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Diet of tadpoles of *Physalaemus biligonigerus* (Leiuperidae) from agricultural ponds in the central region of Argentina

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Abstract. The intensification of agriculture has led an important loss of natural habitats, with significant consequences for biodiversity. In this sense, the studies on anuran amphibian tadpoles inhabiting these environments are relevant, because the larval stage is a phase of population regulation. The aim of this study was to analyze the diet in *Physalaemus biligonigerus* tadpoles, an anuran species widely distributed in South America and that inhabit agroecosystems. Three sites were sampled; two agroecosystems with different alteration degrees (AG1 and AG2) and an uncultured (SM) third place. The captured tadpoles were anesthetized, fixed and preserved in formaldehyde (10%). Subsequently, the complete intestine was removed and analyzed for food items under a binocular microscope. The diet in *P. biligonigerus* tadpoles has a dominance of algae Bacillariophyceae, mainly in agroecosystems, due to the presence of the genera *Navicula*, *Nitzschia* and *Gomphonema*. There was a considerable abundance of the *Gomphonema* genus in the AG2 site. In addition, in the AG1 site several non-diatom algae were particularly abundant in the diet, such as the genera *Euglena*, *Oedogonium* and *Chaetophora*. In the SM site, the non-diatom genus *Oscillatoria* was well represented in the diet. Tadpoles inhabiting the site with abundant crop and livestock (AG1) ingested a significantly smaller amount of food. The presence of certain algae associated with eutrophic environments could indicate some pollution in agroecosystems (AG1 and AG2). Larval diet is suggested as a potential bioindicator of environmental health for these areas.

Keywords. Agriculture, algae, bioindicators, gut content.

Amphibian populations are experiencing worldwide declines (Collins and Crump, 2009), which are caused by multiple factors including trematode parasites, ultraviolet (UV-B) radiation and chemical pollutants (e.g. Blaustein et al., 2003; Henle et al., 2008; Johnson et al., 2008; Peltzer et al., 2011). The conversion of forest to agricultural land is occurring at rapid rates in many Neotropical areas (Pengue, 2005). The central region of Argentina has been greatly affected by agricultural development, mainly to soybean cropland (*Glycine max* Merril) (Schnepf et al., 2001). Thus, agriculture has become the main cause of reduction and fragmentation of natural habitats. Due to environmental degradation, agriculture is also considered an important factor in the decline in amphibians recorded the last decades (Collins and Crump, 2009). Principally, in agriculturally dominated landscapes the amphibians depend on patches of natural vegetation and ephemeral ponds for their survival and reproduction (Peltzer et al., 2003). The decrease in quality and hydroperiod of aquatic environments may influence both time and size at meta-

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morphosis of tadpoles, and may cause increasing mortality or predation pressure (Carey and Bryant, 1995; Altig et al., 2007). In this sense, the adult population would vary in relation to changes in larval success, hence the larval stage is considered a phase of population regulation (Heyer, 1974; Wilbur, 1980). Recent evidence has revealed that the deterioration of agricultural ponds might modify the trophic availability, affecting the development and growth to amphibian tadpoles that are sustained in such environments (Bionda et al., 2012). In addition, the diet of anuran tadpoles is usually diverse, can be composed of plant fragments, protozoa, rotifers, anuran eggs, and even other tadpoles, but most tadpoles are primarily herbivorous consuming a wide variety of algal taxa (Kupferberg, 1997; Altig et al., 2007). Bioindicators include biological processes, species, or communities and are used to assess the quality of the environment and how it changes over time (Holt and Miller, 2011). 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., 2012). The aim this study was analized the diet of *Physalaemus biligonigerus* (Cope, 1861) tadpoles in temporary ponds from agroecosystems for the central region of Argentina.

The study region belongs to the Pampa Plains of central Argentina. Moderately undulating plains and a temperate climate characterize the area, with an annual mean temperature of 23 ºC in January and 6 ºC in July and with mean annual temperature of 18 ºC. The region is characterized by rainy and dry seasons, with rains typically starting in October and continuing through the warm months until March (mean annual rainfall of 800-1 000 mm) (Gatica et al., 2012). We sampled three sites in Rio Cuarto (33º08'S, 64º24'W; 434 m a.s.l.; Córdoba Province, Argentina). Two of these sites were agricultural lands with different alteration degrees (AG1, 9 ha: 33°05'S 64°26'W; AG2, 12 ha: 33°05'S 64°25'W). These study areas are sparsely vegetated with low growing plants (heights <0.5 m) and permanent and temporary ponds. In AG1, the cropland is around ponds (within approx. 10 m), in AG2 is farther (about 200 m). During the study period, both agroecosystems were devoted to soybean crop and were used for cattle grazing. The third site (SM) was a suburban pond located on the Campus of National University of Rio Cuarto (33º06'S, 64º25'W; 465 m a.s.l.). This is an area with permanent and temporary ponds, and a small strip of forest occurred all around the edge of the ponds.

Physalaemus biligonigerus is a species of anuran widely distributed in South America (Kwet et al., 2012), is a representative species of amphibian communities in the region and their populations are abundant in the central region of Argentina (Bionda et al., 2011; 2012). The *P. biligonigerus* tadpoles were captured in December 2007, along transect from the margin of the water body to 2 m therein. The captured tadpoles were anesthetized with chloroethane, fixed and preserved with 10% buffered formalin. Subsequently, for a total of 5-6 tadpoles per site, between stages 36-39 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 400 X, 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). 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. The values of food items consumed for each site were compared within the pairs using Mann-Whitney U tests followed by Bonferroni correction. To describe the dietary diversity among different populations, we calculated the Shannon and Weaver (1949) diversity index (H). Furthermore, we measured the evenness (E) in for each population diet following Magurran (1987).

The diet of *P. biligonigerus* consists primarily of microalgae, mainly diatoms in the sites AG2 and SM. Table 1 shows the results for the more abundant algal genera recorded in the gut contents of the tadpoles for the three sites. In particular, the classes Chlorophyceae (nine genera in total) due mainly to the presence of the genera *Oedogonium* and *Chaetophora*, and Euglenophyceae (four genera) with the genus *Euglena*, were important in the tadpoles diet in AG1. The class Bacillarophyceae (13 genera) mainly represented by the genera *Gomphonema* and *Nitzschia*, was predominant in the diet of AG2. Finally, the class Bacillarophyceae (11 genera) due mainly to the presence of the genus *Navicula*, and Cyanophyceae (five genera) with the genus *Oscillatoria*, were important in the diet of SM.

There were significant differences in the total items consumed by tadpole between AG1 and the other sites (Mann-Whitney, $P < 0.017$). We detected a significantly lower amoung ot food in tadpoles inhabiting the site most modified by crop and livestock (AG1). The amount of food consumed by *P. biligonigerus* in AG1 site was 451 548 ± 238 351 average items by gut, in AG2 was 4 057 099 ± 364 666 and in SM was 3 151 682 ± 2 324 009. Thus, tadpoles of AG1 site consume only 11.1% and 14.3% of food amount consumed by the tadpoles of AG2 and SM, respectively. However, the AG1 site had the diet with higher diversity (AG1: H= 2.32; AG2: H= 1.55 and SM: H= 1.61) and evenness (AG1: E1= 0.69; AG2: E1= 0.43 and SM: E1= 0.50).

Table 1. Percent N (%) and occurrence frequencies FO (%) of the more abundant algal genera recorded in the gut contents of *Physalaemus biligonigerus* tadpoles of two agroecosystems sites AG1 (n= 5), AG2 (n= 5) and the uncultured place SM (n= 6) in Central Argentina. Values followed by different letters differ significantly (Mann-Whitney, P <0.017).

	AG1		AG2		SM	
	N (%)	FO (%)	N (%)	FO (%)	N (%)	FO (%)
Bacillariophyceae						
Craticula	0.12	40	-	-	0.02	80
Cyclotella	0.30	100	0.15	100	0.01	20
Cymbella	0.03	40	0.01	20	-	-
Achnanthes	-	-	0.33	100	0.01	20
Amphora	-	-	0.06	40	0.01	40
Fragilaria	-	-	0.10	40	-	-
Surirella	-	-	0.01	20	-	-
Fallacia	0.03	40	0.12	100	9.21	100
Gomphonema	0.04	20	83.12	100	0.02	40
Hantzschia	0.07	40	0.13	100	0.50	100
Navicula	0.82	100	2.95	100	41.60	100
Nitzschia	11.64	100	11.75	100	8.23	100
Pinnularia	4.01	100	0.01	40	5.84	100
Sellaphora	0.05	40	-	-	-	-
Neidium	-	-	-	-	0.02	20
Chlorophyceae						
Chlorococcal	0.05	40	-	-	-	-
Monoraphidium	0.02	20	-	-	-	-
Ankistrodesmus	-	-	0.01	20	-	-
Chloroficeae	-	-	0.04	20	-	-
Oedogonium	14.29	100	-	-	0.09	17
Microspora	6.04	80	-	-	-	-
Chaetophora	14.92	100	-	-	-	-
Pediastrum	0.02	20	-	-	-	-
Scenedesmus	0.38	100	0.91	100	-	-
Staurastrum	0.02	20			-	-
Tetraedron	0.04	20	0.01	20	-	-
Ulothrix	-	-	0.01	20	-	-
Cladynomonas	-	-	-	-	0.01	20
Cyanophyceae						
Aphanocapsa	0.41	80	-	-	-	-
Chrooccoccus	0.02	20	-	-	-	-
Cyanophyceae	4.56	80	0.06	20	0.40	20
Gomphosphaeria	-	-	0.01	20	0.01	20
Merismopedia	-	-	0.01	20	-	-
Lyngbia	0.34	20	-	-	-	-
Oscillatoria	9.70	100	-	-	29.29	100
Anabaena	-	-	-	-	0.11	40
Spirulina	-	-	-	-	0.82	70
Euglenophyceae						
Phacus	6.35	100	0.01	20	0.55	100
Euglena	18.60	100	0.02	40	1.57	100
Trachelomonas	0.23	80	0.01	20	0.91	100
Strombomonas	-	-	-	-	0.57	100
Lepocinclis	6.84	100	-	-	0.03	50
Mean amount (± SD) of food items consumed by tadpoles	451 548 ± 238 351[a]		4 057 099 ± 364 666[b]		3 151 682 ± 2 324 009[b]	

Average cattle density at wetlands during our study was 11.3 head (SD = 3.05) per wetland ha per month in AG1, and 26.3 head (SD = 3.51) per wetland ha per month in AG2. There were fewer cattle in AG1, but they stayed closer to wetlands than the cattle in AG2.

Amphibian larvae may be at greater risk from the effects of contaminants that adults, since water bodies receive runoff from agricultural lands, and also receive direct aerial fumigation. In anuran larvae, diet is usually indicative of the type and abundance of food resources in their environments (Heyer, 1974; Lajmanovich, 2000). In this sense, our results indicated that the tadpoles of the most altered cropland site (AG1), showed a significantly lower amount of food in their intestine. The surveyed ponds are surrounded by an agricultural matrix and are also used by livestock, these activities may affect food availability in ponds to tadpoles of *P. biligonigerus*, considering that agriculture causes eutrophication of surface waters (Sharpley et al., 2003; Withers and Haygarth, 2007) and the eutrophic environments have lower food quality (Smol, 2002). The AG1 diet showed the highest diversity and evenness, however, the tadpoles consume little food there. Similar results were found by Bionda et al. (2012) for a study performed with tadpoles of the bufonid *Rhinella arenarum* (Hensel, 1867) for these same sites. *Rhinella arenarum* tadpoles also consumed less food items in the AG1 site, despite their diet was most diverse and equitable. In adittion, the *R. arenarum* tadpoles for AG1 reported a lower mass and poorer body condition. Pollution may interfere with the food acquisition and digestion, affecting the amount of food consumed by tadpoles and thus expecting a smaller mass of tadpoles (Carey and Bryant 1995). In this sense, studies have shown that nitrogen fertilizers reduce the feeding activity of tadpoles of some species (Marco et al., 1999; Hatch and Blaustein, 2000). However, further studies are needed to elucidate this question.

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); many of the agrochemicals used by farmers are among its principal components to nitrogen and phosphorus. Among diatom algae, the genera *Navicula*, *Nitzschia* and *Gomphonema* were important in the diet of the tadpoles in agroecosystems (AG1 and AG2). These same algae were most abundant in the diet of *R. arenarum* (Bionda et al., 2012). The genera *Navicula*, *Nitzschia* and *Gomphonema* are considered the main eutrophic indicators among diatoms, and are especially resistant to environments with high electrical conductivity (Palmer, 1977; Kelly et al., 2005). Moreover, several non-diatom algae such as *Euglena*, *Oedogonium* and *Chaetophora* were abundant in AG1 site. The predominance of the genera *Euglena* and *Oedogonium* could indicate elevated content of organic matter in these ponds (Fabrizi, 2012). Furthermore, also the diatom *Nistzchia*, considered among the most widespread algae in sewage ponds (Palmer, 1977), was frequent in the diet of AG1 site. Significant abundance of these genera in the diet of tadpoles of AG1 could correspond to the presence of livestock and its staying close to wetlands. The amount of food in the AG2 site was similar to SM site, although is important to note that the 83.1% of the AG2 diet corresponds to the genus *Gomphonema*. *Gomphonema* is a dominant diatom genus in environments with elevated levels of phosphorus (Nather Khan, 1990; Kelly et al., 2005). In the study that was performed with the toad *R. arenarum*, *Gomphonema* was abundant in the diet of AG1 site; this would raise serious questions about environmental health in these agroecosystems (Bionda et al., 2012).

The *Navicula* algae were predominant in the diet of the tadpoles of SM site. As we mentioned before, the presence of *Navicula* is associated with some pollution, but it can occur at high densities in both unpolluted and polluted sites (Nather Khan, 1990). *Oscillatoria* was another genus of non-diatom algae that was well represented into the diet of SM site. The predominance of *Oscillatoria* in the SM tadpoles might be associated with the presence of higher amount of organic matter in the ponds of this site (Fabrizi, 2012). While crops are absent in the surroundings of SM site and, therefore, it was considered as a reference site to be compared with agroecosystems, this environment shows some degree of disturbance, as it surrounded by residences, which may determine an increase in the amount of organic material and therefore the predominance of this genus.

In summary, the presence of certain algae registered on the tadpoles diet from different agricultural sites could correspond to their levels of alteration. Is possible thus consider these algae as bioindicators, as they are directly associated with eutrophic environments. As similar results were recorded in a previous study realized to these same sites but using the species *R. arenarum*, then the larval diet is suggested that as a potential bioindicator of environmental health for these areas.

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