIFORMES**

**Gasterosteidae**
| | | |
|---|---|---|
| *Gasterosteus aculeatus* Linnaeus, 1758 | 2.06 | 0.08 |
| *Pungitius pungitius* (Linnaeus, 1758) | 0.05 | <0.01 |
| *Spinachia spinachia* (Linnaeus, 1758) | <0.01 | <0.01 |

**Percidae**
| | | |
|---|---|---|
| *Gymnocephalus cernua* (Linnaeus, 1758) | 5.57 | 1.65 |
| *Perca fluviatilis* Linnaeus, 1758 | 26.46 | 26.37 |
| *Stizostedion lucioperca* (Linnaeus, 1758) | 0.85 | 1.28 |

**Zoarcidae**
| | | |
|---|---|---|
| *Zoarces viviparus* (Linnaeus, 1758) | <0.01 | <0.01 |

**PLEURONECTIFORMES**

**Pleuronectidae**
| | | |
|---|---|---|
| *Platichthys flesus* (Linnaeus, 1758) | 0.04 | 0.25 |
| *Pleuronectes platessa* Linnaeus, 1758 | <0.01 | <0.01 |

**Scophthalmidae**
| | | |
|---|---|---|
| *Psetta maxima* | <0.01 | 0.02 |

**SALMONIFORMES**

**Salmonidae**
| | | |
|---|---|---|
| *Coregonus lavaretus* (Linnaeus, 1758) | 0.01 | 0.2 |

**Salmonidae**
| | | |
|---|---|---|
| *Salmo trutta* Linnaeus, 1758 | 0.01 | 0.07 |

**APPENDIX S –** Macrophyte species sampled in Denmark

**Table S2.** List of submerged macrophyte species found in the Danish lakes. *species with submerged forms

| Taxon |
| --- |
| *Vaucheria* spp. |
| **Alismataceae** |
| *Alisma plantago* L.* |
| *Baldellia* spp.* |
| *Sagittaria* spp.* |
| **Aneuraceae** |
| *Riccardia* spp. |
| **Apiaceae** |
| *Berula erecta* (Huds.) Coville* |
| *Cicuta virosa* L.* |
| *Helosciadium inundatum* (L.) Koch* |
| *Sium latifolium* L. |
| **Araceae** |
| *Lemna trisulca* L. |
| **Boranginaceae** |
| *Myosotis scorpioides* L.* |
| **Brassicaceae** |
| *Nasturtium microphyllum* (Boenn) Reichb. |
| *Nasturtium officinale* W.T. Aiton. |
| **Butomaceae** |
| *Butomus umbellatus* L.* |
| **Callitrichaceae** |
| *Callitriche hamulata* Kütz. ex W.D.J. Koch |
| *Callitriche hermaphroditica* L. |
| *Callitriche platycarpa* Kütz |
| *Callitriche* spp. |
| **Campanulaceae** |
| *Lobelia dortmanna* L. |
| **Ceratophyllaceae** |
| *Ceratophyllum demersum* L. |
| *Ceratophyllum submersum*L. |
| **Characeae** |
| *Chara aspera* Willd. |
| *Chara baltica* (Hartm.) Bruz. |
| *Chara canescens* Loiseleur. |
| *Chara connivens* Saltzm. Ex. A. Braun |
| *Chara globularis* Thuill. |
| *Chara hispida* L. |
| *Chara intermedia* A. Braun |
| *Chara* spp. |
| *Chara virgata* Kützing |

*Chara vulgaris* L.
*Nitella flexilis* (L.) C.Agardh.
*Nitella* spp.
*Nitella translucens* (Person) C.Agardh
**Cyperaceae**
*Eleocharis acicularis* (L.) Roem. & Schult
*Eleocharis palustris* (L.) Roem. & Schult.
*Isolepis fluitans* (L.) R.Br.
*Schoenoplectus lacustris* (L.) Palla*
*Scirpus* spp.
**Elatinaceae**
*Elatine hydropiper* L.
**Equisetaceae**
*Equisetum fluviatile* L.
**Feistiellaceae**
*Nitellopsis obtusa* (N.A.Desvaux) J.Groves
**Fontinalaceae**
*Fontinalis antipyretica* Hedw.
*Fontinalis dalecarlica* L.
*Fontinalis* spp.
**Haloragaceae**
*Myriophyllum alterniflorum* DC.
*Myriophyllum spicatum* L.
*Myriophyllum verticillatum* L.
**Hydrocharitaceae**
*Elodea canadensis* Michx.
*Elodea nuttallii* (Planch.) St. John
*Hydrocharis morsus-ranae* L.
*Hydrocotyle vulgaris* L.
*Najas marina* L.
*Stratiotes aloides* L.
*Hydrodictyon reticulatum* (L.) Lagerh.
**Isoetaceae**
*Isoetes lacustris* L.
**Juncaceae**
*Juncus bulbosus* L.*
**Lamiaceae**
*Mentha aquatica* Linn.
**Lentibulariaceae**
*Utricularia australis* R. Br.
*Utricularia minor* Chapm.
*Utricularia* spp.
**Marsileaceae**
*Pilularia globulifera* L.
**Nostocaceae**
*Nostoc pruniforme*

**Nymphaceae**
Nymphaceae*
*Nuphar lutea* (L.) Sm.*
*Nymphaea alba* L.
**Plantaginaceae**
*Hippuris vulgaris* L.
*Plantago uniflora* L.
*Veronica beccabunga*
**Potamogetonaceae**
*Potamogeton* spp.*
*Potamogeton alpinus* Balb.
*Potamogeton berchtoldii* Fieber.
*Potamogeton compressus* L.
*Potamogeton crispus* L.
*Potamogeton friesii* Rupr.
*Potamogeton gramineus* L.*
*Potamogeton lucens* L.
*Potamogeton natans* L.*
*Potamogeton obtusifolius* Mert. & W. D. J. Koch
*Potamogeton perfoliatus* L.
*Potamogeton polygonifolius* Pourr.
*Potamogeton praelongus* Wulfen.
*Potamogeton pusillus* L.
*Potamogeton rutilus* Wolfg.
*Stuckenia pectinata* (L.) Börner.
*Zannichellia major* Hartm.
*Zannichellia palustris* L.
**Primulaceae**
*Hottonia palustris* L.
*Lysimachia thyrsiflora* L.*
**Ranunculaceae**
*Batrachium* spp.
*Ranunculus aquatilis* L.
*Ranunculus circinatus* Sibth.
*Ranunculus flammula* L.*
*Ranunculus hederaceus* L.
*Ranunculus lingua* L.*
*Ranunculus peltatus* Schrank.
**Sparganiaceae**
*Sparganium angustifolium* Michx.
*Sparganium* spp.
**Sphagnaceae**
*Sphagnum cuspidatum* Ehrh. ex Hoffm.
*Sphagnum palustre* L.
*Sphagnum* spp.
**Typhaceae**
*Sparganium emersum* Rehmann

**APPENDIX T –** Environmental variables

**Table S3.** Environmental variables (mean summer) considering 88 lakes and 595 points sampled within the lakes. Min= minimum; Max= maximum; Chla= Chlorophyll *a*; TP= total phosphorus; TN= total nitrogen.

| | Lake scale | Point scale |
|---|---|---|
| N | 88 | 595 |
| **Environmental variables** | **Min – Max** | **Min – Max** |
| Macrophyte coverage (%) | 0 - 86.9 | 0 – 97.5 |
| Mean water depth (m) | 0.54 – 4.1 | 0.19 – 4.5 |
| Lake area (ha) | 4.8 – 1.713 | 4.8 – 1.713 |
| Chla (µg/L) | 4.4 – 411 | 4.4 – 411 |
| TP (mg/L) | 0.02 - 0.82 | 0.02 - 0.82 |
| TN (mg/L) | 0.66 - 5.58 | 0.66 - 5.58 |
| pH | 6.8 - 9.7 | 6.8 - 9.7 |
| Colour (mg Pt/L) | 8.9 - 90.8 | 8.9 - 90.8 |
| Conductivity (mS/m) | 9.4 - 196.4 | 9.4 - 196.4 |

**APPENDIX U –** Combinations of predictors for the models of point and lake scale

**Table S4.** Combinations of predictors for the models considering the point scale. n= number of individuals; w= total weight; Chl= Chlorophyll *a*; Ntot= total nitrogen; Ptot= total phosphorus.

| Response variable | Explanatory variables | $R^2m$ | $R^2c$ |
|---|---|---|---|
| Total fish (n) | **Macrophyte + Lake area + Water depth + Chl** | **21** | **67** |
| | Macrophyte + Lake area + Water depth + Ntot | 8 | 63 |
| | Macrophyte + Lake area + Water depth + Ptot | 9 | 63 |
| Total fish (w) | **Macrophyte + Lake area + Water depth + Chl** | **13** | **50** |
| | Macrophyte + Lake area + Water depth + Ntot | 7 | 49 |
| | Macrophyte + Lake area + Water depth + Ptot | 11 | 49 |
| Fish > 10 cm (n) | **Macrophyte + Lake area + Water depth + Chl** | **15** | **64** |
| | Macrophyte + Lake area + Water depth + Ntot | 6 | 64 |
| | Macrophyte + Lake area + Water depth + Ptot | 8 | 64 |
| Fish > 10 cm (w) | **Macrophyte + Lake area + Water depth + Chl** | **10** | **49** |
| | Macrophyte + Lake area + Water depth + Ntot | 9 | 52 |
| | Macrophyte + Lake area + Water depth + Ptot | 5 | 52 |
| Fish < 10 cm (n) | **Macrophyte + Lake area + Water depth + Chl** | **8** | **56** |
| | Macrophyte + Lake area + Water depth + Ntot | 4 | 56 |
| | Macrophyte + Lake area + Water depth + Ptot | 5 | 56 |
| Fish < 10 cm (w) | **Macrophyte + Lake area + Water depth + Chl** | **16** | **73** |
| | Macrophyte + Lake area + Water depth + Ntot | 6 | 72 |
| | Macrophyte + Lake area + Water depth + Ptot | 9 | 72 |
| Roach (n) | **Macrophyte + Lake area + Water depth + Chl** | **20** | **74** |
| | Macrophyte + Lake area + Water depth + Ntot | 6 | 74 |
| | Macrophyte + Lake area + Water depth + Ptot | 14 | 74 |
| Roach (w) | **Macrophyte + Lake area + Water depth + Chl** | **11** | **69** |
| | Macrophyte + Lake area + Water depth + Ntot | 4 | 69 |
| | Macrophyte + Lake area + Water depth + Ptot | 10 | 69 |
| Perch (n) | **Macrophyte + Lake area + Water depth + Chl** | **17** | **56** |
| | Macrophyte + Lake area + Water depth + Ntot | 2 | 56 |
| | Macrophyte + Lake area + Water depth + Ptot | 2 | 57 |
| Perch (w) | Macrophyte + Lake area + Water depth + Chl | 4 | 60 |
| | Macrophyte + Lake area + Water depth + Ntot | 5 | 60 |
| | Macrophyte + Lake area + Water depth + Ptot | 5 | 60 |
| Bream (n) | **Macrophyte + Lake area + Water depth + Chl** | **48** | **84** |
| | Macrophyte + Lake area + Water depth + Ntot | 36 | 84 |
| | Macrophyte + Lake area + Water depth + Ptot | 44 | 84 |
| Bream (w) | **Macrophyte + Lake area + Water depth + Chl** | **18** | **48** |
| | Macrophyte + Lake area + Water depth + Ntot | 15 | 46 |
| | Macrophyte + Lake area + Water depth + Ptot | 16 | 47 |
| Roach/Perch ratio | **Macrophyte + Lake area + Water depth + Chl** | **13** | **79** |
| | Macrophyte + Lake area + Water depth + Ntot | 13 | 76 |
| | Macrophyte + Lake area + Water depth + Ptot | 7 | 77 |
| Roach/Perch ratio | **Macrophyte + Lake area + Water depth + Chl** | **10** | **75** |
| | Macrophyte + Lake area + Water depth + Ntot | 10 | 71 |
| | Macrophyte + Lake area + Water depth + Ptot | 6 | 71 |

**Table S5.** Combinations of predictors for the models considering the lake scale. n= number of individuals; w= total weight; Chl= Chlorophyll *a*; Ntot= total nitrogen; Ptot= total phosphorus. The best model is highlighted in bold.

| Response variable | Explanatory variables | AIC |
|---|---|---|
| Total fish (n) | **Macrophyte + Lake area + Water depth + Chl** | **510** |
| | Macrophyte + Lake area + Water depth + Ntot | 521 |
| | Macrophyte + Lake area + Water depth + Ptot | 521 |
| Total fish (w) | **Macrophyte + Lake area + Water depth + Chl** | **769** |
| | Macrophyte + Lake area + Water depth + Ntot | 776 |
| | Macrophyte + Lake area + Water depth + Ptot | 771 |
| Fish > 10 cm (n) | **Macrophyte + Lake area + Water depth + Chl** | **412** |
| | Macrophyte + Lake area + Water depth + Ntot | 421 |
| | Macrophyte + Lake area + Water depth + Ptot | 419 |
| Fish > 10 cm (w) | **Macrophyte + Lake area + Water depth + Chl** | **765** |
| | Macrophyte + Lake area + Water depth + Ntot | 770 |
| | Macrophyte + Lake area + Water depth + Ptot | 766 |
| Fish < 10 cm (n) | **Macrophyte + Lake area + Water depth + Chl** | **504** |
| | Macrophyte + Lake area + Water depth + Ntot | 505 |
| | Macrophyte + Lake area + Water depth + Ptot | 505 |
| Fish < 10 cm (w) | **Macrophyte + Lake area + Water depth + Chl** | **658** |
| | Macrophyte + Lake area + Water depth + Ntot | 667 |
| | Macrophyte + Lake area + Water depth + Ptot | 664 |
| Roach (n) | **Macrophyte + Lake area + Water depth + Chl** | **406** |
| | Macrophyte + Lake area + Water depth + Ntot | 415 |
| | Macrophyte + Lake area + Water depth + Ptot | 411 |
| Roach (w) | **Macrophyte + Lake area + Water depth + Chl** | **625** |
| | Macrophyte + Lake area + Water depth + Ntot | 626 |
| | Macrophyte + Lake area + Water depth + Ptot | 626 |
| Perch (n) | **Macrophyte + Lake area + Water depth + Chl** | **380** |
| | Macrophyte + Lake area + Water depth + Ntot | 381 |
| | Macrophyte + Lake area + Water depth + Ptot | 382 |
| Perch (w) | Macrophyte + Lake area + Water depth + Chl | 685 |
| | **Macrophyte + Lake area + Water depth + Ntot** | **684** |
| | Macrophyte + Lake area + Water depth + Ptot | 685 |
| Bream (n) | **Macrophyte + Lake area + Water depth + Chl** | **232** |
| | Macrophyte + Lake area + Water depth + Ntot | 243 |
| | Macrophyte + Lake area + Water depth + Ptot | 236 |
| Bream (w) | **Macrophyte + Lake area + Water depth + Chl** | **373** |
| | Macrophyte + Lake area + Water depth + Ntot | 378 |
| | Macrophyte + Lake area + Water depth + Ptot | 375 |
| Roach/Perch ratio | **Macrophyte + Lake area + Water depth + Chl** | **11** |
| | Macrophyte + Lake area + Water depth + Ntot | 21 |
| | Macrophyte + Lake area + Water depth + Ptot | 27 |
| Roach/Perch ratio | Macrophyte + Lake area + Water depth + Chl | 23 |
| | **Macrophyte + Lake area + Water depth + Ntot** | **9** |
| | Macrophyte + Lake area + Water depth + Ptot | 16 |

**APPENDIX V – Parameters estimates for the slopes from correlation**
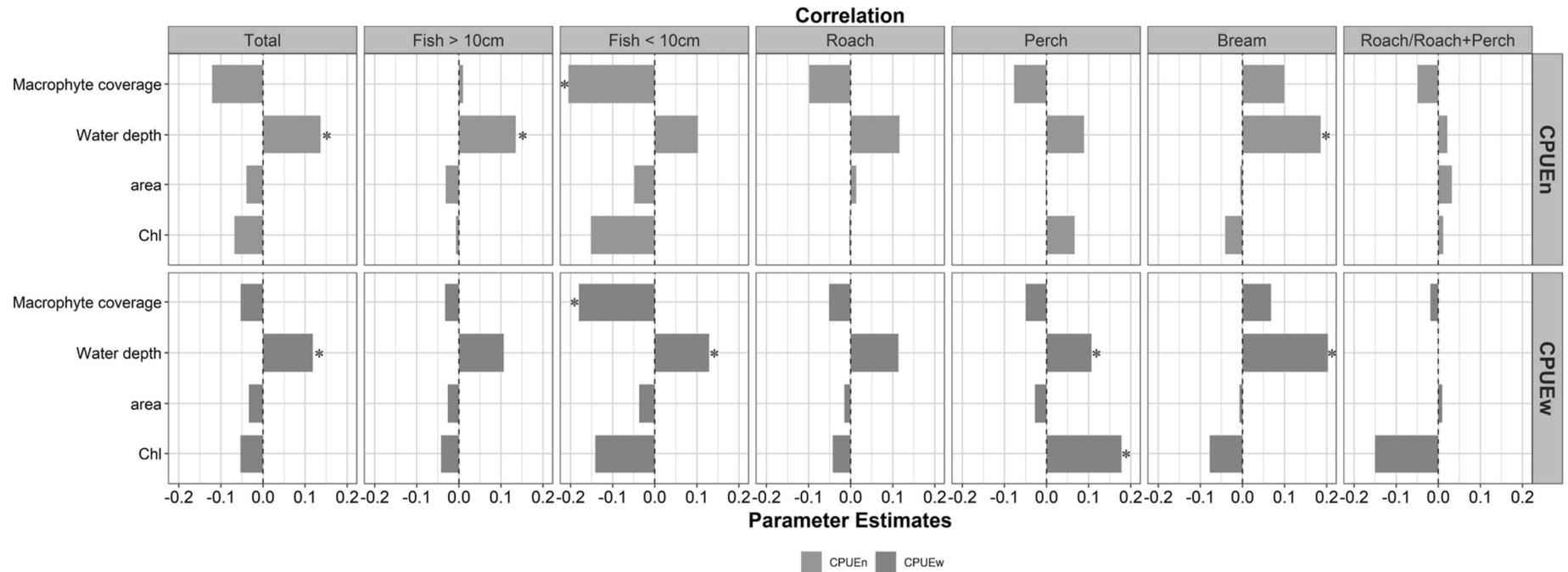


**Figure S1:** Parameters estimates for the slopes from correlation between fish and macrophyte coverage within the lakes considering lakes with at least 8 gillnets.

**APPENDIX X** – Relationship between macrophyte coverage and fish variables
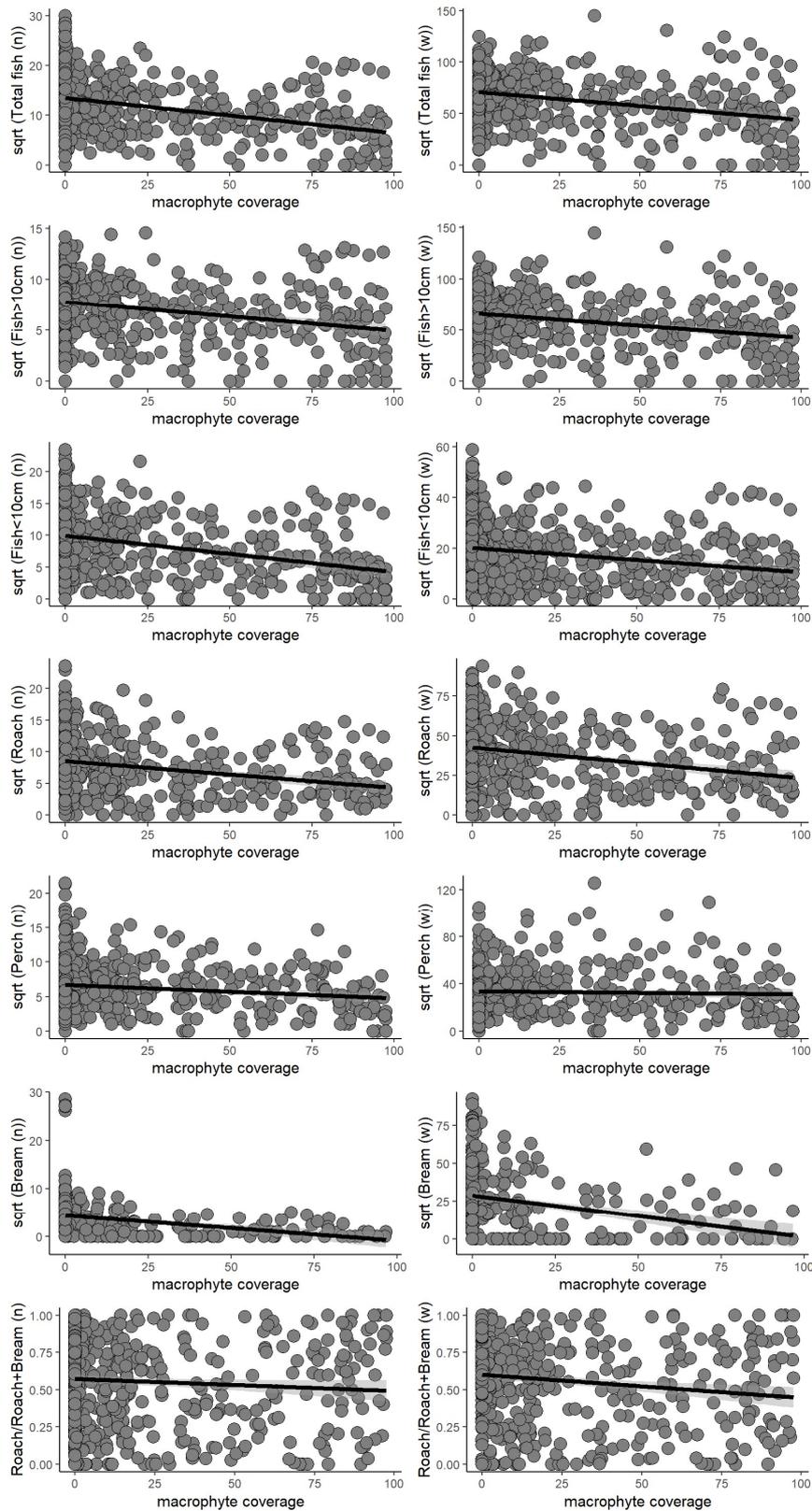


**Figure S2.** Relationship between macrophyte coverage and fish variables considering the point scale.
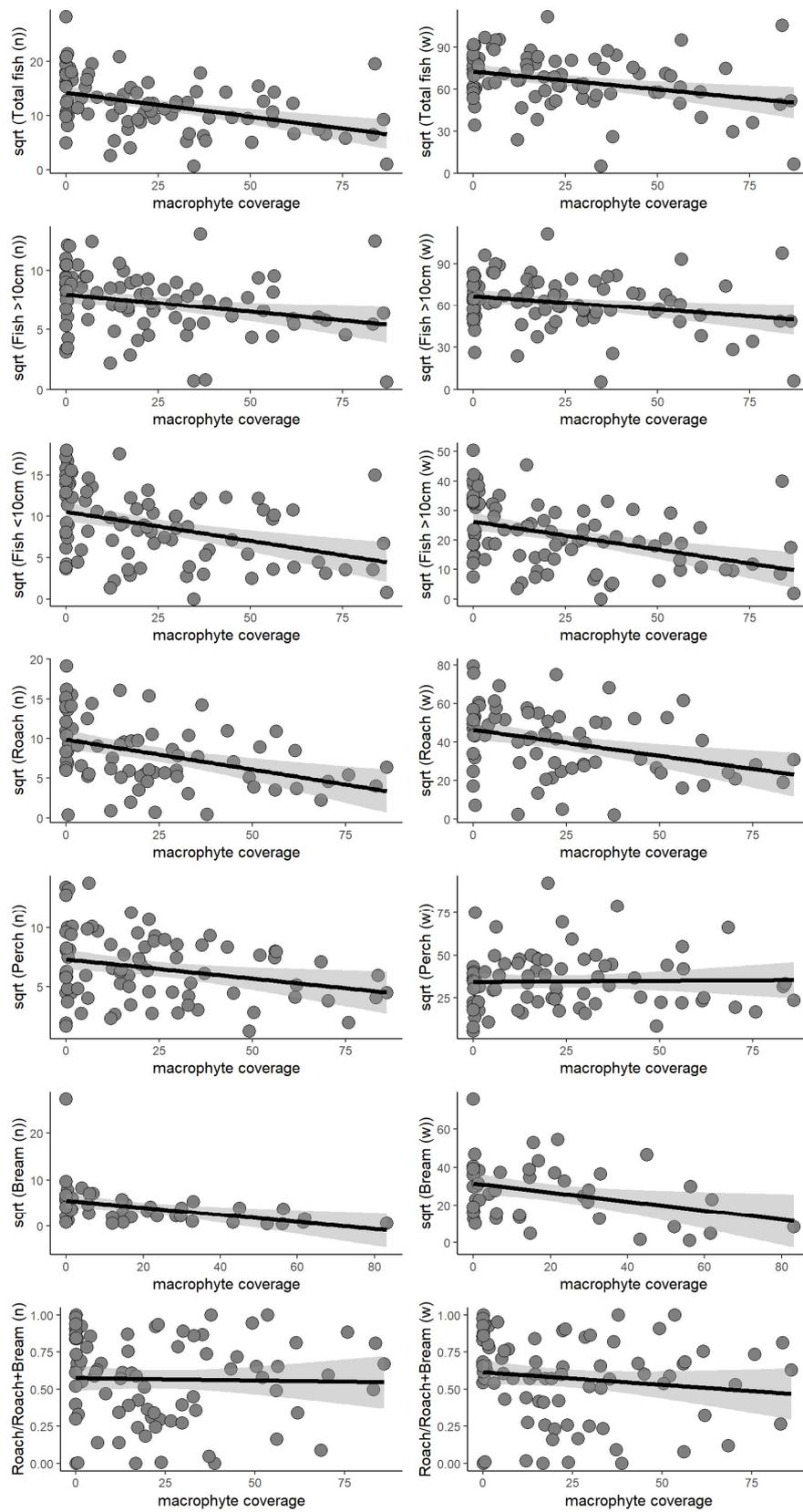
**Figure S3.** Relationship between macrophyte coverage and fish variables considering the lake scale

**APPENDIX W – Parameters estimates for fish abundance and biomass at both scales**
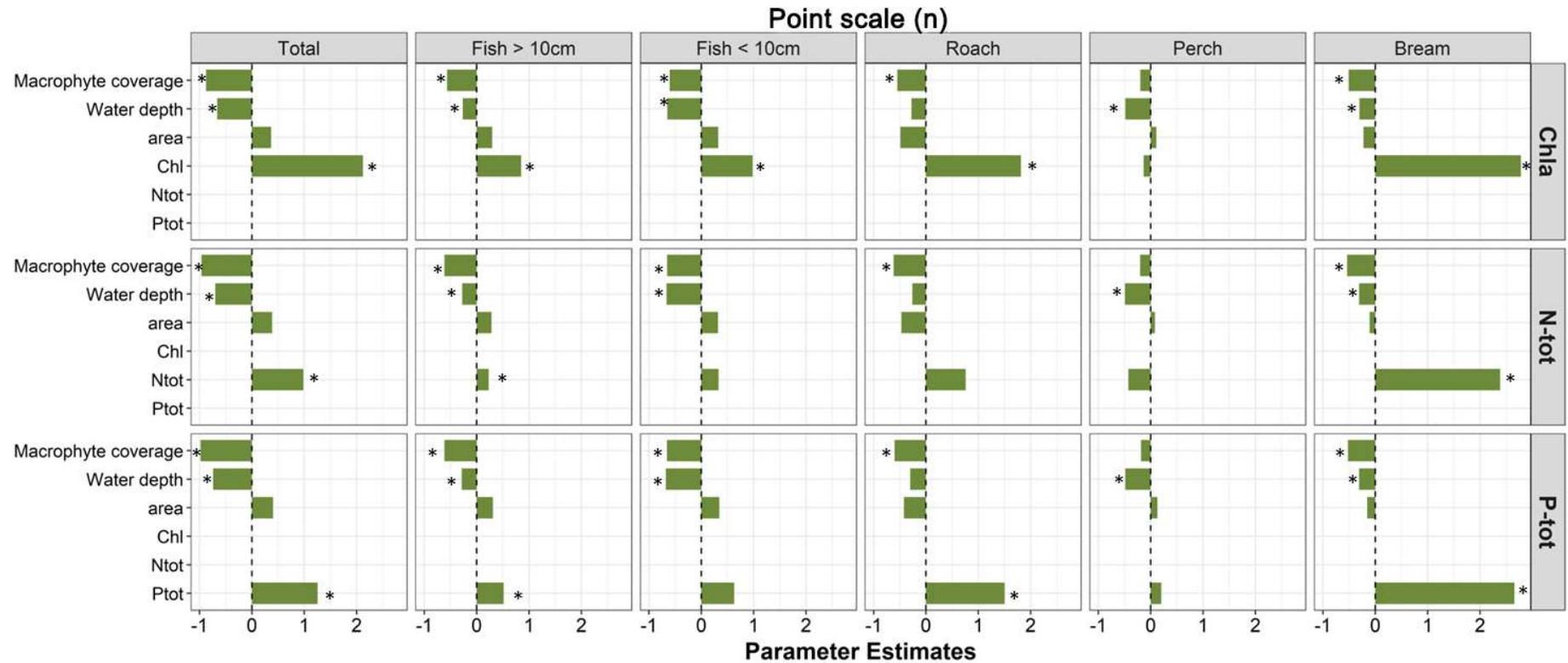


**Figure S4.** Parameters estimates for fish abundance in relation to explanatory variables considering the point scale. The dashed line represents the confidence interval. *significant variables.
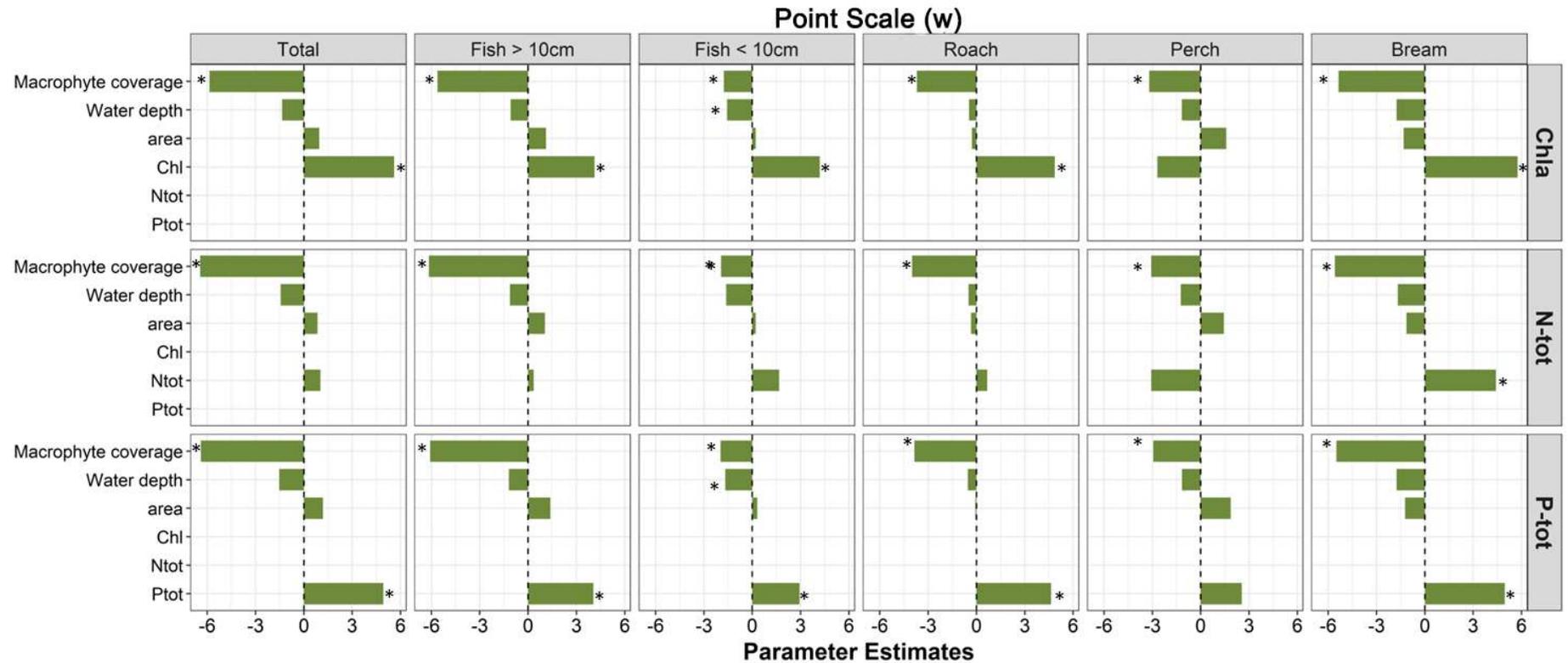
**Figure S5.** Parameters estimates for fish biomass in relation to explanatory variables considering the point scale. The dashed line represents the confidence interval. *significant variables.
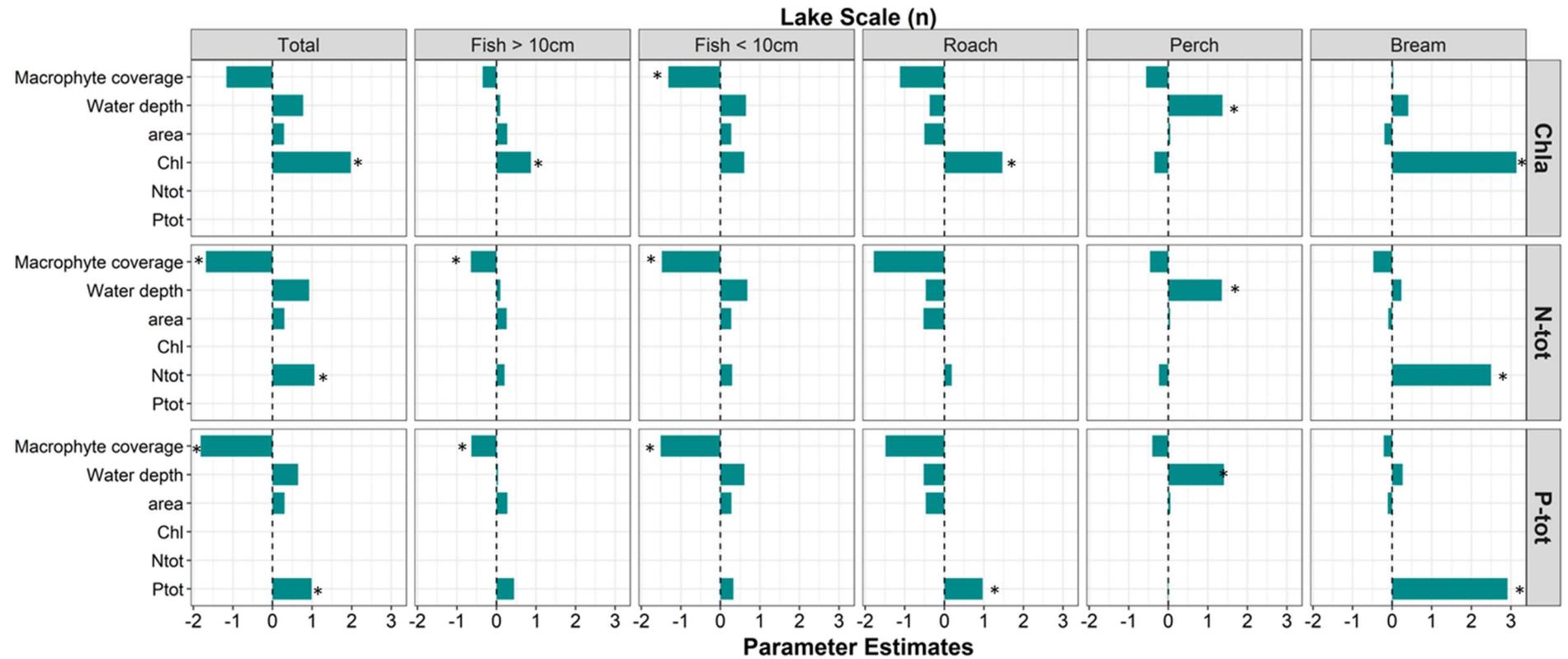
**Figure S6.** Parameters estimates for fish abundance in relation to explanatory variables considering the lake scale. The dashed line represents the confidence interval. *significant variables.
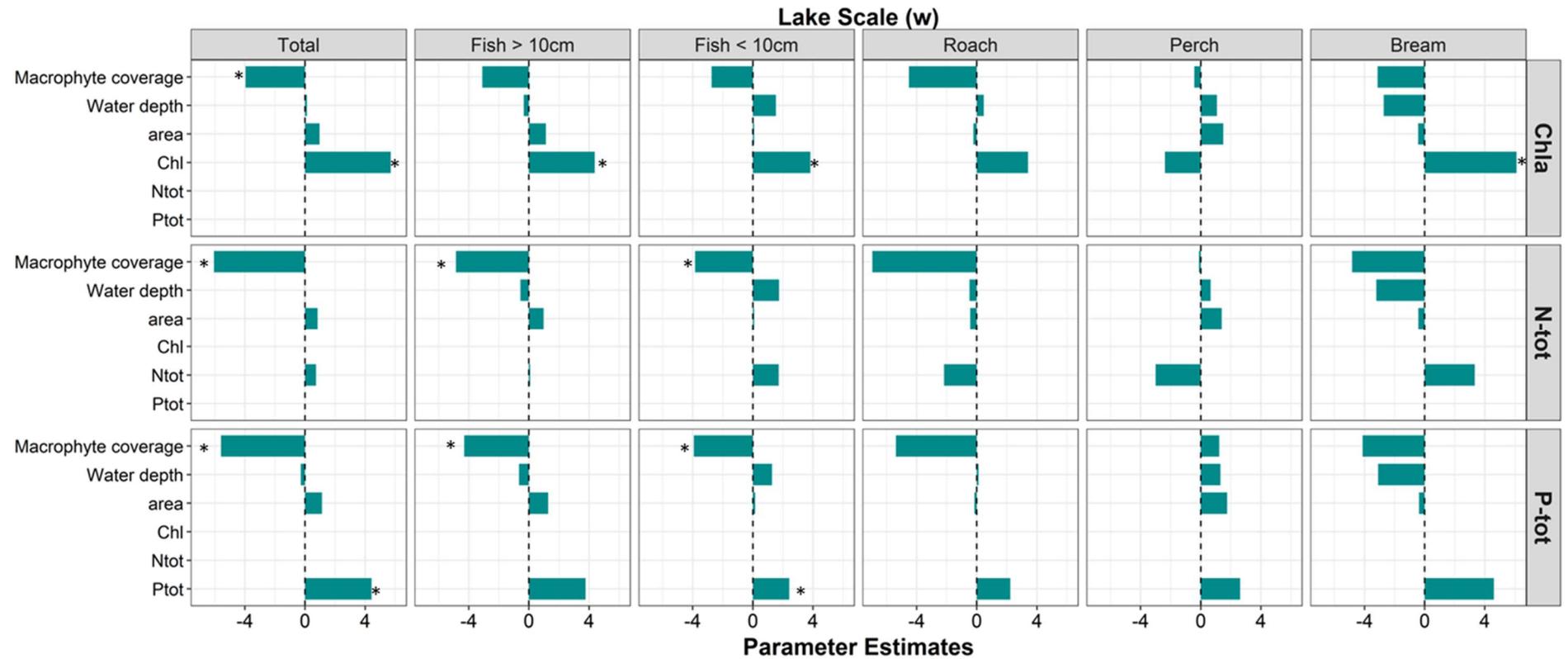
**Figure S7.** Parameters estimates for fish biomass in relation to explanatory variables considering the lake scale. The dashed line represents the confidence interval. *significant variables.
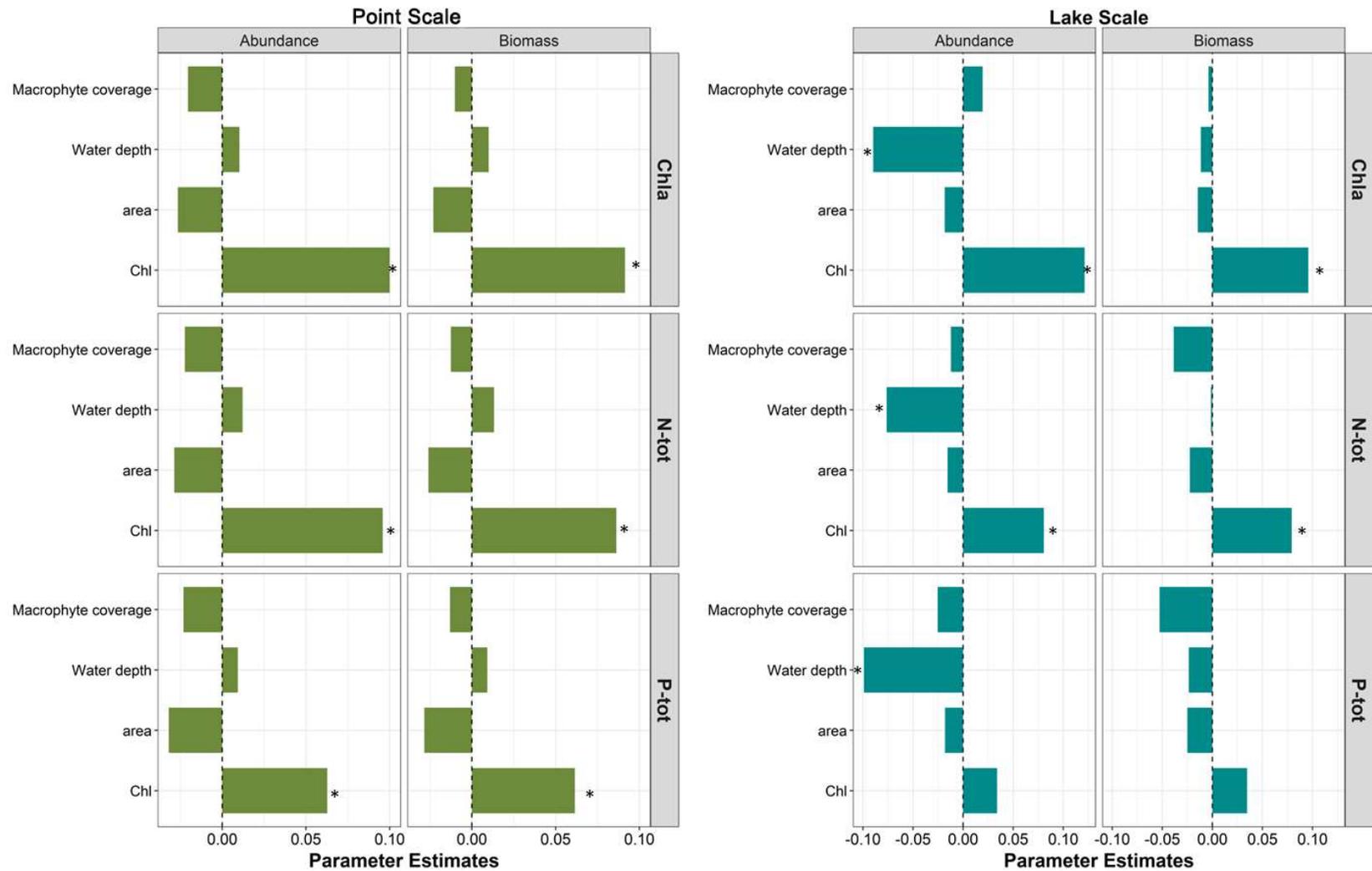
**Figure S8.** Parameters estimates for the ratio between roach and perch and the explanatory variables considering point and lake scales. The dashed line represents the confidence interval. *significant variable.