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ORIGINAL ARTICLE

Anuran larvae diet from agroecosystem's ponds: environmental quality and implications for their populations

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ABSTRACT
Agriculture is considered an important factor for the decline of amphibians recorded in the last decades. Intensive agriculture requires the application of great amounts of pesticides and fertilizers, consequently many aquatic habitats have been altered. The over-enrichment of waters with nutrients from agriculture causes growth of algae and cyanobacteria and the shift of the community composition toward the dominance of tolerant taxa. The aim of this study was to analyze the diet of tadpoles and the size of larvae and metamorphs of the native species Rhinella arenarum *in agroecosystems of central Argentina. Four sampling sites with different degree of human disturbance were selected, three temporary ponds from agroecosystems (A1, A2, A3), and a pond in a semi-modified landscape, not affected by agriculture or cattle. The sites were visited once a week from October 2013 to January 2014. Tadpoles and metamorphs were captured, anesthetize and preserved. Morphometric parameters were measured on tadpoles and metamorphs and for 20 tadpoles the complete intestine was removed and their contents analyzed. Phosphate values were higher in agroecosystem ponds. Body size and mass of tadpoles and metamorphs were different between sites, being smaller in agricultural ponds. Diet analysis revealed that all larvae mainly consumed microalgae. Larval diets allowed differentiating the sites, larvae from A1 and A3 sites showed a diet with a similar composition and structure (with a predominance of* Nitzschia palea*), meanwhile larvae from A2 and SM showed different diets. In SM, the larvae mainly consumed* Trachelomonas *sp. Analysis of structural variables revealed a higher algal abundance in the diet of larvae from A3, a lower richness in SM and that there were no differences between sites in diversity and evenness of diets. Our hypothesis was support in part, given that resistant algae taxa were found in agroecosystem ponds and tadpoles and metamorphs were smaller, but larval diets showed higher abundance and richness. The analysis of the diet of aquatic stages of* R. arenarum *allowed us to know the trophic availability and environmental quality of temporary ponds from agroecosystems.*

Key words: Rhinella arenarum*; bioindicator; algae; diatoms; tadpoles; metamorphs.*

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INTRODUCTION

Amphibian populations are experiencing worldwide declines (Collins and Crump, 2009). Agriculture is considered an important factor for the decline of amphibians recorded in the last decades (Collins and Crump, 2009). The 89% of the amphibian anuran species are affected by agriculture (GAA, 2004; Young *et al*., 2004).

The conversion of forest to agricultural land is occurring at rapid rates in many Neotropical areas (Pengue, 2004). The central region of Argentina has been greatly affected by agricultural expansion (Rossi, 2006). Agriculture is intensive and it requires the application of great amounts of pesticides and fertilizers, consequently many aquatic habitats in this area have been altered (CASAFE, 1999; Lajmanovich *et al*., 2005).

Farming and livestock alter the geomorphology of the aquatic and terrestrial environment and modify the hydroperiod, water quality, vegetation cover, productivity of the environment and food webs (Knutson *et al*., 2004; Nori *et al*., 2013). Likewise, the chemical products used in farming activities can contaminate water bodies through leaching, causing damages to the aquatic environments (Knutson *et al*., 2004, Rimet and Bouchez 2011). High concentrations of elements such as phosphorous and nitrogen have been detected in agricultural areas (Hamer *et al*., 2004). These nutrients cause the eutrophication of the aquatic systems with a drastic decrease of dissolved oxygen and the further death of aerobic organisms (Mitsch and Gosselink, 2000). Moreover, the nutrient availability is one of the most relevant factors that modulate the dynamics of algal communities (Rosemond *et al*. 1993, Tank and Dodds 2003). The over-enrichment of waters with nutrients from agriculture causes growth of algae and cyanobacteria (Correll, 1998; Sabater *et al*. 2000, 2003) and the shift of the community composition

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toward the dominance of tolerant taxa (Whitton and Kelly, 1995; Stevenson and Pan, 1999).

The quality of aquatic environments may influence both time at metamorphosis of tadpoles and the size of metamorphs. In addition, may reduce survival and increase predation pressure (Carey and Bryant, 1995; Altig *et al*., 2007). Recent evidence has revealed that the deterioration of agricultural ponds might modify the trophic availability, affecting the development and growth of tadpoles that are sustained by such environments (Bionda *et al*., 2012; 2013). Impacts on the early stages of development negatively affect the recruitment of metamorphs (Schmutzer *et al*., 2008; Burton *et al*., 2009) and the populations' size (Heyer, 1974; Wilbur, 1980).

To assess the quality of the environment and how it changes over time several bioindicators can be used, including biological processes, species, or communities (Holt and Miller, 2011). Studies on amphibian larvae diet can be a general measure of supply and trophic availability of different habitats. The diet of anuran tadpoles is usually diverse, composed of plant fragments, protozoa, rotifers, anuran eggs, and other tadpoles. However, most tadpoles are primarily herbivorous, consuming a wide variety of algal taxa (Kupferberg, 1997; Altig *et al*., 2007). Diatoms are one of the most common taxa of algae in the diet of tadpoles (Bionda *et al*., 2012). Diatoms are widely used to monitor river organic pollution and eutrophication because they are sensitive to water chemistry, especially ionic content, pH, dissolved organic matter, and nutrients (Potapova and Charles 2007; Gómez and Licursi, 2001). In this way the diet of these organisms may constitute a bioindicator for characterization and monitoring of environmental quality (Blanco *et al*., 2004; Bionda *et al*., 2013).

The aim of this study was to analyze the diet of tadpoles and the size of larvae and metamorphs of the native species *Rhinella arenarum* (Hensel, 1867), that inhabit in ponds from agroecosystems from the central region of Argentina. This anuran species has a wide Neotropical distribution and provide a suitable and useful model for biomonitoring aquatic ecosystems (Vera Candioti *et al*., 2010). The study of the diet of aquatic stages of *R. arenarum* allows us to know the trophic availability and environmental quality of temporary ponds from agroecosystems with different anthropogenic alteration degrees. The study of the diet of aquatic stages of *R. arenarum* can also help us to infer its implication on the size of tadpoles and metamorphs, feature that affects the survival of organisms and regulates the populations of frogs (Kupferberg *et al*., 1994).

We hypothesized that agriculture affects trophic availability to tadpoles, therefore affecting body size, so we expected larval diets in agricultural ponds to be less abundant and diverse, with algal species resistant to pollution, and smaller tadpoles and metamorphs.

METHODS

Study area and sampling sites

The study region belongs to the Pampa Plains of central Argentina. The main socio-economic activities of this area are farming and livestock. Moderately undulating plains and a temperate climate characterize the area. Annual average rainfall ranges between 700 and 800 mm and the maximum rainfall occurs between October and March. Annual mean temperature is 18°C ranging between a mean of 23°C in January and 6°C in July (Bridarolli and di Tada, 1996; PEGRC, 2011; Gatica *et al*., 2012).

Four sampling sites with different degree of human disturbance were selected. Three sites corresponded to temporary ponds from agroecosystems: A1 (33°05'51" S, 64°26'02" W; 471 m asl), rural landscape with a pond near to the crop (about 50 meters) used by cattle to drink water; A2 (33°06'09" S, 64°25'32" W, 467 m asl), rural landscape with pond close to the crop (about 60 meters) and not used by cattle; A3 (33°05'39" S, 64°25'58" W, 468 m asl), rural environment with pond away from the crop (about 900 meters) and used by cattle to drink water. The other site was a semi-modified landscape used as a reference site, not affected by agriculture or cattle: SM (33°06'42 S, 64°18'12 W, 428 m asl) in a protected natural area, the native forest 'El Espinal', located within the National University of Río Cuarto. Ponds of all study sites are temporary and have no fishes. Native species such as *Rhinella arenarum* are known to use these ponds for reproduction and unhealthy tadpoles were found in these sites (Babini *et al*., 2015a, 2015b, 2016).

Bioindicator species

Amphibians possess several characteristics that may make them more sensitive to environmental disturbances than other wildlife (Rowe *et al*., 2003; Hopkins, 2007). Moreover, their aquatic-terrestrial life cycle places them in *doublejeopardy* because a disturbance to the quality or availability of either habitat can disrupt their life cycle and affect populations (Dunson *et al.*, 1992). We selected the native species *R. arenarum* (Hensel, 1867), because its sensitivity as bioindicator was demonstrated in several studies (Howe *et al*., 1998; Venturino*et al*., 2003; Bosch *et al*., 2011; Lajmanovich*et al*., 2014). This anuran species has a wide Neotropical distribution (Frost, 2014) and it is commonly found in forests, wetlands, riversides, urban and agricultural lands. Tadpoles of *R. arenarum* are black and they have benthic habits (Cei, 1980; Echeverria *et al*., 1987; Gallardo, 1987).

Field work

Field work was carried out from October 2013 to January 2014. The sites were visited once a week. The Visual

Encounter Survey (VES) methodology was used for the sampling of individuals. VES consists in walking around the borders of the pond to observe tadpoles and metamorphs. Individuals were captured, anesthetized with a solution at 0.5% of MS 222 or Methanesulfonate Salt (3-Aminobenzoic Acid Ethyl Ester, Sigma-Aldrich, St. Louis, MO, USA) and preserved in Phosphate Buffer for further analysis. Samples of water and riparian macrophytes were taken at each site in order to analyze the algal availability for tadpoles.

The environmental variables that were surveyed at each site per week were: water and air temperature (at 100 cm from the ground), pH, conductivity, total dissolved solids (TDS) and salinity of water bodies using a digital multiparameter 35-series 35425-10 test (Oakton Instruments,Vernon Hills, IL, USA).Water quality analyses were performed to water samples from study sites. These analyses were carried out by the area of Hydrology, Department of Geology, National University of Río Cuarto, according to the standard methods of APHA-AWWA-WEF (1998). Carbonates and bicarbonates were measured by potentiometric titration with Thermo Orion-selective electrode; sulfates were measured by turbidimetry (with centrifugal Macrotronic); chloride was measured by colorimetric titration with silver nitrate; calcium and magnesium were measured by colorimetric titration with EDTA; sodium and potassium were measured by flame photometry (315 Metrolab digital photometer); arsenic was measured with ICP-MS and fluoride by ion selective electrode (Orion-Thermo). Nitrates are determined by potentiometry with ion selective electrode (Orion Model 9307) reference electrode and Orion potentiometer 710 A. To calibrate the potentiometer 6 benchmarks (5, 10, 25, 50, 100 and 300 mg/L NO^{3-}) were used. The detection limit of the determination of NO^{3-} is 0.2 mg L^{-1} and the analytical error of the measurements is 0.5%.

Laboratory analysis

Morphometric parameters measured on tadpoles and metamorphs were: development stage (following Gosner, 1960); mass by means of a Mettler balance (P11N 0-1000 g); total length (TL; length from the snout to the end of the tail on tadpoles and snout-vent length on metamorphs); maximum width (MW; maximum width of the body, dorsal view); tail muscle height (TMH; tail muscle height bundle the vent tube, lateral view, only on tadpoles). For a total of 20 tadpoles (5 per site) in stages 38-40 of Gosner (1960), the complete intestine was removed and their contents analyzed (only first third). The food items were determined under a Zeiss optical microscope at 40X, according to the technique described by Hill *et al.* (2000). Items were identified to the genus level. The algal density estimate was based on quantitative samples, following Villafañe and Reid (1995).

Data analysis

Normality and homogeneity of variance was assessed by Kolmogorov–Smirnov and Levene tests, respectively, using different software packages according to the analysis: InfoStat/P ver. 1.1 (Di Rienzo *et al*., 2012) and Statistica (StatSoft, 2001). Mass of tadpoles and metamorphs was regressed on total length and the residuals were taken as an index of body condition (BC) of individuals (Wood and Richardson, 2009; Pollo *et al*., 2016). Then, BC and morphometric measures of tadpoles were analyzed by analysis of covariance (ANCOVA). Larval stage is the covariate due to the fact that the stage has an influence on the body size of tadpoles linearly (Babini *et al*., 2015a). Morphometric measures of metamorphs were analyzed by ANOVA (all individuals were in stage 45 of Gosner). The Di Rienzo, Casanoves and Guzmán (DCG) *post-hoc* test was used. This test for mean comparison (Di Rienzo *et al.,* 2002) utilizes the multivariate technique of cluster analysis (average chain or UPGMA) on a distance matrix obtained from the sample means (Balzarini *et al*., 2008).

To analyze the composition of larval diets the percentage frequency of occurrence (FO%, percentage of individuals that consumed a given category of prey) and numerical frequencies (N%) for each food item were calculated. In order to compare the composition of larval diets between sites an analysis of similarities (ANOSIM) were performed. ANOSIM is analogous to univariate ANOVA and compare between-group and within-group variation. The statistic (R) is based on rank dissimilarities. R is scaled to be within the range +1 to -1 (Quinn and Keough, 2002). This analysis was performed in R version 3.0.1, using vegan library (Oksanen *et al*., 2013; R Core Team, 2013). Structural attributes of the algal community in the larval diets of the different sites were calculated: total abundance, richness, Shannon's (H') diversity index and evenness (J'), and compared by Kruskal Wallis using InfoStat/P ver. 1.1 (Di Rienzo *et al*., 2012). Rank-abundance curves were constructed to analyze and show graphically the dominance of certain genera, and whether these relationships changed between sites. Principal Component Analysis (PCA) was performed for water parameters of ponds using InfoStat/P version 1.1 (Di Rienzo *et al*., 2012). Given that all the variables have different units they were standardized before plotting the PCA.The correlation matrix indicates high correlation (correlation coefficient: 0.99) between conductivity and salinity and TDS, so these two variables were removed from the PCA. Canonical correspondence analysis (CCA) between the water parameters with highest positive and negative weights of CP1 and CP2 and the abundant taxa (Pi>1%) registered in larval diets was performed. The CCA was performed with Canoco for Windows 4.5 program and the TRIPLOT was performed with Canodraw for Windows program (terBraak and Smilauer, 2002).

RESULTS

Regarding the morphometry of tadpoles, the analysis of covariance (Tab. 1) showed a significant positive linear relation ($P<0.05$; regression coefficients>0.0) between the larval stage and the morphometric variables (except for maximum width –MW-; $P=0.5634$). The analysis showed significant differences ($P<0.05$) among sites for total length (TL), tail muscle height (TMH), weight and body condition (BC). In A2, tadpoles with lower TL and TMH were recorded. The lowest mean weight was in A1 and A2 sites and the lowest BC was in A1 (DCG test; Tab. 1). In relation to the metamorphs (Tab. 2), ANOVAs showed differences for all variables. In A1 and A3 sites, the individuals with lower TL and weight were recorded. In A3, the metamorphic individuals had the lowest MW. Body condition of metamorphs was lower in A1 (DCG test; Tab. 2).

Diet analysis revealed that all larvae mainly consumed microalgae. A total of 52 taxa were registered, 52% corresponding to division Ochrophyta (diatoms), 21% Chlorophyta, 10% Charophyta, 10% Cyanobacteria and 8% Euglenozoa (Supplementary Tab. 1). Analysis of structural variables revealed a higher algal abundance in the diet of larvae from A3 ($F=8.23$, $P=0.04$), a lower richness in SM ($F=9.21$, $P=0.02$) and that there were no differences between sites in diversity and evenness of diets ($P>0.05$).

The analysis of algae taxa registered in larval diets allowed differentiating the sites (ANOSIM statistic $R=0.51$, $P=0.001$). Larvae from A1 and A3·sites showed a diet with a similar composition and structure, meanwhile larvae from A2 and SM showed different diets. Rank-abundance curves show that *Nitzschia palea* was predominant in the diet of larvae from A1 and A3 agroecosystems, with a relative abundance of 47% and 63% respectively (Fig. 1). In A2 *Microspora* sp. was predominant in the diet, with a relative abundance above 50%. In the semi-modified site SM, the larvae mainly consumed *Trachelomonas* with a relative abundance of 30%.

The water parameters from the studied sites are shown in Tab 3. The A1 had the higher values of conductivity, salinity and TDS. Phosphate values were higher in agroecosystem ponds. Nitrate was detected only in A1 and A2 sites. Collinearity was detected between conductivity, salinity and TDS, so salinity and TDS were removed from the analysis. Parameters carbonate, bicarbonate, chloride, calcium, nitrate and phosphate had the highest positive and negative weights of CP1 and CP2 (Tab. 3) and were included in Canonical Correspondence Analysis (CCA). The first factorial plane collects 89% of the total inertia of CCA, well sufficient to summarize the information on the relationship between water parameters and the abundant taxa (Pi>1%) registered in larval diets. The CCA indicated that the accumulated inertia of ratio in the first axis was of 55%. The TRIPLOT shows that the first axis separates the SM and A1 sites of the A3 and A2 sites, while the second axis separates the A1 of the SM and the A3 of the A2, placing each site in a different quartile (Fig. 2). The SM site was associated with *Melosira*, filamentous *Cyanophyceae*, *Trachelomonas* and *Eunotia*, and this site had inverse relation with nitrate, phosphate and carbonate. The A2 site

Tab. 1. Analysis of the covariance for tadpoles (stages 34 to 39) and DGC *post-hoc* test.

Variable	F-value	P-value	P-value Cov: stage	Regression coefficients	*Post-hoc* test
TL	$F_{4, 54}$: 16.9	<0.0001*	<0.0001*	0.88	A2 < A1, A3, SM
MW	$F_{4, 54}$: 0.92	0.4618	0.5634		
TMH	$F_{4, 23}$: 5.72	0.0024*	0.024*	0.05	A2 < A3, A1, SM
Weight	$F_{4, 23}$: 20.3	<0.0001*	<0.0001*	0.03	A1, A2 < A3, SM
BC	$F_{4, 23}$: 5.07	0.0044*	0.0016*	0.01	A1 < A2, A3, SM

**Significant* P-value. *A1, A2 and A3, agroecosystems; SM, semi-modified site; TL, total length; MW, maximum width; TMH, tail muscle height; BC, body condition.*

Tab. 2. Analysis of the variance for metamorphosed (stage 45) and DGC *post-hoc* test.

Variable	F-value	P-value	*Post-hoc* test
TL	$F_{3, 56}$: 12.8	<0.0001*	A1, A3 < A2, SM
MW	$F_{3, 56}$: 8.1	<0.0001*	A3 < A1, A2 < SM
Weight	$F_{3, 56}$: 21.7	<0.0001*	A1, A3 < A2, SM
BC	$F_{3, 56}$: 3.92	<0.013*	A1 < A3, A2, SM

**Significant* P-value. *A1, A2 and A3, agroecosystems; SM, semi-modified site; TL, total length; MW, maximum width; BC, body condition.*

had relation to nitrate and was associated with *Ulothrix, Oedogonium, Microspora*, *Encyonema* and *Scenedesmus*. The A1 was closely associated with *Diatoma* and had the highest conductivity. This site along with the A3 were associated with *Cyclotella*, *Gyrosigma*, *Navicula*, *Nitzschia, Pinnularia* and *Gomphonema*; and these sites had relation to bicarbonate, chloride, carbonate and phosphate.

Analysis of algae in water samples showed some differences with diet composition (Supplementary Tab. 2). In water samples of site A1 *Nitzschia palea* was dominant, in concordance with diet composition, but in the site A2 *Sphaerocystis* sp. was abundant in water and it was not registered in diet of larvae of that site. In the water samples of A3 a genus of order Chroococcales was predominant, but it was not consumed by larvae. In samples from SM *Melosira varians* was predominant, but *Trachelomonas* was the most consumed.

Algae found exclusively in tadpole's gut and in pond water from a single site are highlighted (by underlining) in Supplementary Tab. 2. In the site A2 seven algal taxa were found that were not recorded in the other sites: *Kirchneriella* sp., *Ulothrix* sp., *Pleurotaenium* sp., *Spirogyra* sp., *Staurastrum* sp., *Anabaena* sp. and *Coelosphaerium* sp. In the site A1 two exclusive taxa were found: *Caloneis* sp. and *Arthrospira* sp.; and in the site A3 one alga exclusive was registered: *Diploneis* sp.

DISCUSSION

Amphibian larvae may be at greater risk than adults from the effects of contaminants, since water bodies receive runoff from agricultural lands, and also receive direct aerial fumigation (Lajmanovich, 2000). In ponds with bad conditions for the development of tadpoles, the metamorphosis is accelerated and individuals have smaller body sizes, which impacts the survival of populations (Kupferberg *et al*., 1994). The analysis of the diet of aquatic stages of *R. arenarum* allowed us to know the trophic availability and environmental quality of temporary ponds from agroecosystems. Our results support our hypothesis in part, given that resistant algae taxa were

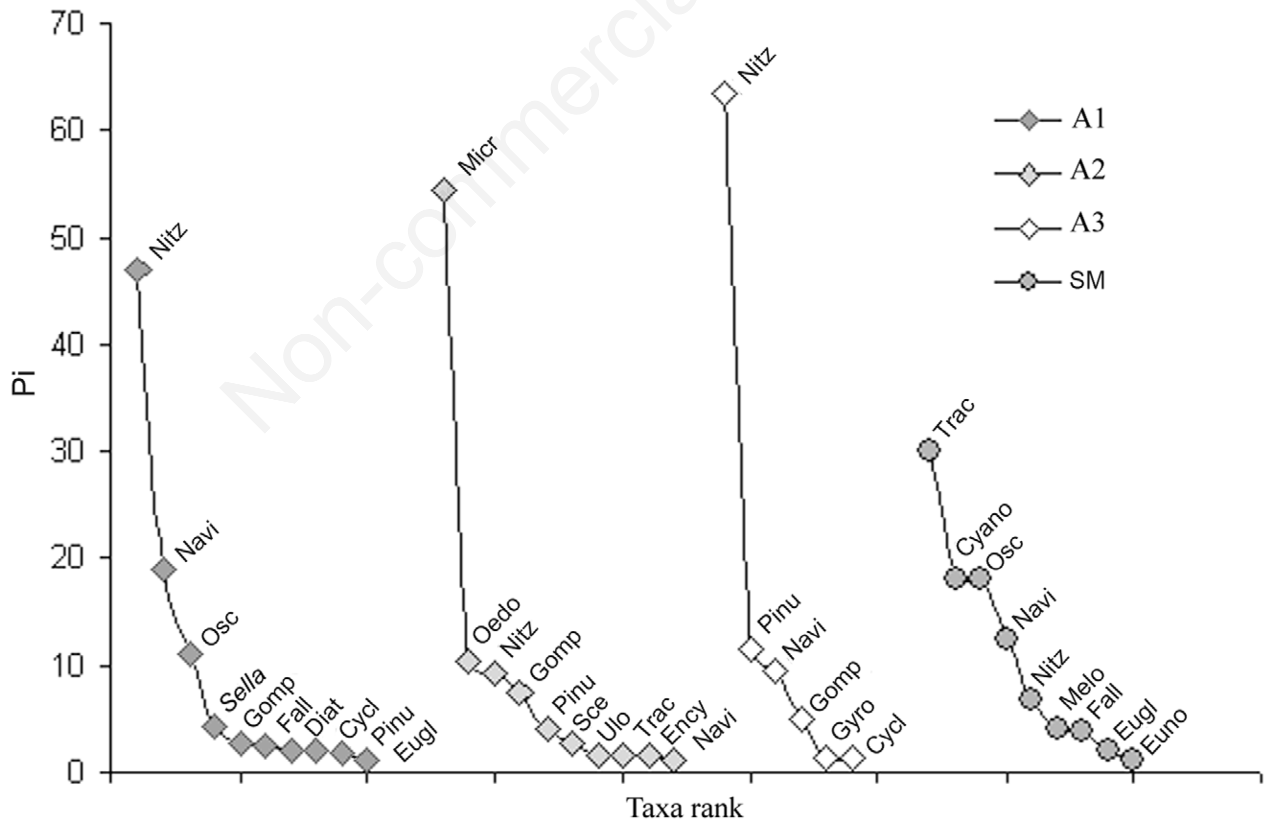

Fig. 1. Rank-abundance curves of abundant taxa (Pi>1%) registered in larval diets from agroecosystems (A1, A2 and A3) and a semi-modified landscape (SM). Cyano, Cyanophyceae filamentous; Cycl, *Cyclotella*; Diat, *Diatoma*; Ency, *Encyonema*; Eugl, *Euglena*; Euno, *Eunotia*; Fall, *Fallacia*; Gomp, *Gomphonema*; Gyro, *Gyrosigma*, Melo, *Melosira*; Micr, *Microspora*; Navi, *Navicula*; Nitz, *Nitzschia*; Oedo, *Oedogonium*; Osc, *Oscillatoria*; Pinu, *Pinnularia*; Sce, *Scenedesmus*; Sella, *Sellaphora*; Trac, *Trachelomonas*; Ulo, *Ulothrix*.

found in agroecosystem ponds and tadpoles and metamorphs were smaller, but larval diets showed higher abundance and richness.

Based on gut analyses of tadpoles in well mixed algal pond environments (Farlowe, 1928; Jenssen, 1967; Heyer, 1972, 1976; Nathan and James, 1972; Seale, 1980; Diaz-Paniagua, 1985; Loschenkohl, 1986), laboratory feeding trials on suspended particles (Wassersug, 1972; Seale and Beckvar, 1980), and on the morphological adaptations associated with suspension feeding (Wassersug, 1975), tadpoles have been considered indiscriminate feeders. About this, in anuran larvae, diet is usually indicative of the quality of food resources in their environments (Lajmanovich, 2000; Rossa Feres *et al*., 2004).

The analysis of composition and structure of larval diets allowed differentiating the sites, although there were no differences in diversity and evenness. This could be due to algal communities have a wide range of sensitivity to chemicals. The input of organic matter and agrochemical to water bodies can damage certain species of algae (Relyea, 2009) while favour resistant algae taxa (Mugni, 2008). This could explain the abundance, richness and diversity high of algal taxa in agroecosystems.

However, composition and structure of larval diets allowed differentiating the agroecosystems of SM site. Presence of some algae registered in the diet of the larvae from agroecosystems may be indicative of altered environments. Diatoms are useful bioindicators of water conditions (Tison *et al*., 2008). Several species of diatoms are associated with the presence of certain nutrients such as nitrogen or phosphorus (Blanco *et al*., 2004); which are the main components of many of the agrochemicals used by farmers. In the three agroecosystems was found in a proportion higher than 1% *Gomphonema parvulum* that is usually found associated to environments with high levels of pollution and high levels of phosphorus (Díaz Quirós and Rivera Rondón, 2004). This is consistent with the highest levels of phosphates recorded in the three agroecosystems. Besides, *G. parvulum* has been reported as resistant to heavy metals, which can be associated with agricultural fertilization (Ivorra *et al.,* 2002; Duong *et al.,* 2008; Roberts, 2014). Also, tadpoles collected in sites A1 and A3 showed diets with similar composition and structure, meanwhile the agroecosystem site A2 differed. This could be explained by the absence of livestock in A2. *Nitzschia palea* was the more abundant species in tadpoles' diet from A1 and A3. This alga is particularly found in environments with organic pollution with high conductivity, salinity and nitrate (Nather Khan, 1990; Ramírez and Plata-Díaz, 2008; Yucra and Tapia, 2008) as was

Tab. 3. Summary of water analysis of ponds and first two components of principal component analysis (PCA). The highest positive and negative weights of CP1 and CP2 are highlighted in bold.

	Ponds				PCA	
	A1	A2	A3	SM	PC1 (60%)	PC2 (27%)
Color	Amber	Dark amber	Amber	Amber light		
Conductivity ($\mu S\ cm^{-1}$)	1089.1	183.6	586.1	349.1	0.12	0.07
Salinity ($mg\ L^{-1}$)	507.6	136.1	288.6	169		
TDS ($mg\ L^{-1}$)	561.2	130.1	392.8	248.4		
pH	7.3	6.9	8.6	7.7	0.31	-0.16
Carbonate ($mg\ L^{-1}$)	ND	ND	21.8	ND	**0.32**	0.13
Bicarbonate ($mg\ L^{-1}$)	182.5	157.5	555	320	**0.32**	-0.14
Sulfate ($mg\ L^{-1}$)	44.4	50.8	89.8	34.2	0.25	0.3
Chloride ($mg\ L^{-1}$)	7.1	7.1	128.6	28.6	0.32	-0.11
Sodium ($mg\ L^{-1}$)	21.2	30.3	186	37.4	0.31	0.04
Potassium ($mg\ L^{-1}$)	28.4	38.7	80.9	22.6	0.25	0.28
Calcium ($mg\ L^{-1}$)	24	10.4	48	60.8	0.22	**-0.34**
Magnesium ($mg\ L^{-1}$)	13.7	8.3	12.2	5.4	0.11	0.4
Nitrate ($mg\ L^{-1}$)	1	1	ND	ND	**-0.25**	0.31
Nitrite ($mg\ L^{-1}$)	ND	ND	ND	ND		
Arsenic ($mg\ L^{-1}$)	35	15	60	20	0.29	0.14
Fluoride ($mg\ L^{-1}$)	0.4	0.4	2	0.3	0.31	0.17
Phosphate ($mg\ L^{-1}$)	9	9	9	5.7	0.03	**0.49**
Hardness ($m^2\ L^{-1}$)	2.3	1.2	3.4	3.5	0.24	-0.3

The detection limit of the determination of NO^{3-} is 0.2 $mg\ L^{-1}$ and the analytical error of the measurements is 0.5%. A1, A2, A3, ponds in agroecosystems; TDS, total dissolved solids; ND, not detected or observed.

recorded in A1 and A3 sites. In larval diet from A2 the most abundant alga was *Microspora* sp., which is almost always in eutrophic waters (Aboal, 1988). This alga was found exclusively in agroecosystems (in tadpole's gut and in pond water samples) although it had low abundance, it could indicate the conditions of these sites.

Larvae diet in SM showed a predominance of *Trachelomonas*, which tend to prefer waters rich in organic and humic substances (Starmach, 1983; Wydrzycka, 1996). In turn, *Melosira varians* was recorded in high abundance in water of the SM pond. This species is adapted to environments with high flow velocity and oxygen (B-Béres *et al*., 2014) and is a high-profile diatom, adapted to living in biofilms in which there is significant competition for space (Hoagland *et al.*, 1982; Berthon *et al*., 2011). Regarding growth forms of algae, tadpoles mainly consumed motile algae or those exposed in the upper layers of biofilm, similar to the findings of other studies of grazing (Holomuzki *et al*., 2010; Cibils Martina *et al*., 2014).

Adaptations of certain species of algae that enable the successful exploitation of disturbed environments, such as agroecosystems, explain the high abundance and richness recorded in the diets of these tadpoles. However, the food availability does not guarantee that the larvae have better body condition, as was observed in this study. Tadpoles from A1 and A2 had lower TL, TMH, Weigh and BC and metamorphs from A1 and A3 had lower TL, MW, Weigh and BC. Diet manipulations have confirmed that food quantity can determine tadpole growth and timing of metamorphosis (Wilbur, 1977a, 1977b; Alford and Harris, 1988, Berven and Chadra, 1988). Since no lower values of abundance, diversity and evenness were detected in larval diets from agroecosystems ponds, a possible explanation could be that pollution may interfere with the food digestion, affecting the amount of food consumed by tadpoles and thus expecting a smaller mass of tadpoles (Carey and Bryant, 1995). Also, the weight can increase and decrease rapidly in response to a change in stress lev-

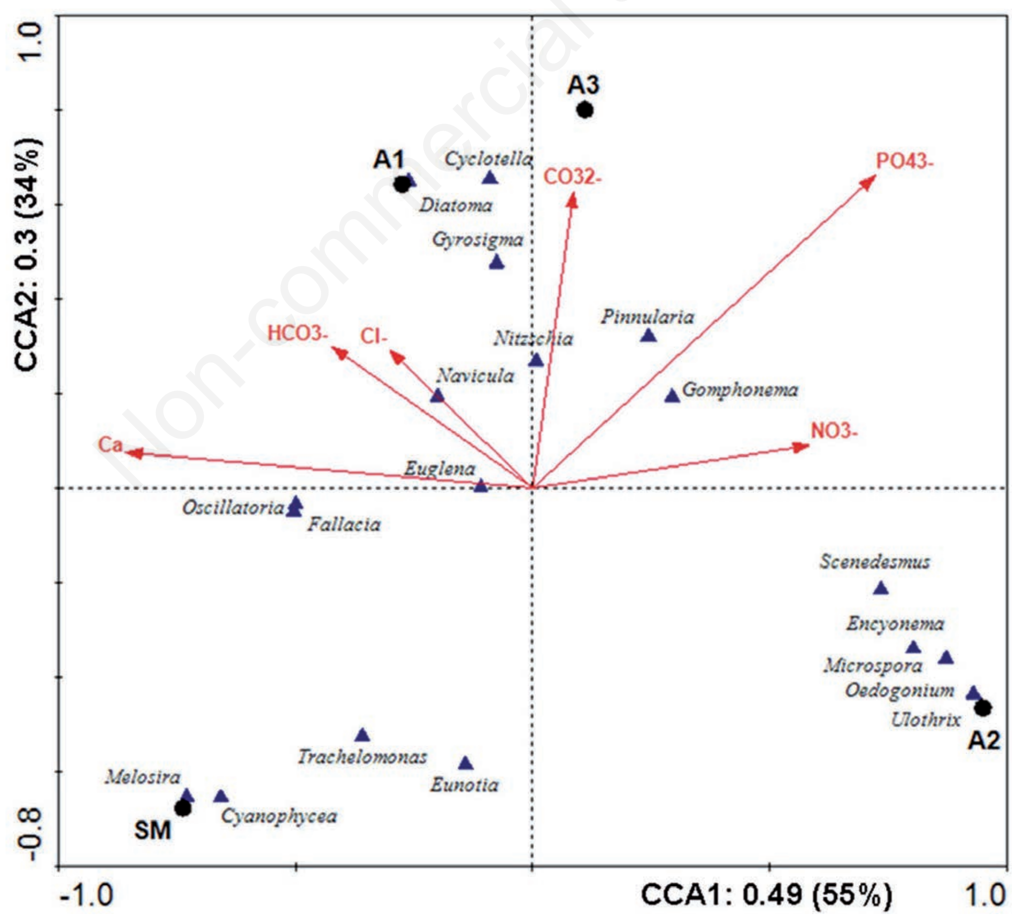

Fig. 2. Triplot (first two CCA axes) showing sites (●), abundant taxa registered in larval diets (Δ) and the water parameters (arrows represent the correlation of the physical variables with the canonical axes): Carbonate -$CO3^{2-}$, Bicarbonate -HCO^{3-}, Chloride -Cl^{-}, Calcium -Ca-, Nitrate -NO^{3-}, Phosphate -$PO4^{3-}$.

els (Reading and Clarke, 1995). In this sense, other studies have shown that nitrogen fertilizers reduce the feeding activity of tadpoles of some species (Marco and Blaustein, 1999; Hatch and Blaustein, 2000).

Moreover, the differences in structure and composition of diet between sites could mean differences in the nutritional values, affecting the size of tadpoles and metamorphs. According to Kupferberg (1997), the natural diet of anuran larva can vary widely in terms of quantity of proteins, carbohydrates, and lipids, factors that influence the function of thyroid hormones, which are essential for metamorphosis. In experimental situations, tadpoles fed with filamentous algae with epiphytic diatoms developed more rapidly and metamorphosed with larger body size than tadpoles submitted to a diet of commercially processed food (Kupferberg *et al*., 1994). Both filamentous algae as well as diatoms are considered important sources of nutrients since the periphytic community, where they occur in abundance, is well explored by the aquatic fauna both invertebrates and vertebrates. The importance of diatoms as a food source has also been observed in other anuran genera such as *Lithobates*, *Dendropsophus*, *Eupemphix* and *Scinax* (Hendricks, 1973; Kupferberg, 1997;Rossa-Feres*et al*., 2004). Diatoms can be richer in calories, mainly as a form of lipids, than the chlorophytes and they are more easily accessible for consumption than filamentous algae (Kupferberg*et al*., 1994). It is worth to note that chlorophytes store the products of photosynthesis as carbohydrates (Bold and Wynne, 1985), which is also an important food resource.

CONCLUSIONS

In summary, the presence of certain algae registered on the tadpoles' diet is related to the characteristics of sites. It is possible that these algae are directly associated with alterations of ponds from agroecosystems and they could be thus considered as bioindicators of environmental quality. The abundance of some food items in the natural diet of tadpoles of *R. arenarum*, especially diatoms, reveals the importance of the periphytic community for the feeding of this species of anuran. Composition and biomass of periphyton may change due to alterations of agroecosystems, and this impair the success of the tadpoles and the ending of metamorphose. Our study suggests that land use alters the availability of several taxa of algae to grazing tadpoles so the individuals would be smaller, which could have significant consequences for the anuran native populations.

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