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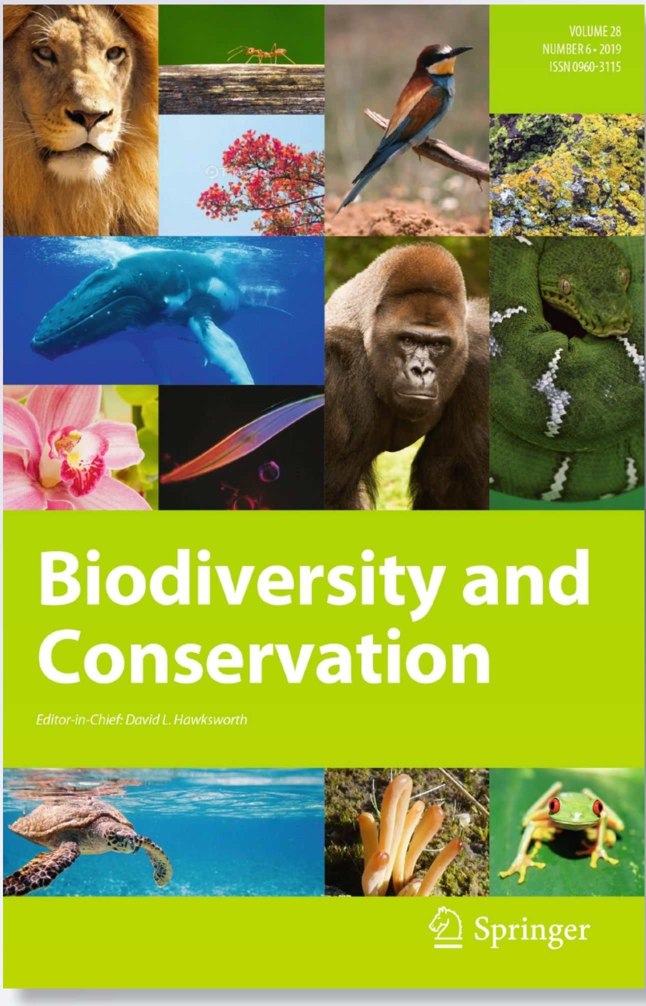
Biodiversity and Conservation

ISSN 0960-3115
Volume 28
Number 6

Biodivers Conserv (2019) 28:1549-1568
DOI 10.1007/s10531-019-01742-7



Springer

Biodiversity and Conservation (2019) 28:1549–1568
https://doi.org/10.1007/s10531-019-01742-7

ORIGINAL PAPER

Urban rain-fed lakes: macro-invertebrate assemblages associated with *Egeria najas* as indicators of biological integrity in wetlands of Corrientes Province (Argentina)

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Received: 21 March 2018 / Revised: 6 February 2019 / Accepted: 11 March 2019 / Published online: 14 March 2019

Abstract

In northeast Corrientes Province, there are more than 50,000 semi-rounded shallow rain-fed lakes. Several lakes have been disturbed mainly because urbanization causes eutrophication due to the illegal discharge of wastewater. We compared 22 metrics based on the structural attributes of macro-invertebrates associated with *Egeria najas* across seasons between five lakes with different human disturbance levels. Sixty-six samples of *E. najas* and associated invertebrates were collected seasonally using a net with an area of 962 cm^2. A total of 17,737 macro-invertebrates of eight major groups, 35 families and 30 genera were recorded. The total macro-invertebrate abundance (number of individuals per plant dry weigh) and the family richness were significantly higher in less disturbed lakes than those under human disturbance, but the differences between seasons were not significant. Non-metric multidimensional scaling analysis differentiated the macro-invertebrate abundances between the more and less disturbed lakes; instead, the diversity indices were not useful for measuring the changes in the studied lakes. Besides, total number of taxa, number of EOT (Ephemeroptera, Odonata, Trichoptera) taxa, abundance and proportion of Trichoptera and abundance of Chironomidae reflected significant differences between the more and less disturbed lakes. Our results suggest that seven invertebrate metrics respond to urbanization, and they could be used to assess biological integrity of the studied lakes in complement of chemical monitoring of water quality. Management efforts should focus on the maintenance of macrophyte stands that provide high invertebrate diversity, which serve as food for a wide variety of fish.

Keywords Urbanization · Human disturbances · Invertebrate metrics · Aquatic plants · Shallow lakes

Communicated by David Hawksworth.

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Introduction

The progress of urban development has caused environmental impacts, contributing to the direct or indirect modification of freshwater ecosystems around the world (McKinney 2006), and these developments cause biodiversity decreases and species composition changes (McDonnell and Pickett 1990; Gleason et al. 2003; Hunter 2002). Human activities have different impacts on aquatic ecosystems, and the impacts that stand out include the discharge of untreated waste water from point and non-point sources and the discharge of industrial and agricultural effluents (Tundisi and Matsumura-Tundisi 2008). These disturbances affect the biological integrity of wetlands, which is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Invertebrates are used to determine wetland conditions (US EPA 2002) and the changes in the biological integrity of a wetland in response to human disturbances. However, the direct application of indices designed for lotic systems is complicated by the habitat complexity of wetland ecosystems (Batzer and Boix 2016).

The composition of macro-invertebrate communities reflects the quality of aquatic ecosystems (Roldán-Pérez 2016). Some attributes such as species richness (Awal and Svozil 2010), total family richness (Ortega et al. 2004), total macro-invertebrate abundance and taxon richness (Stewart and Downing 2008) have been used to evaluate the integrity of wetlands. After testing 50 combinations of relative invertebrate densities, Lunde and Resh (2012) found that only eight taxa were useful: Ephemeroptera, Odonata, Trichoptera, Tanypodinae, Chironomidae, Oligochaeta and Coleoptera. The EOT (Ephemeroptera, Odonata and Trichoptera) richness (Stewart and Downing 2008) and the quotient between Coleoptera and Heteroptera richness (Ortega et al. 2004) were used in biological evaluations of wetlands in different countries.

Macro-invertebrates, especially insects, are the organisms most commonly used as indicators of water quality throughout the world. Biotic indices have been used successfully during the observation and monitoring of aquatic ecosystem contamination, principally in rivers. With adaptations for Argentina, indices have been applied to biological water quality analysis in different lotic environments (Rodrígues Capítulo et al. 2001; Fernández et al. 2002; Paggi 2003; Pavé and Marchese 2005; Zilli and Gagneten 2005; Prat et al. 2009; Ocón and Rodrigues Capítulo 2012; Damborsky and Poi 2015).

Corrientes Province has numerous (more than 50,000), small (<500 ha), sub-rounded rain-fed lakes located on sandy hills (height <2 m) that have low salinities and electrical conductivities (Poi and Galassi 2013). Several lakes in the region have been impacted by land use in the surrounding areas, and this human activity causes a eutrophic state in the lakes due to illegal wastewater discharge from the neighboring areas (Poi et al. 2016). *Egeria najas* is a submerged aquatic plant that typically occurs in the highly transparent ponds that are not influenced by the Paraná River.

To assess the effects of urbanization on the biological integrity of lakes, we compared 22 metrics based on abundance, composition, richness and diversity of macro-invertebrate assemblages associated with *Egeria najas* across seasons between 5 lakes with different human disturbance levels. We hypothesized that the total macro-invertebrate abundance, family richness, proportion of EOT (Ephemeroptera, Odonata, Trichoptera), abundance of Ephemeroptera and Trichoptera and α diversity decrease in the more disturbed lakes, whilst the abundance of Chironomidae increases.

Materials and methods

Studied sites

Corrientes Province is characterized by a subtropical climate with long, warm summers and short, generally mild winters (Bruniard 1999). The mean temperature ranges from 26 °C to 28 °C in January and from 14 to 16 °C in July. Although the absolute minimum winter temperature is as low as −5.5 °C, the occurrence of frost is rare.

We selected five permanent, similarly sized, shallow (mean depth between 1 and 3 m), rain-fed lakes with low salinities and electrical conductivities that range between 25 and 150 µS cm^{-1}, and these lakes also had high dissolved oxygen availability. Three lakes, L1 (27°22′13″S–58°16′52″W), L2 (27°22′03″S–58°20′02″W) and L3 (27°32′21.05″S–58°33′17.28″W), correspond to the typical natural wetlands of the region, which have low nutrient levels (Poi and Galassi 2013). Of the remaining lakes, L4 (28°15′55″S; 58°38′34″W) receives runoff from a cattle breeding area, and L5 (28°15′12″S; 58°36′56″W) is adjacent to a city with 24,000 inhabitants. Within L5, we sampled two sites: an area that received discharge from a wastewater treatment system (L5a) and another area away from the previous area (L5b). Lakes 4 and 5 have fluctuated between mesotrophic and eutrophic over the last 30 years (Poi de Neiff et al. 1999; Poi et al. 2016). In addition, L5 is used for recreation; thus, the submerged aquatic macrophytes were removed from the swimming area by mechanical methods. The harvest of *E. najas* induced a turbid state dominated by Cyanobacteria (Poi et al. 2016).

Due to the accumulation of vegetal detritus coming from the breakdown of aquatic vegetation, these lakes presented an organic bottom where few invertebrate taxa, mainly Oligochaeta and Chironomidae, were registered (Bonetto et al. 1978). The low diversity and abundance of macro-invertebrates in the sediment samples made it impractical to use them to compare potential metrics (Kashian et al. 2000). In addition, vegetated areas provide habitats for invertebrate assemblages, and the structure of these assemblages depends on the dominant aquatic plants (Gallardo et al. 2017). Therefore, the sampling sites were located at similar depths in prairies of submerged plants dominated by *Egeria najas*. Thus, the effect of habitat structuring by different species of aquatic plants on the invertebrate assemblages in the studied lakes (Gallardo et al. 2017) is excluded.

Sampling methods

A net area of 962 cm^2 and a 500 µm mesh size (Poi de Neiff and Carignan 1997) was used to collect *E. najas* and associated invertebrates. Between 2010 and 2012, three samples were removed seasonally from L1, L2, L3 and L4, and during the spring, summer and winter from L5 (a and b), for a total of 66 samples.

In the laboratory, the aquatic plants were thoroughly washed to detach the macro-invertebrates, and the obtained suspensions were filtered through sieves. The macro-invertebrates were preserved in 70% ethanol, and the cleaned plants were dried at 105 °C for 48 h and weighed to obtain the constant dry weight. The macro-invertebrates larger than 1 mm were counted and identified to the lowest practicable taxonomic level (usually family) using keys from Trivinho-Strixino and Strixino (1995), Merrit and Cummins (1996), Thorp and Covich (2001), Domínguez and Fernández (2009) and Ramírez (2010). The macro-invertebrate abundance was expressed as individuals per 1000 g plant dry weight. In tropical and subtropical aquatic habitats, the diversity of invertebrates is poorly known, and taxonomic identification is difficult (specifically to the species or genus level) because the descriptions of some taxa are incomplete and specific taxonomic keys are scarce. Therefore, the use of family or even morphospecies richness has been suggested by Jacobsen et al. (2008). According to Bailey et al. (2001), this level of taxonomic resolution is sufficient to evaluate the responses of macro-invertebrate assemblages to environmental features.

On each date, we measured the physical and chemical variables of the water including temperature, depth, dissolved oxygen concentration, pH, salinity, conductivity and transparency using specific measurement instruments. Chlorophyll *a* was measured by the fluorocolorimetric method (APHA 1975) to determinate phytoplankton biomass, and the total nitrogen and phosphorous contents were determined by spectrophotometry measurements at 543 and 882 nm, respectively. Monthly average air temperature and annual rainfall were provided by the Agricultural Experimental Station of the National Institute of Agricultural Technology.

Data analysis

The non-parametric Kruskal–Wallis test was used to detect significant differences between the lakes that were more and less affected by human disturbances across seasons, taking into account the limnological variables (depth, transparency, water temperature, electrical conductivity, dissolved oxygen, pH, depth/photic zone quotient, Chlorophyll *a* and the nitrogen and total phosphorus contents), plant dry weight, and the 22 invertebrate metrics considered in this study.

To assess the spatial and temporal patterns of the macro-invertebrate assemblages in the lakes, the abundances of the different families were ordered using non-metric multidimensional scaling (NMDS). The results were confirmed by a similarity analysis (ANOSIM; Clarke 1993), using the Bray–Curtis distance.

The similarities among the macro-invertebrate assemblages from the different lakes and seasons were measured with the Jaccard distance using the unweighted pair group method with arithmetic mean (UPGMA).The relationships between the densities of the different macro-invertebrate families and the limnological variables were assessed with Spearman's rs correlation coefficients.

Prior to the analysis, all abundance data were log $(x+1)$ transformed to stabilize the variances and normalize the data sets. The diversity of macro-invertebrate families (α diversity) was assessed using the effective numbers proposed by Jost (2006). We used the first-order diversity (^{1}D), which is the exponent of the Shannon entropy and includes all families with weights proportional to their abundance in the assemblage. Additionally, we used the second-order diversity (^{2}D), which is the inverse of the Simpson index and includes only the most abundant species (Gotelli and Chao 2013). To facilitate comparisons with other studies, the Shannon and Simpson indices were also calculated.

Twenty-two attributes of macro-invertebrate assemblages that are cited to have potential use as metrics in bioassessments of lentic systems (Kashian and Burton 2000; King et al. 2000; Ortega et al. 2004; Stewart and Downing 2008; Trigal et al. 2009; Maltchik et al. 2010; Lunde and Resh 2012; Mereta et al. 2013) were calculated for each lake. The metrics based on the assemblages structure, composition and diversity indices consisted of macro-invertebrate abundance, overall richness, number of families, calculation of the relative contribution of some taxa to the total abundance and α diversity indices.

The statistical analyses were performed using PAST 2.08 (Hammer 2001) and InfoStat (Di Rienzo et al. 2013) software.

Results

Studied Sites

During the study period, the monthly average air temperature varied between 34.5 °C in the summer (February 2012) and 7.1 °C in the winter (July 2012). The annual rainfall varied between 368 mm in the spring (October 2012) and 11.2 mm in the winter (July 2011). The water temperatures were generally high, and the mean dissolved oxygen concentration varied between 5.1 and 8.6 mg l^{-1} (Table 1). Electrical conductivity and total phosphorus were significantly higher (H = 16.91, $P = 0.0046$) in the more disturbed lakes (L4, L5a and L5b) than the less disturbed lakes (L1, L2 and L3), Table 1. The nitrogen content was higher at sites L1 and L4, and the differences with respect to the remaining lakes were statistically significant (H = 16.48, $P = 0.0039$). The Chlorophyll *a* values were less than 10 µg l^{-1} in all lakes, even in the lakes that were more influenced by human disturbances. Despite this, significant differences were registered (H = 10.20; $P = 0.0465$) between L1, L2, L3, L4 and the more disturbed lake (L5a and b, Table 1).

Plant dry weight and macro-invertebrate metrics based on abundance

The mean plant dry weight varied between 7.9 ± 0.02 g in L5a (spring) and 38.75 ± 5.65 g in L4 (autumn). This site registered the highest plant biomass in all sampling dates (Table 2), and the differences with respect to the other lakes were statistically significant (H = 35.23, $P \leq 0.0001$). This result could be because of the high total phosphorus content (Table 1), which favors the growth of aquatic plants.

From a total of 66 samples, we obtained 17,737 macro-invertebrates from 8 major groups, 35 families and 30 genera. The mean total macro-invertebrate abundance varied between 1073.37 and 150,045.60 ind.1000 g plant dry weight in L5a and L3, respectively (Table 2). The unit of reference (number of individuals per plant dry weight) allowed for the differences in macro-invertebrate abundance to be found between lakes with different plant infestation volumes. The total macro-invertebrate abundances were significantly higher (H = 47.38, $P \leq 0.0001$) in the less perturbed lakes (L1, L2 and L3) than those under human disturbance, but the difference between seasons was not significant (H = 1.44, $P = 0.6957$).

The NMDS results reflected the differences in the abundances of the macro-invertebrate families between lakes (ANOSIM: R = 0.72, $P = 0.0001$) with low final stress (9.9%). The first axis of the analysis differentiates the macro-invertebrate abundance between lakes, whereas the second axis shows the differences among seasons (Fig. 1). The abundances of the macro-invertebrate families found in lakes L1, L2 and L3 (less disturbed) during the spring, summer and autumn are grouped on the right of the first axis. The different sampling dates in L4 are located in the middle of axis 1 (Fig. 1), and the families registered in L5 are grouped on the left of the first axis. The area that received treated discharge (L5a) is separated from the area away from the treated discharge (L5b).

When the limnological variables were included, the NMDS ordination showed that some variables were correlated with the gradients in macro-invertebrate family abundance,

Table 1 Mean ± 1SD (min–max) Limnological variables and human disturbances registered in the less (L1, L2, L3) and more (L4, L5 **a** and **b**) disturbed lakes; *n.d* no detected

Variables	L1	L2	L3	L4	L5a	L5b
Depth (m)	(1.8 to 2.4)	(1.5 to 2.2)	(0.7 to 1.8)	(0.7 to 1.7)	(1.0 to 1.5)	(1.2 to 1.9)
Transparency/Secchi disc depth (cm)	133.7 ± 61.5	91.2 ± 28.3	85 ± 22.4	88.7 ± 29.0	93.3 ± 64.1	152.5 ± 33.0
Water temperature (°C)	(14.0 to 28.5)	(13.9 to 30.5)	(13.2 to 30.0)	(16.6 to 31.0)	(22.0 to 30.1)	(13.1 to 28.4)
Electrical conductivity (µS cm^{-1})	32.0 ± 9.4	34.7 ± 15.8	25.0 ± 11.6	60.5 ± 6.0**	155.0 ± 26.6**	161.0 ± 14.4**
Dissolved oxygen (mg l^{-1})	5.1 ± 2.6	6.6 ± 1.9	6.6 ± 0.4	8.0 ± 1.7	4.7 ± 3.7	8.5 ± 2.9
pH	(7.2 to 8.4)	(6.7 to 7.6)	(7.2 to 8.2)	(7.1 to 8.5)	(6.9 to 8.8)	(7.9 to 9.9)
Depth/photic zone (D/PZ) quotient range	(1.0 to 3.4)	(1.7 to 2.5)	(1.1 to 2.1)	(1.0 to 2.0)	(1.0 to 4.4)	1.0
Chlorophyll *a* (µg l^{-1})	(< 5 to 5)*	(5 to > 5)*	>5*	(n.d to 10)*	(n.d to 5)	(n.d to < 5)
Nitrogen—NO^{-3} + NO^{-2} (µg l^{-1})	23.7 ± 2.5**	17.5 ± 2.9	18.7 ± 2.5	28.7 ± 4.8**	11.7 ± 10.4	3.7 ± 4.8
Total phosphorus (µg l^{-1})	21.2 ± 4.8	21.2 ± 4.8	22.0 ± 3.6	152.5 ± 61.2**	88.3 ± 43.1**	90.0 ± 60.1**
Human disturbances						
Occupation of the perimeter of the lake					X	X
Discharge of untreated effluents of neighborhood sewer networks					X	X
Cattle grazing				X		

*Significant at *P* value < 0.05

**Significant at *P* value *P* < 0.005

Table 2 Mean ± 1SD values of plant dry weight and total macro-invertebrate abundance per unit of reference in each lake and season

Lakes	Spring		Summer		Autumn		Winter	
	Plant DW(g)	Ind.1000 g plant DW	Plant DW(g)	Ind.1000 g plant DW	Plant DW(g)	Ind.1000 g plant DW	Plant DW(g)	Ind.1000 g plant DW
L1	17.3 ± 3.8	10,666.5 ± 3494.6	14.1 ± 8.9	57,008.1 ± 33,064.0	14.3 ± 5.2	16,055.2 ± 6798.9	15.9 ± 4.8	34,534.8 ± 34,826.3
L2	16.5 ± 4.3	24,341.4 ± 8082.4	9.2 ± 0.5	30,952.3 ± 11,336.3	9.8 ± 0.4	18,198.6 ± 727.5	9.9 ± 1.2	30,193.0 ± 15,844.9
L3	14.6 ± 3.0	19,745.2 ± 15,618.5	9.2 ± 2.0	32,322.1 ± 26,907.7	8.9 ± 0.1	24,545.5 ± 642.8	9.2 ± 2.1	150,045.6 ± 69,321.3
L4	24.0 ± 2.0	17,344.1 ± 5722.1	21.9 ± 1.0	7978.4 ± 953.9	38.7 ± 5.6	6335.4 ± 1750.3	31.0 ± 6.7	6745.2 ± 2935.0
L5a	7.9 ± 0.2	1392.2 ± 691.0	14.2 ± 1.6	1073.4 ± 384.9	–	–	22.3 ± 5.6	4289.5 ± 1790.6
L5b	14.8 ± 1.9	2010.8 ± 1649.3	14.2 ± 1.6	1236.4 ± 894.2	–	–	15.2 ± 1.5	4288.6 ± 2066.3

DW dry weight, – no data

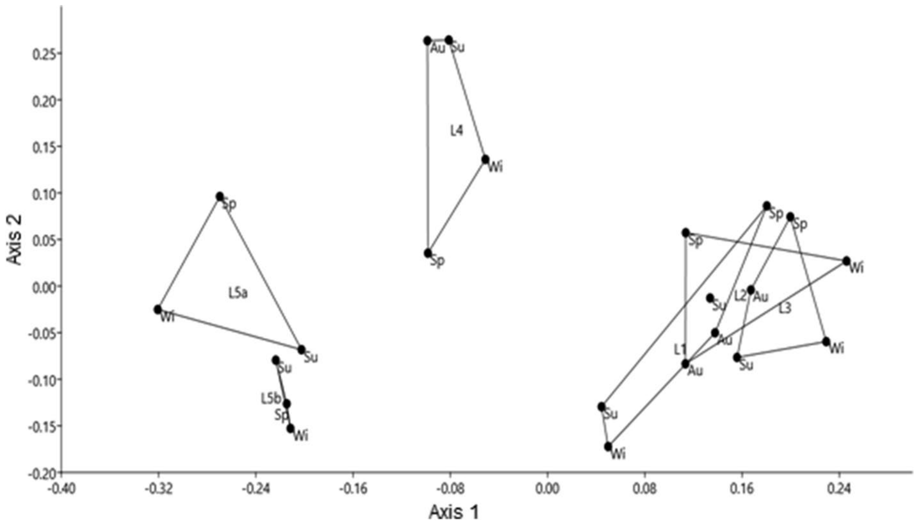

Fig. 1 NMDS ordination of macro-invertebrate abundance (ind.1000 g plant dry weight) in the different lakes and seasons

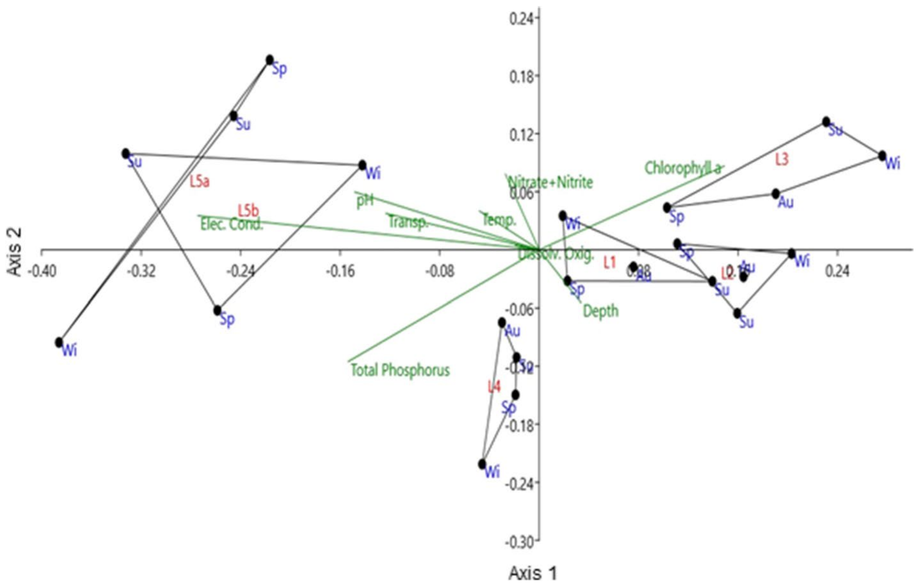

Fig. 2 NMDS ordination of the limnological variables and macro-invertebrate abundance (ind.1000 g plant DW) in the different lakes and seasons

and the ordination had an acceptably low final stress (13%), Fig. 2. Electrical conductivity, pH and total phosphorus were negatively related to macro-invertebrate family abundance, whereas Chlorophyll *a* was positively related (Fig. 2, Table 3).

The percentage of Ephemeroptera varied between 0.7 and 8.3% in L4 and L3, respectively, while the percentages of Odonata and Coleoptera were high in the more disturbed

Table 3 Correlations of densities of macro-invertebrate families and plant biomass with limnological variables using Spearman's rs rank order correlation

	Temp.	Elec. Cond.	Dissol. Oxyg.	pH	Transp.	Depth	Chloroph. *a*	Nitrate + nitrite	Total Phosph.	Macroinv. Abund.	Plant Biomass
Temp.	–										
Elec. Cond.	0.26	–									
Dissol. Oxyg.	−0.06	0.16	–								
pH	0.18	0.44	0.59	–							
Transp.	−0.22	0.31	−0.21	0.21	–						
Depth	−0.28	−0.28	−0.32	−0.22	0.33	–					
Chloroph. *a*	0.21	−0.48	0.10	−0.11	−0.33	−0.03	–				
Nitrate + nitrite	−0.08	−0.23	0.12	−0.19	−0.30	−0.10	0.19	–			
Total Phosph.	−0.11	0.61	0.18	0.11	0.23	−0.33	−0.30	0.12	–		
Macroinv. Abund.	0.19	−0.71*	−0.28	0.47*	−0.34	0.24	0.53*	0.17	−0.67*	–	
Plant Biomass	0.00	0.44*	0.29	0.22	−0.06	−0.07	−0.30	0.18	0.43*	0.26	–

Temp. temperature, *Elec*. Cond. electrical conductivity, *Dissol. Oxyg*. dissolved oxygen, *Transp*. transparency, *Chloroph. a* Chlorophyll *a*, Total Phosph. total phosphorus, *Macroinv. Abund*. macro-invertebrate abundance

*Significant at *P*-value < 0.05

Table 4 Mean ± 1SD (min–max) Macro-invertebrate metrics in more and less disturbed lakes

Measure	Metric	Less disturbed lakes			More disturbed lakes		
		L1	L2	L3	L4	L5a	L5b
Richness	Total number of taxa	16.8 ± 5.5** (8–24)	16.4 ± 5.6** (16–23)	14.3 ± 5.4** (10–21)	11.6 ± 2.4 (8–15)	5.7 ± 1.9 (3–9)	7.1 ± 2.4 (4–11)
	Number of families	13.8 ± 5.2** (5–20)	15.2 ± 2.3** (11–20)	12.1 ± 2.4** (8–16)	10.8 ± 2.5** (7–15)	5.4 ± 1.8 (3–8)	6.9 ± 2.2 (4–10)
	Number of						
	EOT taxa	3.2 ± 1.5** (1–5)	3.8 ± 1.4** (0–6)	5.0 ± 1.2** (3–6)	2.3 ± 1.1 (1–4)	1.0 ± 0.9 (0–2)	2.1 ± 0.8 (1–3)
	Ephemeroptera taxa	1.2 ± 0.9 (0–2)	0.8 ± 0.8 (0–2)	1.5 ± 0.8 (0–2)	0.8 ± 0.6 (0–2)	0.6 ± 0.5 (0–1)	0.9 ± 0.8 (0–2)
	Trichoptera taxa	0.6 ± 0.5 (0–1)	1.4 ± 0.7** (0–2)	2.0 ± 0.9** (1–3)	0.3 ± 0.5 (0–1)	0	0
	Odonata taxa	1.1 ± 0.7 (0–2)	1.5 ± 0.7 (0–2)	1.20–20.7 (0–2)	1.3 ± 0.5 (1–2)	0.4 ± 0.7 (0–2)	1.2 ± 0.7 (0–2)
	Coleoptera taxa	1.2 ± 0.7 (0–2)	0.5 ± 0.7 (0–2)	0.8 ± 0.8 (0–2)	1.2 ± 0.7 (0–2)	0.3 ± 0.7 (0–2)	0.7 ± 0.5 (0–1)
	Percentage of						
	EOT	5.1 ± 4.2 (0–13.0)	12.1 ± 6.4** (0–24.0)	17.6 ± 17.6** (0–55.1)	4.0 ± 2.8 (0.3–10.3)	9.3 ± 9.1 (0–23.5)	16.8 ± 6.0** (8.2–22.2)
	Ephemeroptera	1.9 ± 1.7 (0–4.4)	2.5 ± 4.0 (0–13.7)	11.4 ± 15.5 (0–47.7)	0.7 ± 0.7 (0–1.8)	4.3 ± 5.4 (0–12.5)	4.9 ± 4.7 (0–12.5)
	Trichoptera	2.0 ± 2.1** (0–5.7)	6.0 ± 4.1** (0–13.3)	3.9 ± 4.3** (0–12.8)	0.9 ± 1.4 (0–3.3)	0	0
	Odonata	1.2 ± 0.9 (0–2.8)	3.5 ± 2.3 (0–7.9)	2.3 ± 2.5 (0–9.0)	3.6 ± 3.2 (0.3–10.0)	7.1 ± 14.5 (0–42.9)	11.1 ± 5.8** (0–21.4)
	Coleoptera	1.1 ± 0.9 (0–2.8)	0.2 ± 0.3 (0–0.8)	0.6 ± 0.7 (0–2.0)	1.0 ± 0.9 (0–2.9)	3.8 ± 9.5 (0–28.6)	4.5 ± 5.8 (0–15.4)

Table 4 (continued)

Measure	Metric	Less disturbed lakes			More disturbed lakes		
		L1	L2	L3	L4	L5a	L5b
	Chironomidae	29.1 ± 22.3** (1.8–85.5)	19.9 ± 21.1 (0–63.2)	44.9 ± 26.3** (0–76.5)	42.4 ± 22.5** (20.6–81.4)	4.0 ± 6.2 (0–14.3)	19.7 ± 31.7 (0–76.3)
Abundance (Ind.1000 g plant DW)	Total abundance (see Table 2)	**	**	**			
	Ephemeroptera	466.3 ± 436.0 (0–1234.6)	893.9 ± 1393.5** (0–2834.4)	3964.5 ± 5171.7** (0–18,018.0)	69.9 ± 85.3 (0–272.7)	58.9 ± 60.0 (0–132.4)	104.5 ± 108.2 (0–288.18)
	Trichoptera	591.0 ± 680.4** (0–2098.8)	1552.4 ± 1036.9** (0–3363.6)	1345.2 ± 1117.2** (0–3177.6)	40.6 ± 82.5 (0–242.1)	0	0
	Odonata	250.7 ± 214.2 (0–700.0)	978.3 ± 800.5** (0–1636.4)	698.0 ± 489.0** (0–1621.69	332.5 ± 295.4 (38.5–818.2)	90.3 ± 144.3 (0–384.6)	253.1 ± 214.1 (0–576.4)
	Coleoptera	273.0 ± 275.8 (0–800.0)	57.9 ± 100.3 (0–344.8)	139.1 ± 152.3 (0–450.4)	112.8 ± 102.4 (0–321.0)	42.4 ± 89.7 (0–253.2)	60.5 ± 50.2 (0–131.6)
	Chironomidae	8825.7 ± 17,381.1** (493.8–63,793.1)	7654.3 ± 8683.1** (0–31,454.5)	34,482.5 ± 53,193.8** (0–169,957.7)	5056.2 ± 5489.5 (1538.5–18,681.8)	37.7 ± 58.4 (0–132.4)	935.8 ± 1689.6 (0–4178.7)
Community diversity	Shannon diversity	1.46 ± 0.56 (0.71–2.06)	1.87 ± 0.37 (1.32–2.11)	1,42 ± 0.51 (0.80–1.86)	1.56 ± 0.42 (0.95–1.93)	1,69 ± 0.47 (1.16–2.04)	1.67 ± 0.53 (1.24–2.26)
	Simpson diversity	0.61 ± 0.18 (0.37–0.80)	0.75 ± 0.13 (0.56–0.83)	0.62 ± 0.18 (0.38–0.76)	0.67 ± 0.20 (0.37–0.79)	0.65 ± 0.13 (0.54–0.79)	0.76 ± 0.14 (0.61–0.88)
	Effective number of families (^{1}D)	4.79 ± 2.43 (2.04–7.92)	6.77 ± 2.06 (3.76–8.32)	2.08 ± 4.54 (2.22–6.41)	5.05 ± 1.80 (2.59–6.90)	5.77 ± 2.33 (3.18–7.71)	5.86 ± 3.28 (3.46–9.59)
	Effective number of families (^{2}D)	3.04 ± 1.39 (1.58–4.91)	4.57 ± 1.61 (2.26–6.00)	3.10 ± 1.30 (1.61–4.19)	3.66 ± 1.44 (1.58–4.90)	3.15 ± 1.38 (2.20–4.73)	5.22 ± 2.82 (2.56–8.17)

Significance from paired Kruskal–Wallis test comparing the considered metric between most and less disturbed lakes

**$P < 0.005$

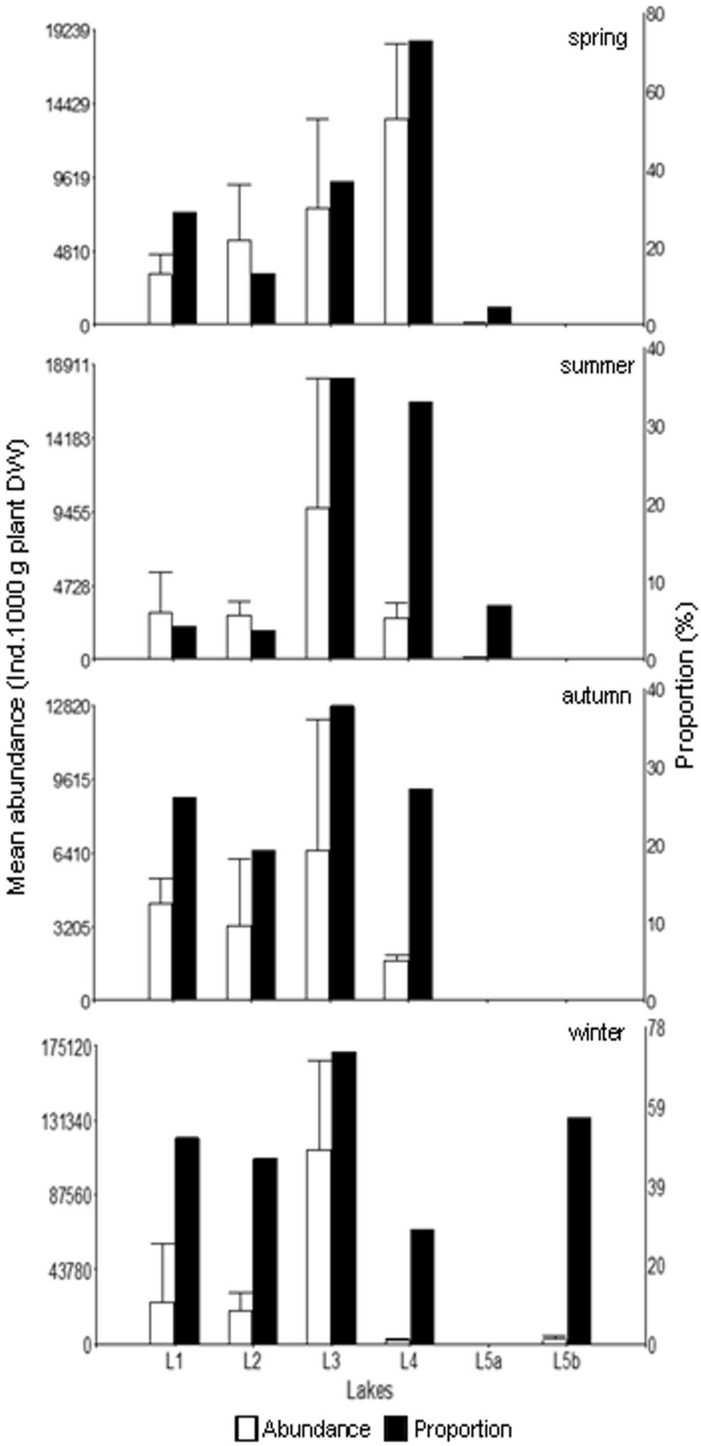

Fig. 3 Chironomidae metrics across seasons in more and less disturbed lakes. The bars show standard deviations

lakes (Table 4). Chironomidae was present at a high proportion, even in the more disturbed lakes (Fig. 3). On the contrary, the families of the order Trichoptera (Polycentropodidae, Hydroptilidae and Leptoceridae) seem to be more sensitive to the changes in water conditions because they were found in very low proportions and abundances in L4, and they were not registered in L5a and L5b (Fig. 4). The proportions of EOT (Ephemeroptera, Odonata and Trichoptera) were higher than 12% in L2, L3 and L5b (Table 4).The majority of the macro-invertebrate metrics based on abundance were higher in the less disturbed lakes (Table 4), but the differences between more (L4, L5a, L5b) and less disturbed lakes (L1, L2, L3) were significant for Chironomidae (H = 33.87, $P \leq 0.0001$) and Trichoptera (H = 40.70, $P \leq 0.0001$) only. The most abundant family of Odonata was Coenagrionidae, which exhibited high values in L2 (1382.11 ind.1000 g plant dry weight) and L3 (1081.08 ind.1000 g plant dry weight). Ephemeroptera with the families Caenidae (*Caenis* sp.) and Baetidae (*Callibaetis* sp.) were abundant in the spring in L3. Trichoptera were dominant in the less disturbed lakes (Fig. 4), and one family (Polycentropodidae) reached its maximum abundance (3084.11 ind.1000 g plant dry weight) in the summer in L3. Chironomidae were very abundant, mainly the subfamily Chironominae, with 70,098.73 ind.1000 g plant dry weigh in the winter in L3. The abundance of the order Coleoptera (Hydrophilidae, Noteridae, Dytiscidae and Curculionidae) did not vary significantly between the more and less disturbed lakes (H = 8.81, $P = 0.0946$, Table 4).

Macro-invertebrate metrics based on richness, composition and diversity

Overall, the macro-invertebrate richness metrics were lower in the more disturbed lakes, except the numbers of Ephemeroptera, Odonata and Coleoptera taxa, which were similar (Table 4). The highest family richness was registered in L1 and L3, and the lowest richness was registered in L5a and L5b (Table 4). The family richness values were significantly higher in less disturbed lakes (H = 37.64, $P \leq 0.0001$) than the lakes more affected by human disturbances, but these values did not differ between seasons (H = 7.30, $P = 0.0619$).

When we analyzed the compositions of the macro-invertebrate assemblages, insects (more than 80% of the total taxa in L3) and gastropods (maximum of 46.16% in L2) had the highest relative abundances in all lakes, except L5a and L5b where insects and one species of decapod (*Pseudopalaemon bouvieri*, which reached 50.19% in L5b) had the highest proportions.

Chironomidae and Oligochaeta (mostly Naididae) alternated in numerical dominance among the seasons in the less disturbed (L1, L2 and L3) and a disturbed (L4) lakes. Ceratopogonidae and Planorbidae were very abundant in the less disturbed lakes, highlighting the elevated relative abundance of Cyclestheriidae (*Cyclestheria hislopii*), which was present in only L1 (59.04%) and L2 (28.50%) in the summer.

In the more disturbed lakes, Ancylidae and Hyalellidae (*Hyalella curvispina*) dominated in the different seasons in L4. In L5a and L5b, a few families were distributed in more equitable proportions. *P. bouvieri* were frequent and dominant, especially in the spring, while Chironomidae and Planorbidae (*Biomphalaria* spp.) exhibited high proportions in the winter.

The cluster analysis based on the presence-absence analysis indicated a clear separation between the macro-invertebrate families present in a more disturbed lake (L5a and b) and the remaining lakes, regardless of the sampling dates (Fig. 5). In turn, L4 (a more disturbed lake) was segregated from L1, L2 and L3 (less disturbed lakes).

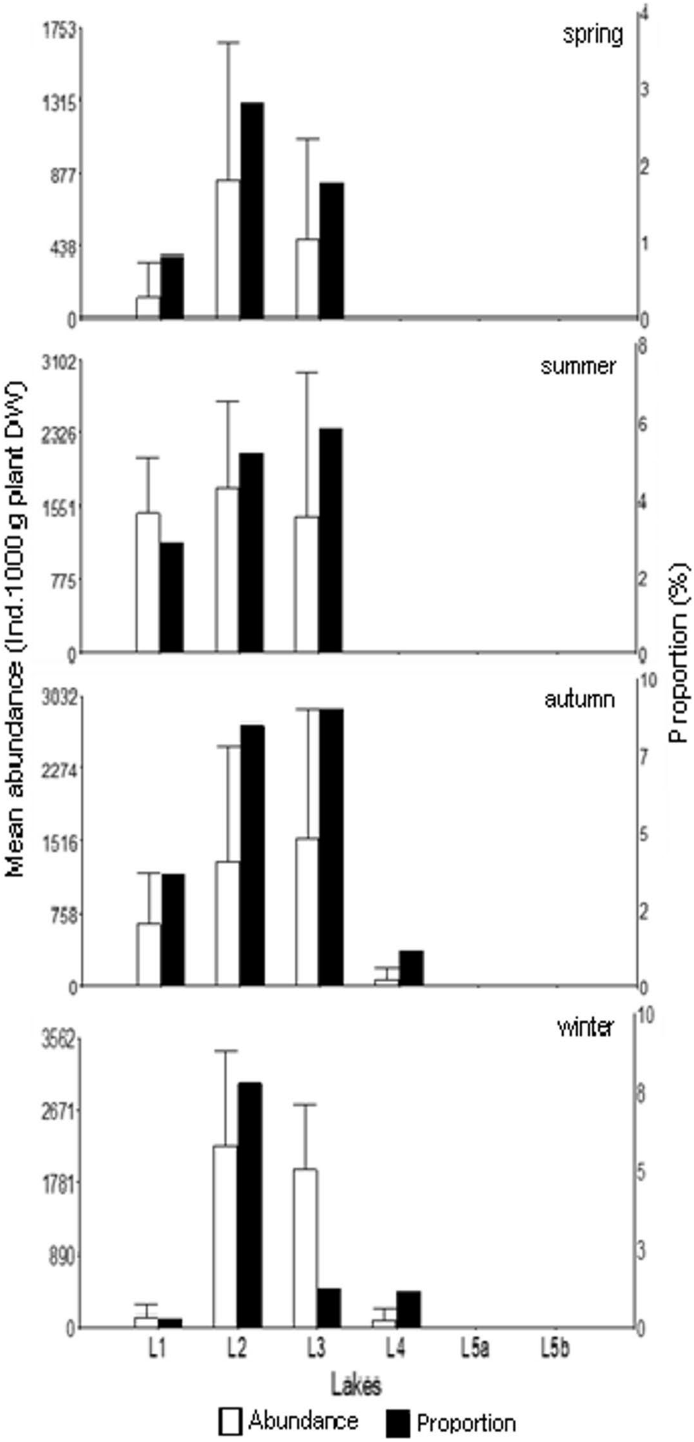

Fig. 4 Trichoptera metrics across seasons in more and less disturbed lakes. The bars show standard deviations

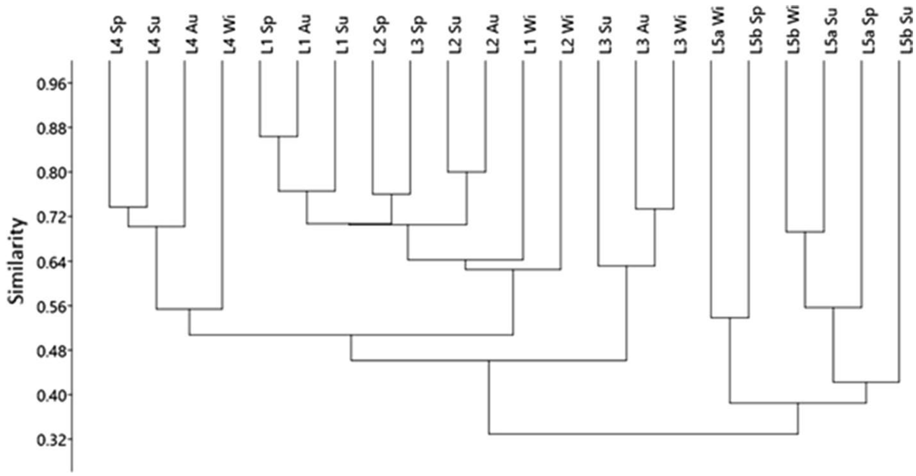

Fig. 5 Cluster analysis based on Jaccard distance (UPGMA method) of the macro-invertebrate assemblages in the studied lakes across seasons. *L1* lake 1, *L2* lake 2, *L3* lake 3, *L4* lake 4, *L5a and L5b* two sampling sites in lake 5. *Sp* spring, *Su* summer, *Au* autumn, *Wi* winter

The macro-invertebrate diversity was similar in both more and less disturbed lakes (Table 4). These results reflect the similar structures of the macro-invertebrate communities (one or two dominant taxa and the lower proportions of the other in the different sampling dates) in both the more and less disturbed lakes. Nevertheless, different families dominated in lakes with different degrees of human disturbance, as described in the previous paragraph. For this reason, the differences between the more and less disturbed lakes were reflected by the NMDS analysis, but not by the diversity indices.

Discussion

Our results indicate that total macro-invertebrate abundance, total number of taxa, number of EOT, total family richness and the abundance and proportions of some taxa decreased in the lakes that were more affected by human disturbances. The facts that lakes with submerged prairies of the same plant species were compared and the plant dry weight was used as the unit of reference validate this decrease. In another way, the differences in the abundance and richness of macro-invertebrates could be confounded by the habitat structure from the different aquatic plant species or the volume of infestation.

The composition of vegetation is a key factor that influences the structure of macro-invertebrate assemblages, as was demonstrated by the comparison of the *E. najas* and *S. biloba* assemblages in lake L4 (Gallardo et al. 2017). For this reason, comparisons between wetlands with different degrees of human disturbances must consider vegetation type to avoid confusing the effects derived from possible human impacts with those derived from the dominant vegetation type in each wetland.

The NMDS analysis showed clear differences in the invertebrate assemblages between the lakes that were more and less affected by human disturbances. In addition, limnological variables, such as electrical conductivity and total phosphorus, allowed for the differentiation between the lakes that were more (L4 and L5 a, b) and less (L1, L2 and L3) affected by human disturbances, which negatively influenced the macro-invertebrate abundance. Conductivity was associated with changes in the macro-invertebrate community structure and correlated with urbanization (Lunde and Resh 2012). In the all studied lakes with dense submerged plant coverages, Chlorophyll *a* was relatively low and it was positively related to macro-invertebrate abundance in the less affected lakes. On the contrary, Mereta et al. (2013) found that Chlorophyll *a* was negatively correlated with the other structural attributes of macro-invertebrate assemblages (family richness, EOT family richness and percentages of filterers/collectors).

The abundance and dominance of the macro-invertebrate taxa found in this study are similar to those described in previous studies carried out in other lakes in the study area (Poi de Neiff 1979, 2003; Gallardo et al. 2017; Poi et al. 2017). These last studies reported the abundance of crustaceans (*H. curvispina* and *P. bouvieri*) and gastropods such as *Gundlachia* spp. and *Biomphalaria* spp., dominance of Chironomidae, high relative abundance of Corixidae (Hemiptera), and other taxa present in smaller proportions (Naucoridae, Caenidae and Libellulidae).

H. curvispina and *P. bouvieri* are frequent and abundant in the wetlands in northeast Argentina. The first species is associated with different aquatic plants and bioforms (Poi de Neiff and Neiff 2006), while the second one is common in the submerged prairies of *E. najas* in lakes of the Riachuelo River basin (Poi and Galassi 2013). According to several authors (Por and Rocha 1998; Poi de Neiff 2003; Carnevali et al. 2016; Tagliaferro and Pacual 2017), these species of crustaceans are sensitive to low concentrations of dissolved oxygen in the water, which causes decreases in the populations. In this study, *H. curvispina* and *P. bouvieri* were numerically dominant in the more disturbed lakes, which is contrary to what was expected, as the availability of dissolved oxygen was high. This result could be due to the alternative clear water state of the lakes on the sampling dates. The water was highly transparent, and the bottoms of the lakes had high coverages of submerged plants, which release oxygen into the water. This situation added to the scarcity of decaying organic matter (which consumes oxygen) and the high nutrient contents, which could favor the increases in these crustacean populations.

Many studies have indicated that some orders of insects, such as Ephemeroptera, Trichoptera (Ode et al. 2005; Arimoro and Muller 2010) and Odonata (Samways and Steytler 1996; Simaika and Samways 2009), are sensitive to human disturbances, and these orders are commonly used in the bioassessment and monitoring of freshwater ecosystems. The relative abundance of Ephemeroptera was suggested as an indicator of the biotic integrity of wetlands (Lunde and Resh 2012), and this suggestion was evaluated by other authors (Mereta et al. 2013) who found that its decrease in lakes was affected by human action. However, in this study, the proportion of this order was similar in all lakes, while its mean abundance was greater in the less disturbed lakes. This result agrees with the result found by other authors (Sharma and Rawat 2009; Shelly et al. 2011), who affirmed that Ephemeroptera are the most abundant insects in submerged vegetation under good water quality, especially at sites with high concentrations of dissolved oxygen. A similar situation occurred with the proportion of Odonata, which was not lower in the more disturbed lakes, as frequently cited in the literature (Batzer and Boix 2016). According to Mereta et al. (2013), odonates can be used as good indicators of water quality, but they are relatively sensitive to pollution, and there is some variation in the tolerance to pollution of the taxa belonging to this group. The results of the present study indicate that in the more disturbed lakes (such as L5a and b), Libellulidae and Coenagrionidae reached high proportions compared to the less disturbed lakes. According to Bouchard (2004), Libellulidae are common and abundant in eutrophic waters, and they are very tolerant to low levels of oxygen and high nutrient contents. Similarly, Coenagrionidae are far less sensitive to pollution (Mereta et al. 2013), and they were abundant in all studied lakes.

Consistent with the results of this study, the proportions and abundances of Trichoptera were useful for differentiating lakes with different human disturbance levels, which coincide with what was indicated by Kashian and Burton (2000) and Lunde and Resh (2012) about the integrity of wetlands. The high relative abundance of Chironomidae in human-disturbed environments has been attributed to the high tolerance of the family to degraded environments (Moya et al. 2007). However, this family is commonly abundant in studied wetlands, and higher abundances have been registered in less disturbed lakes; however, its proportion was variable. Kerans and Karr (1994) suggest that Chironomidae must be identified to the genus or species level to use them as a water quality indicator because of they are a very diverse group that is constituted of species with different pollution sensitivities.

The different metrics (total macro-invertebrate abundance, family richness, total number of taxa, number of EOT taxa, abundance and proportion of Trichoptera and abundance of Chironomidae) reflected the differences between the lakes that were subject to more and less human disturbances. On the contrary, the diversity indices were not useful for the measurement of the changes in the studied lakes, as the macro-invertebrate assemblages had one or two dominant families and the rest of the families were present in smaller proportions. Those indices give weight to the family richness and the more equitable distribution among these families and not the absence of some sensible families to human disturbances, which was shown by the NMDS analysis.

It should be noted that the taxonomic level (family) used in this study was enough to obtain results (some metrics) that allowed for the differentiation between lakes that were more and less disturbed by human action in the study area. This method could be very useful to reduce the time spent on the classification of invertebrates to lower levels and, in this way, may provide a faster tool for evaluating the biotic integrity of these wetlands.

The macro-invertebrate assemblages associated with *E. najas* can be useful to assess and monitor the studied lakes and can complement the chemical monitoring of water quality. Management efforts should focus on the maintenance of macrophyte stands that provide high invertebrate diversity, which serve as food for a wide variety of fish.

Acknowledgements This work was supported by the Project "Analysis of ecological condition of peri-urban ponds (Corrientes, Argentina)" PI 2011Q001 SGCYT of the National University of Northeast (UNNE), Corrientes, Argentina. The authors thank the technical assistants of the Centro de Ecología Aplicada del Litoral (CECOAL) for field assistance and for water chemical analysis.

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