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Do cage fish farms interfere with the food aspects of the wild species *Metynnis lippincottianus* (Characiformes, Serrasalmidae)?

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ABSTRACT

We evaluated the differences in the diet and trophic guild of *Metynnis lippincottianus* under the influence of cage fish farms in the Neotropical reservoir. We collected samples from two areas (cage farm and control) in March and June 2019. Stomach contents were examined, and food items were identified and quantified using the volumetric method. Differences in diet composition were evaluated using PERMANOVA and SIMPER analyses, while trophic niche breadth was determined using PERMDISP. The trophic guild for each area was also determined. Significant differences in diet between cage farm and control areas were observed, due to consumption of pelleted feed, microcrustaceans, *Egeria* sp., and filamentous algae. In both sampling areas, *M. lippincottianus* was classified as algivorous. Despite the pelleted feed consumption in the cage farm area, no differences were observed in trophic niche breadth and the trophic guild. In addition, algae and macrophytes still accounted for the majority of this species' diet in both areas, indicating partitioning of resources. This resource partitioning may favor coexistence, but it is worth mentioning that pelleted feed consumption still indicates the influence of cage fish farms on the diet of wild fish.

Keywords: aquaculture; environmental impact; non-native species; pacu-prata; pelleted feed; trophic guild.

Pisciculturas em tanques-rede interferem sobre os aspectos alimentares da espécie silvestre *Metynnis lippincottianus* (Characiformes, Serrasalmidae)?

RESUMO

Avaliamos as diferenças na dieta e guilda trófica de *Metynnis lippincottianus* sob a influência de pisciculturas em um reservatório neotropical. Coletamos amostras de duas áreas (tanques-rede e controle) em março e junho de 2019. O conteúdo estomacal foi examinado e os itens alimentares foram identificados e quantificados usando o método volumétrico. As diferenças na composição da dieta foram avaliadas usando as análises PERMANOVA e SIMPER, enquanto a amplitude do nicho trófico foi determinada usando PERMDISP. A guilda trófica para cada área também foi determinada. Foram observadas diferenças significativas na dieta entre as áreas tanques e controle, devido ao consumo de ração, microcrustáceos, *Egeria* sp. e algas filamentosas. Em ambas as áreas de amostragem, *M. lippincottianus* foi classificado como algívoro. Apesar do consumo de ração na área tanque, não foram observadas diferenças na amplitude do nicho trófico e na guilda trófica. Além disso, algas e macrófitas ainda representam a maior parte da dieta desta espécie em ambas as áreas, indicando partilha de recursos. Essa partilha de recursos pode favorecer a coexistência, mas vale ressaltar que o consumo de ração ainda indica influências das pisciculturas em tanques-rede sob a dieta dos peixes silvestres.

Palavras-chave: aquicultura; espécies não nativas; guilda trófica; impacto ambiental; pacu-prata; ração.

INTRODUCTION

Brazil is currently among the largest producers of fish in the world (FAO, 2020) due to its predominantly tropical climate and the high availability of continental water resources (Nobile et al., 2020). Brazil has about 4.2 million hectares of backwater, which favor a freshwater cultivation model, especially using net cages (Sidonio et al., 2012). However, the high density of cultivated fish, the need for a constant flow of water, and the dependence on a high input of pelleted feed into the system can affect the environment and, in extreme cases, interfere in ecosystem processes (Cyrino and Conte, 2004; El-Sayed, 2006). In addition, cage fish farms are installed on artificially dammed rivers, changes in the natural course of water cause

additional disruptions (Hahn and Fugi, 2007), such as changes in chemical-physical compositions and water velocity (Júlio et al., 1997), and the composition of the aquatic ecosystem community (Agostinho et al., 1999).

Studies in Brazilian reservoir show that up to 18% of the organic matter used in cage fish farming (pelleted feed, fish waste, and feces) is made available to the aquatic environment (Montanhini Neto and Ostrensky, 2015; Montanhini Neto et al., 2015). Organic matter in the aquatic environment has the potential to trigger a cascade of environmental changes. According to Nobile et al. (2020), eutrophication can occur increasing in primary production and consequently reducing oxygen concentration and causing an of parasitic diseases in farmed and wild fish. In addition, there are reports of that the presence of cage fish farms interfere with community structure (Nobile et al., 2018), histopathological changes (Kliemann et al., 2018), reproductive influences (Brandão et al., 2014; Nobile et al., 2020), and changes in dietary aspects of wild fish (Barrett et al., 2018; Nobile et al., 2020; Kliemann et al., 2018, 2022).

Changes in wild fish feeding associated with cage fish farming are mainly reported for fish with opportunistic/generalist feeding behavior (Brandão et al., 2012, 2013, 2014; Demétrio et al., 2012; Ramos et al., 2008, 2013; Kliemann et al., 2018, 2022). Species with opportunistic/generalist behavior use the most available food resources, regardless of the origin (plant, animal, autochthonous, and/or allochthonous) (Gerking, 1994; Abelha et al., 2001). Therefore, the presence of food items from cultivation cages, such as pelleted feed, may cause a decrease in the consumption of natural foods, affecting other trophic levels of the food chain (Demétrio et al., 2012; Ramos et al., 2013; Kliemann et al., 2018, 2022).

Fish feeding is directly related to the availability of environmentally dependent food resources (Schoener, 1974; Ross, 1986; Prejs and Prejs, 1987; Russo et al., 2002). Thus, studies on the diets of wild fish species associated with cage fish farms are of paramount importance to demonstrate the influence of organic matter input on the ichthyofauna in these areas (Ramos et al., 2013; Edwards, 2015). Determining the feeding guild of a wild species as affected by cage fish farms can also contribute to food web data and provide a foundation for work on the management and conservation of aquatic ecosystems (Ximenes et al., 2011).

Among the fish species found in the Upper Paraná River, *Metynnis lippincottianus* (Cope, 1870), popularly known as "pacu CD, pacu-marreca or pacu-prata" exhibits opportunistic/ generalistic feeding behavior (Hahn et al., 2002; Santos, et al., 2004; Ramos et al., 2008; Demétrio et al., 2012; Froese and Pauly, 2019). This species is native to the Amazon River, but its distribution covers South America (Froese and Pauly, 2019). According to studies carried out in Upper Paraná River basin the diet of *M. lippincottianus* is composed mainly of filamentous algae, terrestrial and aquatic plants, items of animal origin, and detritus (Ramos et al., 2008; Kliemann et al., 2022). Furthermore,

depending in food availability, species of the genus *Metynnis* may use various tactics for feeding and exploring other trophic levels, demonstrating high trophic plasticity (Ramos et al., 2008; Kliemann et al., 2022).

Considering the trophic plasticity of *M. lippincottianus* and the influence of cage fish farming in the aquatic environment, we evaluated the effect of cage fish farms in a reservoir dam on the feeding and trophic guild of *M. lippincottianus*. We hypothesized that the nutrient load from the cultivation cages promote changes in the trophy of the environment, with consequent changes in the feeding aspects of *M. lippincottianus*. Thus, we tested the following predictions:

- the diet composition of *M. lippincottianus* differs between the area around the cultivation cages (cage farm area) and the area without the influence of cage fish farms (control area);
- wild individuals from the cage farm area consume food provided by the cultivation cages, mainly pelleted feed;
- cage fish farms influences the foraging of *M. lippincottianus* and promote a trophic niche contraction in the cage farm area;
- the influence of cage fish farms results in a change in the trophic guild of *M. lippincottianus*.

MATERIAL AND METHODS

Study area

The Ilha Solteira reservoir, formed in 1978, is located on the Upper Paraná River, between the states of São Paulo, Mato Grosso do Sul, and Minas Gerais. This reservoir is of the accumulation basin type, with an average depth of 17.6 m, a maximum volume of 21.06 x 106 m³, a hydrographic basin of 1,195 km² in area, and a residence time of 47.67 days (Garcia et al., 2014). There are approximately 72 cage fish farms in activity, producing mainly *Oreochromis niloticus* (Linnaeus, 1758) and its strains (CDRS, 2021).

The study was carried out at five sampling points, grouped into two areas: cage farm and control. The cage farm area comprised three cage fish farms (CF1, CF2, and CF3). The control area comprised two areas upstream from the cage fish farms (approximately 10 km), free from the influence of cage fish farms (CT1 and CT2) (Figure 1).

Collection of biological material

The collection period comprised the rainy and dry periods of 2019 (March and June). Specimens were collected using gill nets (3 to 18 cm between non-adjacent nodes) in both areas (SISBio License 64763-2, CEUA/FEIS Authorization 006/2019; SisGen Certificate A908D5F). All specimens collected were euthanized (benzocaine 5%), identified, measured for standard length (cm) and total mass (g), and the stomachs were removed, fixed (formaldehyde solution 4%) and preserved in 70% alcohol for further laboratory analysis.



CT: control; CF: cage farm.

Figure 1. Sampling areas in the Ilha Solteira reservoir, Upper Paraná River basin, São Paulo, Brazil.

Laboratory analysis

Stomach contents were examined under an optical stereomicroscope, and food items were identified to the lowest possible taxonomic level using the identification keys such as Bicudo and Bicudo (1970) and Mugnai et al. (2010). Food items were quantified according to the volumetric method (Hyslop, 1980): the volume of each item was obtained by displacement of liquid in a beaker or millimeter petri dish (food items were compressed with glass slides of up to 1 mm in height, and the number of quadrants occupied by food item was multiplied by 0.001 to obtain the volumes in milliliters) (Hellawell and Abel, 1971).

Data analysis

To summarize the composition of the diet of *M. lippincottianus* in each sampling area (cage farm and control), we created a table with percentage values of the volume of each food item. For statistical analysis, we used a matrix of raw volume data of all consumed food items.

To compare the significance of differences in the food composition of M. *lippincottianus* between the cage farm and

control areas, we used one-way PERMANOVA (Multivariate Permutation Analysis of Unidirectional Variance) was used based on the Bray-Curtis distance with 999 random permutations (Anderson, 2001). To confirm whether the observed differences were related to the factors analyzed (differences in diet composition between the sampling areas) (p > 0.05) or only related to the dispersion or heterogeneity of the samples (p < 0.05) (Anderson, 2006), a Permutation Analysis of Multivariate Dispersions (PERMDISP) was applied to the same dataset. In addition, to test whether individuals in the cage farm area consumed the food (mainly pelleted feed) provided by the cultivation cages, the SIMPER overall pool similarity analysis was applied (Clarke, 1993) with the same data matrix and Bray-Curtis distance (Hammer et al., 2001).

To test whether cage fish farms influence the foraging of *M. lippincottianus* and promote trophic niche contraction in the cage farm area, PERMDISP was also used. The trophic niche breadth was measured through the dispersion of the diet in space. The differences in distance between the specimens indicate that some specimens have more restricted or broader diets than others. PERMDISP measures the distance of the multivariate median of the group (similar to the centroid), in this case, the

populations in each sampling area, through a principal coordinate analysis (PCoA) (Silva et al., 2017). The calculation of the group median is performed using the Bray-Curtis dissimilarity measure, which allows the mean dissimilarity comparison in n individual observations within the group. A permutation test was calculated to compare the mean distance of each sample to the group median, to test the null hypothesis that trophic niche breadth did not differ between groups (cage farm and control). The permutation test is a test statistic is a pseudo-F-ratio, similar to the F-ratio in ANOVA. The p-value was obtained through 999 permutations of least squares residuals (Anderson, 2006).

Finally, to verify whether cage fish farms promote a change in trophic guild, the trophic guild in each area was calculated using stomach content matrices and classification. For example, more than 50% algae in the stomach: algivorous; more than 50% detritus/sediments: detritivorous; more than 50% aquatic insects: aquatic insectivorous; more than 50% various invertebrates and fish in the stomach: carnivorous; similar proportions of detritus and aquatic insects: detritivorous/ insectivorous; none of the above statements valid and contents included items from different origins (plants and animals): omnivorous (Neves et al., 2015).

PERMANOVA, SIMPER, PERMDISP and permutation test were performed using the software R (R Core Team, 2021) with the aid of the vegan package (Oksanen et al., 2021) and the functions *adonis*, *simper*, *betadisper*, and *permutest*. *betadisper*, respectively. The significance level adopted for all analyses was p < 0.05.

RESULTS

The stomach contents of 69 specimens were analyzed (Table 1). The main items consumed in the cage farm area were pelleted feed (25.93%), Rhodophyceae (14.74%), and *Egeria* sp. (14.56%) (Table 1; Figure 2). In the control area, the main items consumed were unidentified macrophytes (21.29%), Chlorophyceae (20.14%), and Rhodophyceae (14.69%) (Table 1; Figure 2). *Metynnis lippincottianus* was classified as algivorous in both areas, due to the consumption of 61.5% of algae in the control area and 50.9% in the cage farm area (Figure 2).

There was a significant difference in diet composition between the sampling areas (PERMANOVA, Df = 1; F = 4.14; p < 0.05). The food items that most contributed to this difference were pelleted feed, microcrustaceans, *Egeria* sp., and filamentous algae such as Rhodophyceae, Cladophoraceae, and Chlorophyceae (Table 2). No significant difference was found in the trophic niche breadth between the areas (PERMDISP, Df = 1; F = 0.689; p > 0.05).

Plastic fragments was found in 19 fishes from both areas in the cage farm area (11 stomachs) and control area (8 stomachs). The plastic fragments presented blue, green, and black coloration, with fibrous textures or fragments.

 Table 1. Diet composition of *Metynnis lippincottianus* in the control and cage farm areas, Ilha Solteira reservoir, Upper Paraná River basin, São Paulo, Brazil. Values-based on a percentage of food item volume data. Values in bold indicate the most consumed items.

Local	Cage farm	Control	
Number of stomachs analyzed	40	29	
Food items	Volumetric percentage (%)		
Autochthonous (80.64%)			
Unidentified macrophyte	1.86	21.29	
<i>Egeria</i> sp.	14.56	9.61	
Chlorophyceae	7.23	20.14	
Ulothricaceae	5.23	1.20	
Cladophoraceae	9.86	9.85	
<i>Spyrogira</i> sp.	0.01	0.15	
Zygnemataceae	7.80	6.08	
Rhodophyceae	14.74	14.69	
Bacillariophyceae	2.20	1.93	
Cyanophyceae	3.85	7.49	
Nematoda	*	*	
Microcrustacean	0.70	0.50	
Aquatic insect	0.13	0.04	
Cycloid scale	0.09	0.04	
Allochthonous (14.48%)			
Terrestrial vegetable	0.57	2.41	
Acari		*	
Terrestrial insect	*	0.04	
Pelleted Feed	25.93		
Undetermined (4.88%)			
Detritus	5.22	4.55	

*Values below 0.01.



Figure 2. Volumetric percentage (%) of consumed food items and trophic guild of *Metynnis lippincottianus* in the cage farm and control areas of the Ilha Solteira reservoir, Upper Paraná River basin, São Paulo, Brazil.

	Overall average dissimilarity	Contribution %	Cumulative Contribution %	Average abundance CT	Average abundance CF
Pelleted feed	92.98	16.75	16.75		0.24
Microcrustacean		12.91	29.66	0.16	0.02
<i>Egeria</i> sp.		12.51	42.17	0.08	0.14
Rhodophyceae		11.29	53.46	0.09	0.14
Cladophoraceae		7.74	61.2	0.08	0.09
Chlorophyceae		7.72	68.93	0.16	0.07

Table 2. Result of the dissimilarity analysis (SIMPER) for the proportion of food items consumed by *Metynnis lippincottianus* between the sampling areas (CT and CF) in the Ilha Solteira reservoir, Upper Paraná River basin, São Paulo, Brazil.

CT: control; CF: cage farm.

DISCUSSION

Our hypothesis that nutrient load from the cultivation cages promotes changes in the trophy of the environment, with consequent changes in the feeding aspects of *M. lippincottianus*, was corroborated. Diet composition differed between sampling areas, and pelleted feed consumption was observed only in the cage farm area, although no change in the trophic guild was observed between areas. According to reports from studies from different geographic areas, *M. lippincottianus* is opportunistic trophic and consumes a variety of food items, such as aquatic plants, seeds, phytoplankton, mollusks, crustaceans, and detritus (Moreira et al., 2009; Yamada et al., 2012; Hoshino and Tavares-Dias, 2014). Here, we observed the consumption of mainly aquatic vegetables and algae, in addition to insects and microcrustaceans, and pelleted feed in the cage farm area.

The consumption of a wide variety of food items indicates that the species has the potential to use energetic and abundant food resources in the environment (Abelha et al., 2001). Thus, we infer that plasticity and trophic opportunism explains the pelleted feed consumption in the cage farm area, as it is a resource available in abundance and easily acquired. Studies show that a significant percentage of the pelleted feed available to cage fish farms is not consumed and is available in the aquatic environment as food for wild fish (Montanhini Neto and Ostrensky, 2015; Cacho et al., 2020). The consumption of pelleted feed can result in changes in the nutritional status and food chain of the local aquatic ecosystem (Brandão et al., 2012; Ramos et al., 2013; Edwards, 2015). For the species M. lippincottianus, as it is a secondary consumer (Dias et al., 2005), the consume of pelleted feed changes can cause a cascade of effects in the trophic levels related to the increased density of producers and consequent imbalance in the aquatic ecosystem (Ramos et al., 2010).

Despite the pelleted feed consumption in the cage farm area, there was no change in trophic guild classification between the sampling areas. *Metynnis lippincottianus* was classified as algivorous in both areas, however, recent studies have classified this species as a herbivore (Yamada et al., 2012; Hoshino and Tavares-Dias, 2014; Sá-Oliveira et al., 2014; Kliemann et al., 2022). The higher consumption of algae by *M. lippincottianus* in this study may be associated with the proliferation of macrophytes observed in reservoirs (Hahn and Fugi, 2007). According to the authors of the previously mentioned study, macrophytes act as substrates for the development of various algae. In addition, the study area (Ilha Solteira reservoir) contained a predominance of submerged plants represented by the species *Egeria najas* Planch. and *Eichhornia crassipes* (Mart.) Solms (CESP and DRENATEC Engenharia Ltda, 2009). Considered along with the opportunistic behavior of *M. lippincottianus*, we assume that the categorization of algivore obtained in this study is due to the greater availability of algae, favored by presence of macrophytes in both areas (second largest item consumed by the species).

Although there was a difference between the diet composition of *M. lippincottianus* in the cage farm cultivation and control area, mainly due to the pelleted feed, the trophic niche breadth did not vary. This result indicates that the diet between individuals within each population and between populations are homogeneous. Studies report that homogeneous diets may indicate the partitioning of food resources favoring intraspecific coexistence and population stability (Neves et al., 2018; Soares et al., 2020; Kliemann et al., 2021). Thus, we assumed that the homogeneity in the diets and the high consumption of algae and macrophytes indicate the partitioning of resources.

Our study also found plastic fragments in the stomachs of fish in both areas. This concerning because these synthetic objects can cause negative effects on fish when ingested (Pinheiro et al., 2017) such as obstruction of the digestive tract, inflammation, laceration of gastrointestinal tissues and can lead to the death of fish (Lusher et al., 2013; Rochman et al., 2013; Pedà et al., 2016; Pappis et al., 2021). These plastic fragments are light and have different sizes and colors, so it is easily ingested by fish that confuse them as pieces of food (Pinheiro et al., 2017). Furthermore, plastic fragments easily lodge in filamentous algae and aquatic plants (Costa et al., 2011). Thus, we suggest that the accidental ingestion of these plastic fragments is associated with the types of food consumed by the species. Still, considering the materials used in the containment of the cage fish farm, such as ropes, these plastic fragments may have come from the cage fish farm, demonstrating another influence of these farming models (Sandra et al., 2020; Skirtun et al., 2022; Tian et al., 2022). These findings point to the growing problem of plastic products contaminating aquatic environments and being consumed by the biota with adverse consequences.

CONCLUSION

In summary, we observed interferences of cage fish farm on the trophy of the environment due to pelleted feed consumption by M. lippincottianus only cage farm area. Thus, there were changes in food between areas but not to the point of altering the trophic guild. We confirmed that these farming models change the dietary aspects of wild fish. Another important result is the high consumption of macrophytes and algae in both sampling areas. We infer that occur trophic partitioning due to this high abundance of algae, macrophytes, and pelleted feed, favoring intraspecific coexistence. However, despite this favoring of pelleted feed availability for partitioning resources, the longterm effects of pelleted feed consumption are uncertain. We suggest future studies that evaluate the effects on wild fish in a longer time frame to verify if the population size is being affected. Therefore, we emphasize the importance of studies on the trophic ecology of wild fish under the influence of cage fish farms. The reported consumption of plastic fragments in both sampling areas further reinforces the importance of these studies. Thus, the data reported here can deepen our knowledge of fish farming activities and assist in the management of cage fish farms and excess pelleted feed in the surroundings.

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CONFLICT OF INTERESTS

Nothing to declare.

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AUTHORS' CONTRIBUTIONS

Ramos, J.K.K.: Funding acquisition, Project administration, Writing — original draft, Writing — review & editing. Bonfim, V.C.: Writing — review & editing. Kliemann, B.C.K.: Conceptualization, Methodology, Project administration, Writing — review & editing. Garves, J.D.S.: Writing review & editing. Delariva, R.L.: Writing — review & editing. Ramos, I.P.: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing review & editing.

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