







## Analysis of the zooplanktonic community in rice fields during a crop cycle in agroecological versus conventional management

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Received: 11/05/20

Accepted: 22/04/21

### ABSTRACT

#### Analysis of the zooplanktonic community in rice fields during a crop cycle in agroecological versus conventional management

This study investigated the zooplankton community in rice fields with different management practices: conventional crop (CC) and agroecological crop (AE). In both wetlands, physicochemical parameters and pesticide residues in water and sediment were measured, and the structure of zooplankton was analyzed. Environmental parameters did not show significant differences between the wetlands. In AE samples, no pesticide residues were recorded in water; only bentazone residues were found in sediment. In CC water samples, bentazone, glyphosate, and AMPA were found, while bentazone, AMPA, clomazone, imidacloprid, and tebuconazole were detected in sediment. AE rice field presented significantly ( $p < 0.01$ ) higher richness of Cladocera taxa, which is considered the most sensitive group among zooplankters, as well as abundance of rotifers, diversity, and evenness of taxa ( $p < 0.05$ ). In contrast, in CC, only the dominance was significantly ( $p < 0.05$ ) higher than in AE, mainly explained by the dominance of smaller species and copepods, considered more tolerant. The percentage of dissimilarity between crops was high (79.9 %). Through different biological indicators and ecological indices, we conclude that the AE rice field showed better environmental quality than CC. This study contributes to understanding the effect of pesticides on zooplankton and alerts about the importance of diversifying crop management practices and diminishing the use of agrochemicals in rice production.

**Key words:** zooplankton, bioindicators, community indices, agroecological rice crop, conventional rice crop

### RESUMEN

#### Análisis de la comunidad zooplanctónica en cultivos de arroz durante un ciclo de producción bajo manejo agroecológico versus convencional

Este estudio investigó la comunidad zooplanctónica en dos campos de arroz con diferentes prácticas de manejo: cultivo convencional (CC) y cultivo agroecológico (AE). En ambos humedales se midieron parámetros fisicoquímicos y residuos de plaguicidas en agua y sedimentos y se analizó la estructura del zooplancton. Los parámetros ambientales no mostraron diferencias significativas entre los humedales. En las muestras de AE no se registraron residuos de plaguicidas en agua; sólo se encontraron residuos de bentazon en sedimento. En las muestras de agua de CC se encontró bentazon, glifosato y AMPA, mientras que en sedimento se detectó bentazon, AMPA, clomazone, imidacloprid y tebuconazole. AE presentó mayor riqueza de cladóceros ( $p < 0,01$ ), considerado el grupo más sensible, así como mayor abundancia de rotíferos, diversidad

y equitatividad de taxones ( $p < 0.05$ ). En contraste, en CC, solo la dominancia fue significativamente mayor que en AE ( $p < 0.05$ ), explicado principalmente por la dominancia de especies pequeñas y copépodos, considerados más tolerantes. El porcentaje de disimilitud entre cultivos fue alto (79.9%). A través de diferentes indicadores biológicos e índices ecológicos, se concluye que AE mostró mejor calidad ambiental que CC. Este estudio contribuye a comprender el efecto de los plaguicidas en el zooplancton y alerta sobre la importancia de diversificar las prácticas de manejo de cultivos y disminuir el uso de agroquímicos en la producción de arroz.

**Palabras clave:** zooplancton, bioindicadores, índices de comunidad, cultivo de arroz agroecológico, cultivo de arroz convencional

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## INTRODUCTION

Rice (*Oryza sativa* L., Poales: Poaceae) is the most widespread irrigated crop worldwide (Yoon, 2009); it is grown in at least 114 countries (Pernollet *et al.*, 2015) and occupies about 13 % of the arable land (164 million ha) (FAO, 2013). Rice fields are among the most productive and highly dynamic agroecosystems because the physical and chemical characteristics of water change continuously during cultivation (Fernández Valiente & Quesada, 2004). Also, the great heterogeneity of rice fields contributes to their richness and varied biodiversity (Bambaradeniya *et al.*, 2004). During the crop cycle, irrigation water sources and drainage make the system intimately connected with natural wetlands that harbor a large number and diversity of terrestrial and aquatic organisms, providing supplementary habitats for vertebrate and invertebrate species (Fernando, 1993; Schoenly *et al.*, 1998; Zhang *et al.*, 2013).

However, rice production has harmed the areas where it is grown due to the excessive use of water, pesticides, methane emissions, and the conversion of natural habitats into simplified agroecosystems (FAO, 2004; IPCC, 2007; Rizo-Patrón *et al.*, 2013).

Rice management practices range from organic farming to methods that include the intensive use of agrochemicals for pest control (Abdullah *et al.*, 1997). Agroecological management contributes to improving the sustainability of agroecosystems, reducing the use of external and nonrenewable inputs with the greatest potential to damage the environment and is based on ecological processes and ecosystem services—nutrient cycling, biological N fixation, carbon sequestration, natural regulation of pests, soil and water

conservation, and biodiversity conservation (Al-tieri & Nicholls, 2005; Wezel *et al.*, 2014). Many authors highlight that the maintenance of water quality achieved by agroecological practices increases food safety levels, biodiversity conservation, and ecosystem integrity (Brussaard *et al.*, 2010; Phalan *et al.*, 2011).

On the other hand, conventional rice cultivation could contribute to pollution scenarios because it must be flooded in one stage of the crop cycle, and in another one, the water is returned to the receiving course. Thus it can potentially contaminate wetlands or adjacent watercourses, generating a potential risk to non target aquatic species (Marques *et al.*, 2011). Currently, the agrochemicals used in rice farming are quite diverse, many of them with very low levels of toxicity and rapid degradation in the environment, but some pose high environmental risks.

The zooplankton community plays a fundamental role in trophic networks (Jernberg *et al.*, 2017) by cycling nutrients and stabilizing aquatic ecosystems. It is also sensitive to environmental fluctuations, species-specifically responding, which results in different dominance between species (Adamczuk *et al.*, 2015). Zooplankton communities have small body sizes and short generation times, responding quickly and with high sensitivity to environmental changes (DeLorenzo *et al.*, 2001; Hanazato, 2001).

There are several field studies that analyze the zooplankton community in relation to agricultural practices (e.g., Dodson *et al.*, 2007; Iturburu *et al.*, 2019; Regaldo *et al.*, 2018). Nevertheless, this community responds to several environmental factors, which makes it difficult to relate its response to pesticide contamination in the fields. In this sense, the selection of two rice fields no far apart (70 Km),

belonging to the same watershed, offers promising opportunities to deepen the knowledge on agrochemical effects on zooplankton communities of rice fields under different management practices.

Due to its importance in aquatic communities, the loss of zooplankton species by exposure to pesticides can alter top-down and bottom-up mechanisms, and modify the structure and function of biological communities (Carpenter & Kitchell, 1996).

Living organisms as indicators of pollution have been used for the conservation of biodiversity in rice fields (Ueno, 2013). Although zooplankton is a key component of the aquatic fauna of rice fields (Lim et al., 1984), only in very few countries, such as Italy and Southeast Asian countries, was zooplankton used as a bioindicator of contamination in paddy fields (Chittapun et al., 2009; Ferrari et al., 1991; Ferrari et al., 1984; Leoni et al., 1998). Especially in developing countries, where control policies are limited, it is important to conduct risk assessments in rice

fields based on site-specific measured environmental concentrations (MECs) to identify possible risks to aquatic organisms (Stadlinger et al., 2018). The knowledge about relevant ecological aspects of temporary rice wetlands is still scarce. Moreover, no works were found simultaneously comparing the zooplankton community structure under different crop management practices. We hypothesize that the biological indicators (abundance, density) and ecological indices: Diversity (Shannon Index), Dominance (Simpson Index), and Evenness (Pielou Index) show better ecological conditions in AE than in CC.

To address this hypothesis, the objective of this research was to carry out an exhaustive analysis of the structure of the zooplankton community of rice fields under two different management practices: with the application of pesticides (conventional crop, CC) and without them (agroecological crop, AE) in San Javier Department (Santa Fe, Argentina) to better understand the possible effects of agrochemicals on zooplankton under both cultivation practices.

## METHODS

### Study area

The study area is located in the central-eastern region of Argentina, in San Javier Department, Santa Fe province (Fig. 1). While in Argentina soy is the predominant cultivated crop (18 056 462 ha, 2016/17 harvest, Ministerio de Agricultura, Argentina), rice production is incipient (206 500 ha 2016/17 harvest, Ministerio de Agricultura, Argentina). Rice fields are mainly located in the flood plains of the Paraná River (Alvisio, 1998), occupying a north-south area 15-20 km wide and 100 km long with a total cultivated area of 30 000 ha. In the area, native grasslands and natural wetlands coexist with land used for intensive farming and cattle breeding (Begenesic, 1998). Unlike dry-land crops, rice requires irrigation during some of its growth stages; the water for the irrigation of rice fields comes from the San Javier River, a tributary of the Paraná River (Castignani, 2011). The sowing season extends from August to December, and the harvest takes place in February and March (GRiSP, 2013). The climate is hot



**Figure 1.** Location map of sampling sites. a- Argentina; b- Santa Fe Province, San Javier Department. Agroecological crop (AE) and Conventional crop (CC). *Mapa de los sitios de muestreo. a- Argentina; b- Provincia de Santa Fe, departamento de San Javier. Cultivo Agroecológico (AE) y Cultivo Convencional (CC).*

and humid, with rainfall exceeding 1000 mm per year and an average annual temperature of 18 °C.

The samplings were carried out between December 2015 and February 2016, during the rice-growing season. This period includes both the seeding and harvesting of rice. Rice fields under different management practices were selected: conventional crops (30° 05' 13.56" S - 59° 53' 19.98" W), with the use of pesticides in the production process, and agroecological crops without the application of pesticides (30° 36' 38" S - 59° 57' 55" W). Conventional and agroecological rice fields were separated by a distance of 70 km. In both rice fields, water, sediment, and zooplankton samples were taken simultaneously.

### **Determination of environmental parameters and screening of pesticides in water and sediment**

In each rice field, the following environmental parameters were surveyed in situ: water temperature (°C), conductivity (µmhos/cm), dissolved oxygen (DO) (mg/L), and pH with YSI Professional Plus Multiparameter Water Quality Meter.

Water samples of each rice field were collected for physical and chemical determinations and were kept refrigerated until analysis. In the laboratory, the parameters measured were: turbidity (TNU), conductivity (µmhos/cm), dry residues (mg/L dried at 180 °C), alkalinity (mg/L), carbonates (mg/L), bicarbonates (mg/L), total hardness (mg/L), calcium (mg/L), magnesium (mg/L), total iron (mg/L), sulfate (mg/L), fluorides (mg/L), chloride (mg/L), nitrates (mg/L), nitrites (mg/L), ammonium (mg/L), arsenic (mg/L) and phosphorus (reactive phosphorus mg/L) following the methodology of the American Public Health Association (APHA, 2012).

Screening of pesticides in water and sediment of both rice fields was carried out. For this purpose, a liquid-liquid partition was used to extract pesticides from sediment samples with acetonitrile, following the QuEChERS approach (Anastassiades *et al.*, 2003) with minor modifications. Briefly, 5 g of sediment was soaked with 10 mL of water (0.2 % formic acid) for 15 min, then 10 mL of acetonitrile was added, and the sample was shaken for 30 min on a horizontal mechanical shaker. Then

partition was induced by adding 4 g MgSO<sub>4</sub> and 1 g NaCl, and completed with one-minute vigorous manual agitation followed by ten-minute centrifugation at 15 000 rpm at room temperature. Then, 500 mL water samples were subjected to a preparation and cleaning process by solid-phase extraction (SPE) with C18, based on the methodology described by (Picó *et al.*, 2007) before the chromatographic injection. Separate determinations of glyphosate, AMPA (the primary metabolite of the microbial degradation of glyphosate), and glufosinate-ammonium were performed as described by Sasal *et al.* (2015).

For LC-amenable pesticide determination, an ultra performance liquid chromatograph was employed (ACQUITY UPLC™, Waters, Milford, MA, USA) coupled to a triple quadrupole mass spectrometer (Micromass TQ Detector from Waters, Manchester, UK) through an orthogonal-Z-spray ionization source (ESI+ and ESI-). Moreover, aspects related to chromatographic methodology, mobile phase composition, ionization conditions, and operating variable detection in multiple-reaction monitoring (MRM) mode of the triple quadrupole mass spectrometer were evaluated. The separation was carried out in a rapid resolution column (C18, 2.1× 100 mm, 1.7 µm) using gradient elution, with an acetonitrile and water mix mobile phase, both with 0.1 % (v/v) formic acid. For mass detection, two transitions from each compound pseudomolecular ion ([M+H]<sup>+</sup> or [M-H]<sup>-</sup>) were used for identification in addition to the retention time, while for quantification, the most abundant transition was used. Gas chromatography (GC) analysis of non-polar pesticides was performed with a GLC system coupled to a 63Ni electron capture detector (Hewlett Packard Model 5890) using two different columns: Pas 5 (25 m, 0.32 mm ID, film th. 0.52 mm) and Pas 1701 (25 m, 0.32 mm ID, film th. 0.25 mm).

Validation was carried out following the SANTE/11945/2015 document (2015), determining recovery, selectivity, limits of quantification (LOQ) and detection (LOD), linearity, precision, and accuracy. The specifications of each pesticide are detailed in Table S1 and S2 of the supplementary data. (Supplementary information, available at <http://www.limnetica.net/en/limnetica>)

### Zooplankton: sampling, taxonomic analysis, and community structure

Zooplankton sampling in CC and AE rice fields was carried out 4 times, 20 days apart, covering the entire water cycle of rice production. The first sampling was performed after the rice field was flooded and at the beginning period of rice plant growth; the second and third samples were taken in the mid-cycle period, and the last sampling was carried out in the pre harvest period, just before the fields were drained. In each rice crop, sampling was performed in the perimeter and central areas; for this purpose, one 100 m long transect was established in each rice field, covering the

different microhabitats. An equal area was sampled in each rice field to avoid the area and spatial autocorrelation effects.

At each rice field and sampling date, a qualitative sample of zooplankton was taken with a plankton net (45  $\mu\text{m}$ ) to later determine the species richness. Also, three quantitative samples (replicates) of 100 mL were taken for the quantitative analysis of the zooplankton community by filtering 12 L of rice field water for each replicate. All the samples were stained with erythrosine and fixed with 10 % formalin in the field.

A total of 32 zooplankton samples were analyzed (4 qualitative and 12 quantitative samples for each rice field). The taxonomic identification

**Table 1.** Analysis of water quality of agroecological crop (AE) and conventional crop (CC). *Análisis de la calidad del agua del cultivo agroecológico (AE) y del cultivo convencional (CC).*

	AE	CC
<b>Turbidity</b> (U.N.T.)	15	40 $\pm$ 7.1
<b>Conductivity</b> ( $\mu\text{mhos/cm}$ )	130.6 $\pm$ 32	113.9 $\pm$ 16.7
<b>Dry waste</b> (mg/L)	87.6 $\pm$ 18.9	81.6 $\pm$ 7.4
<b>Alkalinity</b> ( $\text{CaCO}_3$ ) (mg/L)	43.0 $\pm$ 11.4	39.2 $\pm$ 4.7
<b>Carbonates</b> ( $\text{CO}_3^{2-}$ ) (mg/L)	< 0.5	< 0.5
<b>Bicarbonates</b> ( $\text{HCO}_3^-$ ) (mg/L)	34 $\pm$ 12.3	47.9 $\pm$ 5.8
<b>Total hardness</b> ( $\text{CaCO}_3$ ) (mg/L)	31.4 $\pm$ 1.1	34.9 $\pm$ 15.7
<b>Calcium</b> (Ca) (mg/L)	8.2 $\pm$ 3.2	5.3 $\pm$ 0.1
<b>Magnesium</b> (Mg) (mg/L)	4.6 $\pm$ 4.6	5.2 $\pm$ 3.7
<b>Total iron</b> (Fe) (mg/L)	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
<b>Sulfate</b> ( $\text{SO}_4^{2-}$ ) (mg/L)	13.3 $\pm$ 0.2	14.8 $\pm$ 3.7
<b>Fluoride</b> ( $\text{F}^-$ ) (mg/L)	< 0.2	< 0.2
<b>Chloride</b> ( $\text{Cl}^-$ ) (mg/L)	14.5 $\pm$ 5.1	12.6 $\pm$ 2.6
<b>Nitrates</b> ( $\text{NO}_3^-$ ) (mg $\text{NO}_3\text{-N/L}$ )	1.2 $\pm$ 0.8	1.3 $\pm$ 0.1
<b>Nitrites</b> ( $\text{NO}_2^-$ ) (mg $\text{NO}_2\text{-N/L}$ )	< 0.002	< 0.002
<b>Ammonium</b> ( $\text{NH}_4^+$ ) (mg $\text{NH}_4\text{-N/L}$ )	0.6 $\pm$ 0.2	0.9 $\pm$ 0.1
<b>Arsenic</b> (As) (mg/L)	< 0.01	< 0.01
<b>Soluble reactive phosphorus</b> (mg/L)	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1



was made with a binocular microscope (Motic SMZ-168), an optical microscope (Olympus Cx31), and specific keys for each of the main taxa: Cladocera, Copepoda, and Rotifera (Ahlstrom, 1940, 1943; Koste & Shiel, 1989; Lopretto & Tell, 1995; Paggi, 1995; Ringuelet, 1958). The quantitative samples were subsampled using a 1 mL pipette and transferred to a Sedgewick Rafter counting chamber. The mean of three subsamples was used to calculate the abundance of zooplankton in 1 L water sample replicate from both rice fields. Cladocerans and rotifers were identified to the lowest possible taxonomic level (except for bdelloid rotifers). Copepods were identified up to order level (Cyclopoida, Calanoida, and Harpacticoida—mature stage), nauplii, and copepodite (immature stages) (Reimche *et al.*, 2015).

The richness, abundance, density, diversity, evenness, and dominance of species for each rice field were calculated with the obtained data. The richness (S) was calculated by counting the number of recorded species or taxa, the abundance by counting the number of individuals in each sample, and the density by counting the number of individuals/L of each species or taxa in both rice fields, the diversity of rotifers and cladocerans was calculated using the Shannon-Weaver diversity index  $H$  (Makoto & Tsutomu, 1984). The evenness (Pielou  $J'$ ) was assessed for each zooplankton community, and the dominance of species was calculated using the Simpson index following Begon *et al.* (1986).

The three diversity indices were calculated using the statistical program PAST version 4.05 (Hammer *et al.*, 2001). The Shapiro-Wilk test was used to test for the normal distribution of residuals. ANOVA was used to test for significant effects of crop management (CC *versus* AE) on the number of taxa, abundances, and the three diversity indices of the zooplankton community. All the tests were performed using Infostat (Di Rienzo *et al.*, 2018).

An analysis of similarities (ANOSIM) was used to test for any differences between the rice fields (CC and AE) based on their zooplankton composition of Cladocera and Rotifera (Clarke & Green, 1988). Because ANOSIM revealed significant differences between rice crops, a similarity percentage analysis (SIMPER) was applied to

determine which species contributed most to the differences in zooplankton communities between CC and AE. The analysis of similarities and the similarity percentage are based on the decomposition of the Bray-Curtis dissimilarity index (Clarke, 1993) and were analyzed using the program PAST version 4.05 (Hammer *et al.*, 2001).

## RESULTS

### Water quality parameters

In CC, the mean physicochemical parameters of the water were: dissolved oxygen, 7.2 mg/L; temperature, 29.6 °C; pH, 5.8; conductivity, 118.7  $\mu$ mhos/cm. In AE: dissolved oxygen, 4.6 mg/L; temperature, 33.4 °C; pH, 5.5; conductivity, 133.3  $\mu$ mhos/cm. Although slightly acidic, these pH values are similar to others reported for rice fields (Ahmad *et al.*, 2014). Water quality parameters did not show significant differences between CC and AE (Mann-Whitney test,  $p > 0.05$ ). The results of the laboratory physical and chemical analyses are listed in Table 1.

### Pesticide concentrations in water and sediments in CC and AE rice fields

In AE, pesticide concentrations were under the LOD in water, while in sediment, residues of bentazone were found ( $1.1 \pm 0.3$   $\mu$ g/kg). Conversely, in the CC water samples, three compounds were recorded: bentazone ( $0.4 \pm 0.1$   $\mu$ g/L), glyphosate ( $0.9 \pm 0.2$   $\mu$ g/L), and AMPA ( $8 \pm 2$   $\mu$ g/L), while in the sediment samples, five compounds were found: bentazone ( $1.3 \pm 0.4$   $\mu$ g/kg), AMPA ( $25 \pm 8$   $\mu$ g/kg), clomazone ( $15 \pm 5$   $\mu$ g/kg), imidacloprid ( $9 \pm 3$   $\mu$ g/kg), and tebuconazole ( $135 \pm 20$   $\mu$ g/kg).

### Zooplankton community structure in CC and AE rice fields

#### *Species composition*

The highest species richness was recorded in AE, with a total of 94 taxa of zooplankton identified in this crop. Of these, 27 were cladoceran species, 65 were rotifer species, and 2 taxa were copepods. In CC, the number of zooplankton taxa was

lower (79 taxa): 17 cladoceran species, 59 rotifer species, and 3 orders of copepods. When analyzing the relative richness (%), it became evident that the major component of zooplankton in each crop was rotifers (69.1 % in AE and 74.7 % in CC); however, AE presented a higher percentage (around 10 %) of cladoceran species.

In AE, *Polyarthra* was the dominant genus among rotifers, and *Chydorus* among cladocerans; meanwhile, in CC, *Lecane* and *Diaphanosoma* were the dominant genera, respectively.

### Zooplankton abundance and density

#### Abundance

The difference in abundance (total number of individuals) between AE and CC was mainly due to rotifers (5022.2 in AE *versus* 1058.3 in CC, see Table 2) and copepods (267.6 in AE *versus* 828.7 in CC). Despite these particular differences, the

overall abundance of total taxa was greater—almost double—in AE (6979.6 in AE *versus* 3733.3 in CC, Table 2). The abundance of rotifers was higher in AE, and the difference was statistically significant ( $p = 0.0286$ ). For the copepod abundance, no statistical differences were found between rice fields ( $p > 0.05$ ).

#### Density

In AE, 16.9 ind./L was the average abundance of cladoceran species, and 20.5 ind./L the average abundance of rotifer species. In CC, we found an average abundance of 27.5 ind./L for cladocerans and 5.4 ind./L for rotifers. The comparison of total density (Ind./L) between AE and CC showed significant differences of rotifers ( $p = 0.0413$ ). In both cultures, the nauplii had the highest density (AE: 1784.3 ind./L; CC: 1399.1 ind./L), indicating active reproduction in AE, followed by *Polyarthra* spp. among rotifers (average abundance

**Table 2.** Values for abundance, taxa richness, and diversity indices in the zooplankton community in the agroecological (AE) and conventional crops (CC). *Abundancia, riqueza de taxones e índices de diversidad de la comunidad zooplanctónica en el cultivo agroecológico (AE) y convencional (CC).*

		Tx	Ab	H	Simpson	J'
Cladocera	AE	<b>27*</b>	1689.8	<b>2.03**</b>	0.19	<b>0.74**</b>
	CC	17	1846.3	1.15	<b>0.42*</b>	0.52
Rotifera	AE	65	<b>5022.2*</b>	2.7	0.13	0.72
	CC	59	1058.3	2.6	0.14	0.75
Copepoda	AE	2	267.6	-	-	-
	CC	3	828.7	-	-	-
Total taxa	AE	94	6979.6	<b>3.0*</b>	0.09	<b>0.73*</b>
	CC	79	3733.3	2.37	<b>0.17*</b>	0.62

Bold numbers, significant differences; Ab, abundance; Tx, taxa number; H', Shannon-Wiener index; Simpson, Simpson's index; J, Pielou's evenness index. \* Significant difference at  $p < 0.05$ ; \*\* Significant difference at  $p < 0.01$ .

**Table 3.** The 8 highest-ranking taxa contributing to the dissimilarity between agroecological (AE) and conventional (CC) rice fields. *Los 8 taxones de mayor contribución a la diferencia entre los campos de arroz agroecológico (AE) y convencional (CC).*

Taxa	Contr %	Cum %	Mean abundance	
			AE	CC
Bdelloidea	11.4	11.4	280	55.6
<i>Diaphanosoma spinolosum</i>	8.1	19.5	9.5	181
Orden Calanoida	7.0	26.5	6.9	147
<i>Moina minuta</i>	6.0	32.5	14.1	120
<i>Diaphanosoma brevireme</i>	5.5	38	53.5	112
<i>Polyarthra</i> spp.	4.6	42.6	120	28.7
<i>Chydorus</i> sp.	4.1	46.7	97.7	0
<i>Plationus patulus</i>	4.0	50.7	95.6	1.8

Contr%, average contribution to overall dissimilarity; Cum% ordered cumulative contribution.

478.7 ind./L) and *Chydorus* spp. among cladocerans (390.74 ind./L) in AE and *Lecane papuana* Murray, 1913 (139.81 ind./L) and *Diaphanosoma spinolosum* Murray, 1913 (average abundance 722.2 ind./L) in CC.

#### *Zooplankton diversity, dominance and evenness*

Considering all taxa, the highest Diversity Index ( $H = 3.0$ ) and Evenness Index ( $J' = 0.73$ ) were reported for AE. In contrast, the Dominance Index (Simpson Index = 0.17) was higher in CC; the ecological conditions in CC favored the abundance of fewer taxa. In all three indices tested, the differences were statistically significant (Table 2).

#### *Zooplankton dissimilarity*

The ANOSIM test revealed statistical differences between the two rice fields based on species composition and abundance ( $R = 0.93$ ,  $p = 0.026$ ). Of

the total taxa recorded, 44 % were species present only in AE samples, while in CC, only 13 % of exclusive taxa were recorded. The SIMPER analyses identified the taxa that contributed most to the observed difference (Table 3): Bdelloidea, *Plationus patulus* Müller, 1786; *Polyarthra* spp., *Chydorus* sp., *Diaphanosoma brevireme* Sars, 1901; *Diaphanosoma spinolosum* Herbst, 1975; *Moina minuta* Hansen, 1899, and the order Calanoida. Together, these 8 taxa accounted for more than 50 % of the dissimilarity between AE and CC rice fields. The percentage of dissimilarity between crops was high = 79.9 %.

## DISCUSSION

The aim of this study was to analyze whether the water quality—measured through nutrient and pesticide concentrations—of two rice fields in eastern Argentina differs according to conventional or agroecological management.



The analysis of physicochemical environmental parameters showed slight differences between CC and AE in some parameters but with no statistical differences, so it can be ruled out that they caused the differences recorded in the zooplankton.

Our results showed that the AE rice field presented greater richness than CC, a more significant proportion (%) of cladoceran species, which is considered the most sensitive group among zooplankters, as well as greater abundance, diversity, and evenness of taxa. In contrast, in CC, only the dominance was higher than in AE, mainly explained by the dominance of fewer species and copepods. The CC was dominated by small species, as was pointed out by Relyea & Hoverman (2006), who argued that some herbicides could interact with a range of different natural stressors.

Agrochemicals can reach freshwater bodies by drift, leaching, or runoff from agricultural lands to surface waters, affecting freshwater quality and non-target organisms (Andrade et al., 2021; Etchegoyen et al., 2017; Jergentz et al., 2005; Sasal et al., 2015). The main effects may usually be (a) dominance of smaller species, (b) reduction of species richness and diversity, and (c) reduction in the efficiency of energy transfer from primary producers to higher predators (Hanazato, 2001).

Although the taxonomic composition differed between rice crops, the presence of many taxa that only occur in the agroecological field indicates that this management practice favors the establishment of some exclusive zooplankton taxa of cladocerans and rotifers compared to CC. Also, total abundance, density, diversity, and evenness were favored.

The present results show that cladocerans were more sensitive to agrochemicals than rotifers and copepods. The abundance of cladoceran populations could be controlled by one or several factors related to pesticide exposure: sublethal toxicity affected the physiological functioning; especially their reproductive capacity, habitat selection, and food preferences resulted in a differential exposure to some species and changes in predator-prey relationships and interspecific competition (Lim et al., 1984). In this survey, Rotifera was less affected than Cladocera, in agreement with Neves et al. (2003), who stated that rotifers possess a wide tolerance to environmental variability due

to their small size and short life cycle. Also, little influence of agrochemicals was observed for nauplii and copepodites.

Specifically, in rice crops, during the dry phase, agrochemicals can move from treated rice field waters to natural water bodies (Heong et al., 1995) and quickly spread to other areas through spray drift and irrigation channels. In this scenario, the pesticides recorded in CC could damage zooplankters and other aquatic organisms of the receiving water bodies, in this case, the San Javier River. This wetland is used by local people for fishing, recreation, and as drinking water supply.

Regarding the pesticides recorded in CC, Rizo-Patrón et al. (2013) highlighted that one technique used in conventional rice fields is to apply glyphosate and clomazone (both found in this work) that cause mortality and changes in the behavior, reproduction, and development of aquatic organisms. Among herbicides, glyphosate stands out because of its massive use, as in Argentina and other developing countries (Stadlinger et al., 2018). Many studies assessed glyphosate toxicity to the non-target organism, such as zooplankton, and suggest that the commercial formulation of glyphosate can be more toxic than the active ingredient (Reno et al., 2018; Reno et al., 2014). Moreover, clomazone acts as inhibiting pigments, causing indirect adverse effects on the zooplankton, diminishing its abundance or inducing changes in its taxa composition due to reduced feed availability (Relyea, 2005, 2009).

Among other pesticides found in the present study, imidacloprid is one of the most widely used neonicotinoid insecticides. Several studies have demonstrated harmful effects on a wide range of non-target communities—zooplankton, benthic, neuston, and macroinvertebrates (Hayasaka et al., 2012; Sánchez-Bayo & Goka, 2006; Van Dijk et al., 2013). Moreover, the effects of bentazone on zooplankton species were studied by Barata et al. (2007), who reported severe effects on the grazing rate and enzyme inhibition on *Daphnia magna*. The recorded residues of bentazone in AE could be due to residues of previous conventional practices of which there are no records. Bentazone could also reach AE by drift from surrounding fields, as suggested by Ronco et al. (2016).

Especially in developing countries where controls are scarce or insufficient, pesticides pose a health risk. Lajmanovich & Peltzer (2008) and Attademo *et al.* (2014) drew attention to the risks posed by the use of pesticides in wetland areas of high conservation value. At a global level, there are increasing demands for good water quality and healthy food products. Agroecological practices should be encouraged by managing rice fields within a framework of “rational use” by developing practices that reconcile economic sustainability with high biodiversity levels. Pesticide risk assessment at the community level based on long-term monitoring is imperative for protecting biodiversity and food safety.

## CONCLUSION

From the results obtained after comparing two rice fields under conventional and agroecological practices, we can conclude that in AE, the parameters that showed better values than in CC were the richness of Cladocera taxa, diversity, evenness of taxa, and abundance of rotifers. In contrast, in CC, only the dominance was higher than in AE, mainly explained by the dominance of smaller species and copepods. In synthesis, the obtained results supported the initial hypothesis regarding better ecological conditions in AE than in CC, and highlighted that the zooplankton structure is a good indicator of water quality in rice fields.

## ACKNOWLEDGMENTS

We thank the owner of the fields for allowing us to conduct the research. This work was carried in part by “Importancia de los bordes de campo de arroz orgánico en el rol de los vertebrados en el control biológico de plagas” and “Elaboración de un índice de sostenibilidad (indicadores ecológicos, económicos y sociales) de sistemas acuáticos de la provincia de Santa Fe” supported by Curso de Acción para la Investigación y Desarrollo (Programa de I + D Orientado a Problemas Sociales y Productivos, UNL) whose directors are A.M. Attademo and A.M. Gagneten. We especially thank Florencia Gutierrez for collaboration with SIMPER and Paola Peltzer, Candela Martinuzzi, and Agustin Basso for their help in fieldwork.

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