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Influence of seasonality on macroinvertebrate diversity associated with the aquatic fern *Salvinia biloba* Raddi

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Abstract. The genus *Salvinia* is composed of fast-growing floating ferns, capable of surviving in different environmental conditions. Some authors suggest that the relationships between this genus and macroinvertebrates may serve as water quality indicators. The present study aimed to determine the influence of seasonality and water quality on macroinvertebrate diversity associated with the *Salvinia biloba* Raddi. Water and fern were collected in rainy and dry seasons and was conducted a microbiological analysis of the water, as well as, the area of fern cover on the water, richness, and density of macroinvertebrates. Microbiological analysis of the water detected > 5,700 CFU/mL (rainy season) and 175 CFU/mL (dry season) of heterotrophic bacteria and was positive for total thermotolerant coliforms. The *S. biloba* cover on the water surface was 100% in the rainy and 30% in the dry season. In the rainy season, 142 macroinvertebrates were identified, divided into 12 morphospecies, with a density of 434 individuals/m^3. In the dry season, there were 419 individuals in 14 morphospecies, with a density of 2,076 individuals/m^3 exhibited. The highest species density recorded in the rainy season was for Chironomidae sp.1 (Diptera) (57.71%) and Odonata Zygoptera sp.1 (17.44%), and in the dry season, Chironomidae sp.2 (73.98%) followed by Gerromorpha sp.1 (Hemiptera), with 9.54%. The Sørensen similarity index between the two seasons was 53.84%. The higher density of Gerromorpha sp.1 in the dry season may indicate an increase in environmental integrity.

Keywords: Bioindicators; Insect-fern interaction; Water microbiology.

Aquatic macrophytes are plants whose photosynthesizing structure is floating, permanently or periodically submerged (Cook 1996; Irgang & Gastal 1996). Aquatic macrophytes provide fauna shelter, a refuge from predators, oviposition sites, and diversified food sources, given that they are also substrate for peripheral algae, and filter organic particles that can be used by detritivores (Trivinho-Strixino *et al.* 2000; Dornfeld & Fonseca-Gessner 2005).

Aquatic macroinvertebrates, such as arachnids, insects, crustaceans, and mollusks, are groups of invertebrates over one-millimeter-long at the end of the larval stage or in the imaginal phase and can be seen with the naked eye (Mugnai *et al*. 2010; Buss *et al*. 2016). Aquatic macroinvertebrates can be classified based on their habitat, as follows: planktonic, nektonic, pleustonic or benthic, with variations such as epibenthic, which inhabit both water bodies and the substrate, normally in different stages of life (Mugnai *et al*. 2010).

The macroinvertebrate community contributes to water quality since they are efficient bioindicators that describe all environmental stresses (Dornfeld & Fonseca-Gessner 2005). They are more effective than biological inferences made by comparing values obtained in laboratory trials or instantaneous field measures.

Aquatic ferns belong to the genera *Salvinia* and *Azolla* (Salviniaceae); *Marsilea*, *Pilularia* and *Regnellidium* (Marsileaceae) and *Isoëtes* (Isoëtaceae) (PPG I 2016). Species of the genus *Marsilea* are popularly known as four-leaf clovers or lucky clovers. Mauz & Reeder (2009) reported the occurrence of weevils (small beetles) that use sporocarps of *Marsilea mollis* B.L. Robins & Fern as a breeding site. In the Pantanal region of Mato Grosso state and Central Amazonia, Brazil, Sousa (2008) recorded aquatic and semi-aquatic species of Curculionoidea (Insecta, Coleoptera) associated with species of *Azolla*, *Salvinia auriculata* Aubl. and *Salvinia minima* Baker.

Most studies on the interaction between insects and aquatic ferns involve species of the genus *Salvinia* (Pelli & Barbosa 1998a, 1998b; Callisto *et al*. 2002; Prellvitz & Albertoni 2004; Dornfeld & Fonseca-Gessner 2005; Bervian *et al*. 2006; Sousa 2008). These species exhibit very fast growth, sometimes completely covering the water surface (Beyruth 1992), making them an important substrate for establishing associations with fauna (Callisto *et al*. 2002).

The genus *Salvinia* contains 12 species worldwide (PPG I 2016), 10 of which can be found in Brazil (Salviniaceae in Flora do Brasil 2020). Interactions with aquatic insects were recorded in four of these: *S. auriculata* (Callisto *et al*. 2002; Prellvitz & Albertoni 2004; Bervian *et al*. 2006; Sousa 2008), *S. herzogii* de la Sota (Prellvitz & Albertoni 2004), *S. minima* (Sousa 2008) and *S. molesta* D.S. Mitch (Pelli & Barbosa 1998a, 1998b).

However, there is no record of interactions with *S. biloba*, a plant native to Brazil, found in all the regions of the country.

The present study aimed to (1) analyze the microbiological quality of water in the dry and rainy seasons in a stretch of the Aldeia River, municipality of São Gonçalo, Rio de Janeiro state, Brazil; (2) record aquatic macroinvertebrates associated with the aquatic fern *S. biloba*; (3) record seasonal variation (dry and rainy season) in the richness and abundance of macroinvertebrates associated with *S. biloba*; and (4) assess the influence of microbiological water quality on the community of macroinvertebrates associated with *S. biloba* in the dry and rainy seasons.

MATERIAL AND METHODS

Study area. The Aldeia River is one of the tributaries of the Caceribu River, one of the main contributors to Guanabara Bay, Rio de Janeiro state, Brazil (Souza-Lima *et al*. 2012). The 16km-long Aldeia River, whose source is between Serra da Tiririca and Serra de Itaitindiba, flows through rural and urban areas. The main negative impacts are silting caused by agribusiness and mining, as well as improper sewage and waste disposal (Souza-Lima *et al*. 2012).

Collections were conducted in a 10m-wide lake (22°52'39.4" S and 42°57'21.0" W), on a stretch of the Aldeia River influenced by agribusiness activity, located in the municipality of São Gonçalo, Rio de Janeiro state, Brazil.

Water collection and analysis. Samples of the river water were collected in two sterile Nasco bags containing 100 mL each. After collection, the bags were sealed and refrigerated until analysis in the Microbiology Laboratory of the Faculdade de Formação de Professores (Teacher Training School) of the UERJ. The analysis was carried out according to Collegiate Directorate Resolution (RDC) 357, of March 17, 2015, established by the National Council for the Environment (CONAMA).

The Colibert system (patented by IDEXX Laboratories) was used to detect the microbiological indicators. The system is applied for simultaneous detections, specific identifications and confirmation of total coliforms and *Escherichia coli* T. Escherich in natural or treated continental water. Colibert uses nutrients (sugars linked to chromogenic organic radicals) that make the microorganisms of interest present in the sample produce a change of color (or fluorescence) in the inoculated system (IDEXX 2018).

Collection of macroinvertebrates associated with *Salvinia biloba*. Water and *S. biloba* were collected in January 2016 (rainy season) and August 2017 (dry season). Plant sampling occurred in two 25 x 25 cm transects (sampling area of 0.125 m^2). The volume occupied by submerged leaves was obtained by calculating the average leaf length multiplied by the transect area. The area of fern cover on the water surface (10 m-wide lake) was visually assessed.

The ferns were stored in plastic bags, with 70% alcohol and sent to the Biodiversity Laboratory of the Faculdade de Formação de Professores (FFP - Teacher Training School) of the Universidade do Estado do Rio de Janeiro (UERJ - Rio de Janeiro State University). The plants were washed with 70% alcohol in a 0.225 mm mesh sieve and examined under a stereoscopic microscope. The macroinvertebrates found were identified into taxonomic groups and separated by morphotypes using the identification keys of Mugnai *et al.* (2010).

The number of individuals, richness, density per m^3, diversity (Simpson 1-D and Shannon H) and Sørensen similarity index (Brower *et al*. 1997) were determined for each season. Relative density was calculated using the formula DR= (ns/N) x 100, where ns is equivalent to the number of individuals sampled per species, and N the total number of individuals sampled.

The statistical tests were conducted using the PAST program (PAleontological STatistics), version 3.10.

RESULTS AND DISCUSSION

Microbiological analysis of the Aldeia River water. Microbiological analysis of the water detected > 5700 CFU/mL (rainy season) and 175 CFU/mL (dry season) of heterotrophic bacteria and was positive for total thermotolerant coliforms. Heterotrophic bacteria count, genetically defined as microorganisms that require organic carbon as a nutrient source, provides extensive information on the bacteriological quality of water. The test includes nonspecific detection of bacteria or bacterial spores of fecal origin, components of natural water flora or resulting from biofilm formation (Domingues *et al*. 2007). High concentrations of organic matter provide favorable conditions for heterotrophic bacteria development (Oliveira 2012).

Cover, leaf area and density of macroinvertebrates associated with *Salvinia biloba*. *Salvinia biloba* cover on the water surface was 100% in the rainy and 30% in the dry season (Table 1, Figure 1). The length of submerged leaves, as well as their estimated area (m^3), was greater in the rainy season (Table 1, Figure 2). The decline in *S. biloba* cover in the dry season caused higher macroinvertebrate density (2,076 individuals/m^3) on their submerged leaves, compared with the rainy season (434 individuals/m^3). However, species richness was similar in the two seasons analyzed (Table 1).

Microbiological analyses demonstrated a difference in water quality between the dry and rainy seasons, the latter exhibiting a high heterotrophic bacteria count (>5,700 UFC/mL), which may indicate high organic matter concentrations in the water. High concentrations of organic matter provide favorable conditions for heterotrophic bacteria development. Several factors can interfere in aquatic plant growth; among them the nutrients level (Medeiros *et al.* 2017). The higher percentage of *S. biloba* cover in this season is likely related to the poor water quality since this species benefits from the greater nutrient supply in the water (Robinson *et al*. 2010).

According to Boschilia *et al*. (2006), analysis of *Salvinia herzogii* de la Sota leaf length in relation to stand size indicated that at high densities the length of submerged leaves increased

Table 1. Cover, *Salvinia biloba* Raddi leaf area and density of associated aquatic macroinvertebrates in different seasons of the year.

	Rainy 2016	Dry 2017
Cover area on the water surface	100%	30%
Submerged leaf length	5.235 ± 2.55 cm	3.23 ± 0.94 cm
Estimated submerged leaf area	0.3268 m^3	0.2018m^3
No. of macroinvertebrates	142	419
Density	434 individuals/m^3	2,076 individuals/m^3
Richness	12	14

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Figure 1. Water surface cover by the aquatic fern *Salvinia biloba* Raddi. A: Rainy season; B: Dry season. (Source: M. Guerra Santos).

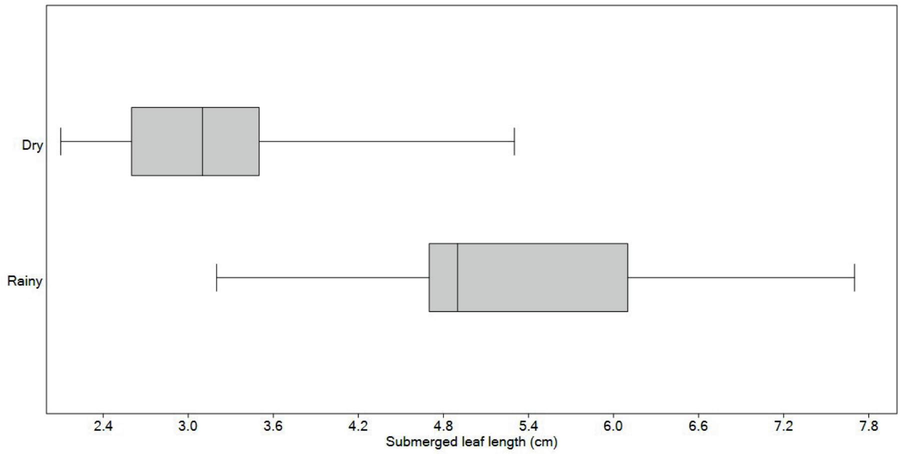

Figure 2. Submerged *Salvinia biloba* Raddi leaf length in the dry and rainy seasons. According to the Mann-Whitney test, the two measures were significantly different (p = 0.00001988).

compared to low and medium densities, likely caused by competition for nutrients in the water and the need to raise absorption. Medeiros *et al.* (2017) demonstrated experimentally higher *S. auriculata* in conditions of moderate shade compared to other light conditions, concluding that a shaded environment does not limit the clonal growth of this plant, but does produce more branches.

The rapid growth of some *Salvinia* species can pose a serious environmental threat by enabling them to spread across the entire water surface, restricting light penetration and removing nutrients. As a result, they compete with native plants, reducing habitat diversity and limiting food sources in the food chain, especially fish. However, they can also be used as bioremediators in sewage treatment (Robinson *et al.* 2010).

Macroinvertebrates associated with *Salvinia biloba*. The Simpson's Diversity Index (1-D) didn't show difference between rainy (0.9167) and dry (0.9286) seasons (Table 2). However, in the rainy season, 142 macroinvertebrates were identified, divided into 12 morphospecies, with a density of 434 individuals/m^3. In the dry season, there were 419 individuals in 14 morphospecies, with a density of 2,076 individuals/m^3 (Table 2). Prellvitz & Albertoni (2004), who analyzed a population of *Salvinia* spp. (*S. auriculata* e *S. herzogii*) for one year, found an decrease in macroinvertebrate density during the dry compared to the rainy season, correlating this fact to the hydrodynamics of the area affected by high rainfall and the non-destructuring of the community associated with the lack of substrate for short periods.

The Sørensen similarity index indicated a similarity of 53.84% in macroinvertebrate diversity between the dry and rainy seasons (Table 2). The morphospecies Chironomidae sp.1 (Diptera) had higher relative species density in the rainy season (57.71%), followed by Odonata Zygoptera sp.1 (17.44%). In the dry season, Chironomidae sp.2 displayed the highest density (73.98%), followed by Gerromorpha sp.1 (9.54%) (Table 2). Dornfeld & Fonseca-Gessner (2005) report a predominance of mosquitoes from the family Chironomidae associated with aquatic macrophytes, including aquatic ferns. Silveira *et al.* (2016) also report Chironomidae as an indicator taxon in different stages of leaf decomposition in *S. auriculata*.

Hemiptera Gerromorpha density was higher in the dry season, a period with the lowest pollution index (175 UFC/mL). According to Silva (2009), the richness of the family Gerromorpha showed a positive relation with the Habitat Integrity Index, that is, a rise in environmental integrity raises the species richness of this family.

The family Culicidae (Diptera) are known to be vectors of human and animal diseases, typically associated with the family Flaviviridae, which cause diseases such as yellow fever, dengue fever and Nile fever (Clairouin 2009; Flores & Weiblen 2009). The family Ceratopogonidae is associated with *Salvinia* infestation (Parys & Johnson 2013).

Dornfeld & Fonseca-Gessner (2005) compared the eating habit predominance of Diptera associated with *Salvinia* sp. and *Myriophyllum* sp. (Haloragaceae), where predators and detritivore collectors predominate in the *Salvinia*, due to the facility of submerged leaves to retain organic matter, which justifies the abundance of Odonata and Chironomidae, predators and detritivores, respectively.

Table 2. Number of individuals and relative density of macroinvertebrates found in *Salvinia biloba* Raddi in the Aldeia River, São Gonçalo, RJ, Brazil.

Classes	Morphospecies	No. of individuals			
		Rainy season	Relative density	Dry season	Relative density
Arachnida	Araneae sp. 1	1	0.67	1	0.23
	Acariformes sp.1	0	0	1	0.23
	Acariformes sp.2	0	0	1	0.23
Hexapoda	Coleoptera Dystiscidae (*Celina* sp.)	1	0.67	0	0
	Coleoptera sp.1 larva	0	0	1	0.23
	Coleoptera sp.2 larva	0	0	1	0.23
	Diptera Ceratopogonidae	4	2.68	0	0
	Diptera Chironomidae sp.1	86	57.71	20	4.77
	Diptera Chironomidae sp.2	9	6.04	310	73.98
	Diptera Culicidae sp.1	1	0.67	9	2.14
	Hemiptera (Hebridae)	1	0.67	0	0
	Hemiptera Gerromorpha sp. 1	0	0	40	9.54
	Hemiptera Gerromorpha sp. 2	0	0	14	3.34
	Hemiptera sp.3	0	0	1	0.23
	Lepidoptera larva	3	2.01	0	0
	Odonata Zygoptera sp.1	26	17.44	10	2.38
	Odonata Anisoptera sp.1	9	6.04	4	0.95
	Odonata Anisoptera sp.2	1	0.67	6	1.43
Maxillopoda	Copepoda	7	4.69	0	0
	Total of individuals	**149**		**419**	
	Richness	**12**		**14**	
	Simpson's Diversity Index (1-D)	**0.9167**		**0.9286**	
	Sørensen similarity index	**53.84%**			

Microbiological analyses demonstrated a difference in water quality between the dry and rainy seasons, the latter with worse water quality. There was a greater percentage of *S. biloba* cover in the rainy season, likely due to the poor water quality, since this species benefits from the higher organic matter content in the water. Greater species richness associated with *S. biloba* stands was found during the dry season. However, the decline in stand cover in the dry season promoted higher macroinvertebrate density. The family Chironomidae exhibited greater relative density in both seasons, but in the dry season, with less pollution, the morphospecies with the second highest density was Gerromorpha sp. 1 (Hemiptera). Studies indicate that an increase in environmental integrity raises the species richness of this family, corroborating the results found here. A greater sampling effort should be undertaken to broaden knowledge regarding the association between macroinvertebrates and *Salvinia* species, in order to determine their correlation with water bodies.

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