Chemosphere 135 (2015) 24–30

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Plasma retinoids concentration in *Leptodactylus chaquensis* (Amphibia: Leptodactylidae) from rice agroecosystems, Santa Fe province, Argentina

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## HIGHLIGHTS

- Retinoic acid, 13-Cis-retinoic acid, and retinol were quantified in *Leptodactylus chaquensiś* plasma.
- Plasma retinoic acid and 13-Cis-retinoic acid were significantly higher in the rice field that a reference site.
- Retinoic acid/retinol and 13-Cis-retinoic acid/retinol ratios were significantly higher in the rice field.

## ARTICLE INFO

*Article history:*
Received 30 June 2014
Received in revised form 27 March 2015
Accepted 29 March 2015

Handling Editor: David Volz

*Keywords:*
Amphibians
Retinoic acid
Pesticides
*L. chaquensis*

## ABSTRACT

Retinoids are known to regulate important processes such as differentiation, development, and embryogenesis of vertebrates: Alteration in endogenous retinoids concentration is linked with teratogenic effects. Retinol (ROH), retinoid acid (RA), and isoform 13-Cis-retinoic acid (13-Cis-RA), in plasma of a native adults frog, *Leptodactylus chaquensis* from a rice field (RF) and a forest (reference site; RS) were measured. ROH did not vary between treatment sites. RA and 13-Cis-RA activities were higher ($93.7 \pm 8.6$ μg $mL^{-1}$ and $131.7 \pm 11.4$ μg $mL^{-1}$, respectively) in individuals collected from RF than in those from RS ($65.5 \pm 8.6$ μg $mL^{-1}$ and $92.2 \pm 10.2$ μg $mL^{-1}$, respectively). The ratios retinoic acid-retinol (RA/ROH) and 13-Cis-RA/ROH revealed significantly higher values in RF than in RS. RA and 13-Cis-RA concentrations in plasma on wild amphibian's species such as *L. chaquensis* would be suitable biomarkers of pesticide exposure in field monitoring. Finally, the mechanism of alteration in retinoid metabolites alteration should be further explored both in larvae and adult, considering that the potential exposition and uptake contaminants vary between the double lives of these vertebrates.



## 1. Introduction

Retinoids include to those chemicals that are structurally or functionally similar to retinol (ROH), or vitamin A, which is an indispensable biomolecule for embryonic development and adult body homeostasis. In this sense, different studies reveals have examined the pharmacokinetics of various retinoids in pregnant animals and the embryonic exposure to retinoids to assess the role of metabolism, the extent of transfer to the embryo, and the proximate/ultimate teratogen in retinoid-induced teratogenesis (Nau et al., 1994; Sass et al., 1999). Retinoic acid (RA), a metabolite of ROH, is a signaling molecule involved in many important pathways in every cell (Dollé and Niederreither, 2015). The effects of RA may be mediated through the retinoid receptor signaling pathways (Lee et al., 2005). Accumulated evidence has shown that inadequate endogenous concentration (excess or deficiency) of RA cause a wide range of malformations and during human pregnancy and in experimental embryos of chicken and amphibian models (Dollé and Niederreither, 2015), being recently linked with pesticides exposure (Paganelli et al., 2010). RA mediated gene transcription depends on the rate of transport of RA to target cells and the timing of exposure of RA to Retinoic Acid Receptors (RARs) in the target tissues (Das et al., 2014). In addition, during early embryonic development, other bioactive form of retinoids, all-trans retinoic acids (atRA), precursors of the corresponding RA isomers are

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http://dx.doi.org/10.1016/j.chemosphere.2015.03.063
0045-6535/

attracted attentions for their potent dysmorphogen effects on developing mammals (Nau, 1993), and amphibians embryos (Pijnappel et al., 1993; Kosian et al., 2003). The bioactive trans-retinoic acid (RA) isomerizes at several cis-retinoic acid (Cis-RA) such as 13-Cis-RA. This isomers is mainly circulation retinoids in plasma (Horst et al., 1995), and an increase in its values due to slower metabolization can exert teratogenic action (Sass et al., 1999)

Retinoid homeostasis in wildlife is known to be affected by contaminants such as pesticides used for crop production (Bérubé et al., 2005). Accordingly, plasma ROH concentrations in wild birds and mammals have been positively correlated with exposure to agrochemical contaminants (Shaw, 1998; Champoux, 2000); however, information on retinoids of wild amphibians is still scarce in the literature (Boily et al., 2005; Novák et al., 2007). Agricultural pesticides in general in the nearest of water bodies and the pluvial pesticide runoff being the principal reasons for amphibian declines (Boone et al., 2007; Peltzer et al., 2008; Mann et al., 2009). Mainly, amphibians may be sensitive to these compounds, principally during the aquatic embryonic and larval stages (García Muñóz et al., 2011), and recently altered phenotypes mainly as consequence of the increase of endogenous retinoid activity were pointed (Paganelli et al., 2010).

In the present study, concentrations of retinol (ROH), retinoic acid (RA), and 13-Cis-RA in plasma were quantified in adult of *Leptodactylus chaquensis* frogs plasma from rice fields and forests from mid-eastern Argentina. These evidences contribute to characterize the biological risk of amphibians when occur in agricultural sites where it is frequently used pesticides that could exert alteration in retinoids in vertebrates (Mann et al., 2009; López et al., 2012).

## 2. Materials and methods

### 2.1. Study area

The study area was situated in Santa Fe province, in the mid-eastern region of Argentina. The area is primarily devoted to irrigated rice production, with a rainy season from October to March and a dry season from April to September. Expansion of rice production, particularly in Santa Fe, Entre Ríos, and Corrientes provinces, involves deforestation and destruction of native forests. In Santa Fe province, rice is produced mainly on the floodplains of the Paraná River (Alvisio, 1998). Such intensive agriculture has led to an increase in the use of herbicides (glyphosate, atrazine) (López et al., 2012) and insecticides (e.g. organophosphorus, carbamate, and pyrethroid) (CASAFE, 2005). In general, rice fields are surrounded by several forest fragments characterized by native vegetation of the Deltas and Islands of Paraná River and Espinal ecoregions (Burkart et al., 1999).

### 2.2. Fields survey

We selected two sites (Fig. 1), a reference site (RS) and a rice field (RF). RS was located within a private native reserve of the Paraná River floodplain in Cayastá, Garay department, Santa Fe province (31°10′ 21.10″ S–60°15′31.73″ W). We considered the RS free of pesticides because no agricultural activities or pesticide use have been observed in the nearby areas (Lajmanovich et al., 2012). RF was a rice (*Oryza sativa*) plantation located in San Javier department (RF: 30° 01′ 5.92″ S–59° 50′35.7″ W).

### 2.3. Anuran species selected

*L. chaquensis* is widely distributed across Paraguay, Bolivia Uruguay, and Paraná River ecosystems (Cei, 1980). In Argentina, this frog is categorized as "not threatened" (Vaira et al., 2012) and is distributed in the provinces of Buenos Aires, Formosa, Chaco, Corrientes, Santiago del Estero, Tucumán, Jujuy, Salta, Entre Ríos, and Santa Fe. *L. chaquensis* is a large-sized frog that belongs to the *L. latrans* group (Frost, 2007). This species was selected because it is frequently found in rice fields (Duré et al., 2008; Attademo et al., 2011) and individuals were commonly observed in unpaved roads across rice fields during the night (22:00 and 24:00 h). In addition, in this species, in the last 10 years several individuals collected from agricultural sites accounted development abnormalities (Peltzer et al., 2011), so the hypothesis that retinoid could be affected by agrochemicals application (Bérubé et al., 2005) is considered also in the selection of this species.

### 2.4. Animal sampling

Twenty-seven adult males of *L. chaquensis* were collected by hand from the sampling areas (RS = 12 and RF = 15) on 25 January 2012. After capture, animals were rapidly transported to the laboratory in darkened buckets containing water to minimize stress. The number of amphibian followed the same protocol about the number of samples necessary to studies the effect of pesticide *in situ* fields (Attademo et al., 2011, 2014). Blood samples (0.4 mL) were collected by cardiac puncture using a heparinized syringe (Lajmanovich et al., 2004; Attademo et al., 2007). No anesthesia was used because it might have interfered with enzymatic activity (Vernadakis and Routledge, 1973). Busk et al. (2000) also reported that anaesthesia might be more stressful to amphibians and reptiles than cardiac puncture. Likewise, Tyler (1999) suggested that cardiac puncture is a reliable method. Therefore, we considered the practical experience of our personnel with amphibians, the health of animals after blood sampling, and minimum blood volume required for experimental purposes as the criteria for selecting cardiac puncture as the most appropriate blood sampling technique in the frogs studied. Blood was centrifuged at 10.000 rpm for 15 min, and the plasma was separated and immediately frozen (−80 °C). Snout-vent length and body mass were recorded, and a condition factor (CF) for each animal was then calculated and expressed as $100 \times [\text{body weight (g)}]/[\text{length (cm)}]^3$ (Bagenal and Tesch, 1978). The mean condition factor is an indicator of health status, so analysis of variations in this indicator between populations or individuals is used to demonstrate the effects of different factors, such as environmental quality and food resources (Gislaine et al., 2011). Frogs were toe-clipped for later individual identification, and after determining their health status, they were released at the capture sites.

### 2.5. Retinoid analysis

#### 2.5.1. Laboratory equipment and specific software

All experiments were performed using an Agilent 1100 Series liquid chromatography equipped with a quaternary pump, degasser membrane, thermostatted column compartment, autosampler and diode array detector (DAD) (Agilent Technologies, Waldbronn, Germany). Chromatograms were recorded at 350 nm. The Chemstation version B 0103 was used for data acquisition and processing. The HPLC column was a Zorbax C18 (4.6 × 75 mm, 3.5 μm particle size) from Agilent.

#### 2.5.2. Chemicals and reagents

Retinoids standards were purchased from Sigma–Aldrich Inc. (St Louis, MO, USA). Hexane p.a. and ethyl acetate p.a. were supplied by Anedra (San Fernando, Argentina), and tetrahydrofuran p.a. and acetic acid p.a. by Cicarelli (San Lorenzo, Argentina). Acetonitrile and methanol HPLC-grade were obtained from Merck

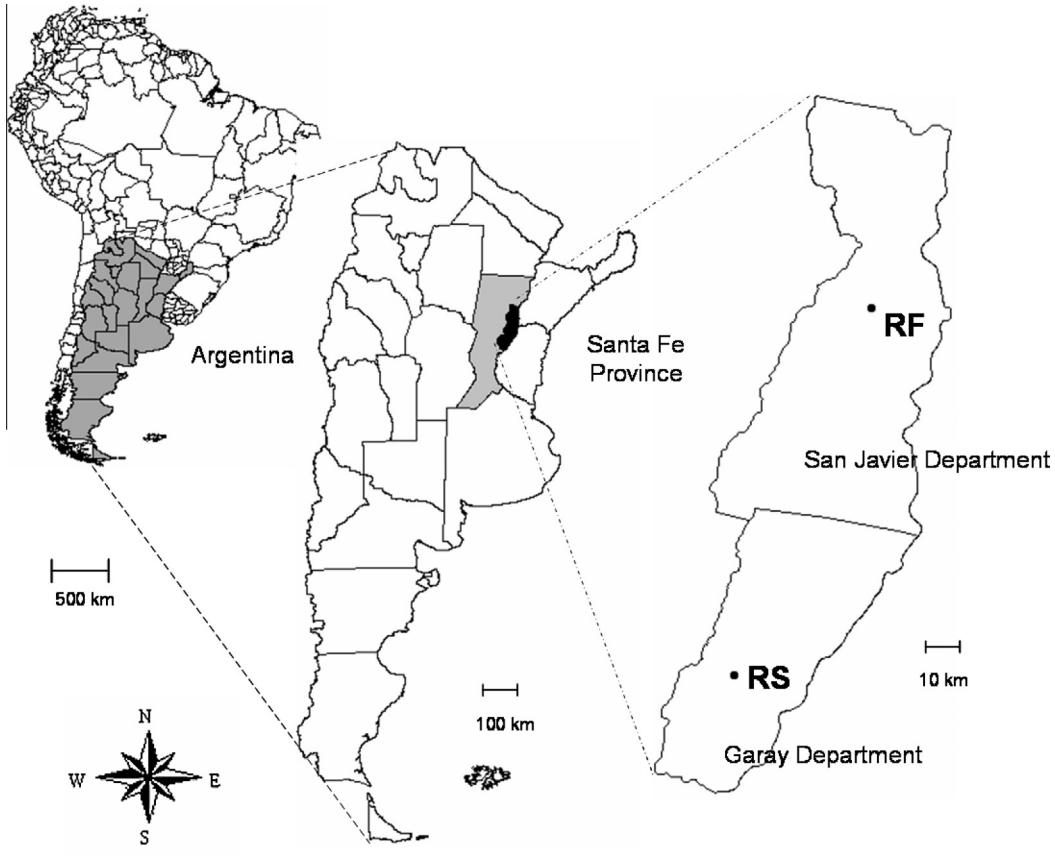


**Fig. 1.** Location of sampling sites in mid-eastern Argentina. RS: reference site and RF: rice field.

(Darmstadt, Germany). HPLC-grade water was obtained from a Milli-Q Biocel System (Millipore SAS, Molsheim, France).

Solutions and solvents for mobile phase were always filtered through 0.45 μm nylon filters. Standard and sample solutions were also filtered through syringe 0.20 μm nylon membrane before injection in the chromatographic system.

*2.5.3. Standard solutions and samples*

A RA stock standard solution of 0.600 mg $mL^{-1}$ was prepared by exactly weighing and dissolving a portion of the standard in methanol. The solution was conserved at 4 °C in light-resistant containers and was allowed to reach room temperature before use. Standard solutions were prepared at the moment of use by diluting an appropriate volume of the stock standard solution in methanol, according to Teglia et al. (2014).

*2.5.4. Sample preparation*

Aliquots (50 μL) of sample plasma were transferred into a 1.5 mL centrifuge tubes using an Eppendorf® pipette and 100 μL of acetonitrile were added. The samples were vortexed for 10 s and 300 μL of a solvent mixture composed of ethyl acetate (50%) and hexane (50%) was added. Finally, the samples were vortexed for 10 s, centrifuged at 6000 g for 2 min and the organic phase was transferred to glass tubes. The extraction was repeated thrice and the organic phases were collected and mixed and, finally, evaporated to dryness under a gentle stream of nitrogen gas. The residue was dissolved in 50 μL acetonitrile and 15 μL of final solution were injected into the HPLC.

*2.5.5. High-performance liquid chromatography conditions*

The HPLC conditions were those described by Teglia et al. (2014). The peaks of retinol (ROH), retinoic acid (RA), and its isomer (13-Cis-RA) were identified by comparing chromatograms of standard solution and samples. Retinol coeluted under these high-performance liquid chromatography conditions and were quantified together as ROH.

*2.6. Data analysis*

Concentrations of ROH and retinoid metabolites (RA and 13-Cis-RA) were calculated following the calibration curve provided by Teglia et al. (2014). Data was expressed as the mean ± SEM. Differences in circulating of RA and 13-Cis-RA were compared between samples of two sites. The relation between RA/ROH and 13-Cis-RA/ROH were also calculated using Student's *t*-test. Data were previously tested for homogeneity and normality of variance (Kolmogorov–Smirnov test and Levene test). Statistical analyses were performed using INFOSTAT/P 1.1 for Windows software (Grupo Info Stat Professional, FCA, Universidad Nacional de Córdoba, Argentina).

## 3. Results

The mean condition factor (CF) values for *L. chaquensis* collected from the rice field (RF) was 9.54 ± 1.83, which were similar to those for frogs from the reference site (RS = 10.94 ± 2.61; *Student t* = 1.94, $p = 0.17$).

Chromatograms of standard and values for retinol and retinol metabolites of the two different samples (RS and RF) of *L. chaquensis* are shown in Fig. 2.

ROH was similar frog samples of the two sites (RS = 94.9 ± 14.5 and RF = 70.0 ± 7.2; $t = 1.54$, $p = 0.1360$; Fig. 3). RA and 13-Cis-RA concentrations were higher for individuals collected from RF (93.7 ± 8.6 μg $mL^{-1}$ and 131.7 ± 11.4 μg $mL^{-1}$; respectively) than

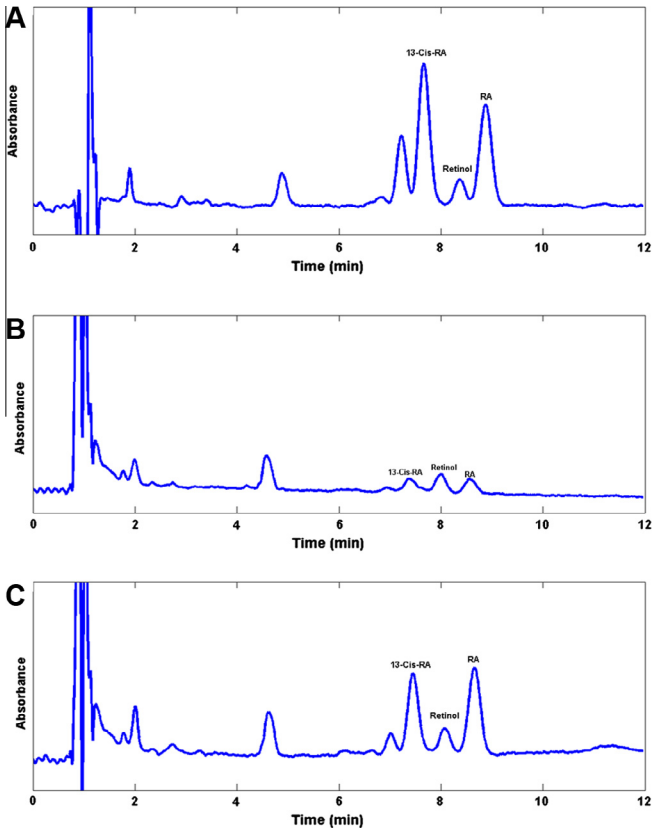


**Fig. 2.** Chromatograms corresponding to (A) a standard solution of 0.100 μg $mL^{-1}$ and (B) plasma samples of *L. chaquensis* from the reference site (RS) and (C) plasma sample of *L. chaquensis* from a rice field (RF).

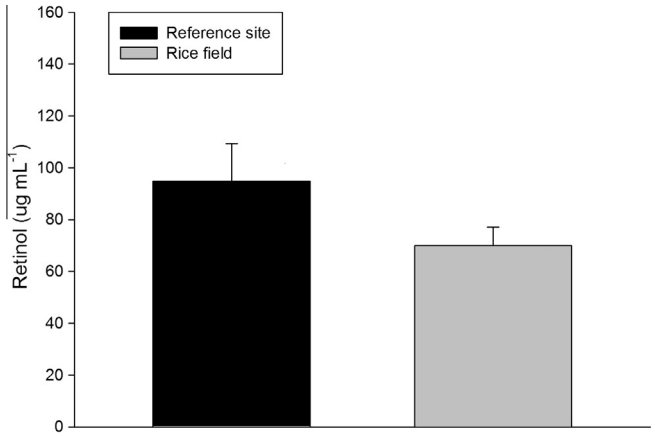


**Fig. 3.** Comparative values of retinol in individuals of *L. chaquensis*. Bars represent the mean ± SEM. *$p < 0.05$ compared with RS.

for those from RS (65.5 ± 8.6 μg $mL^{-1}$ and 92.2 ± 10.2 μg $mL^{-1}$, respectively). The retinoid metabolites varied significantly between the two sampling sites (Student test $t$ RA = 2.32, $p = 0.0266$; and $t$ 13-Cis-RA = 2.57, $p = 0.0148$; Figs. 4 and 5).

The relation between RA/ROH and 13-Cis-RA/ROH reached significantly higher values ($t = 4.60$, $p = 0.0001$ and $t = 4.20$, $p = 0.0004$, Figs. 6 and 7) in individuals collected from RF (1.38 ± 0.11 μg $mL^{-1}$ and 1.94 ± 0.20 μg $mL^{-1}$, respectively), than in those from RS (0.79 ± 0.06 μg $mL^{-1}$ and 1.05 ± 0.08 μg $mL^{-1}$, respectively).

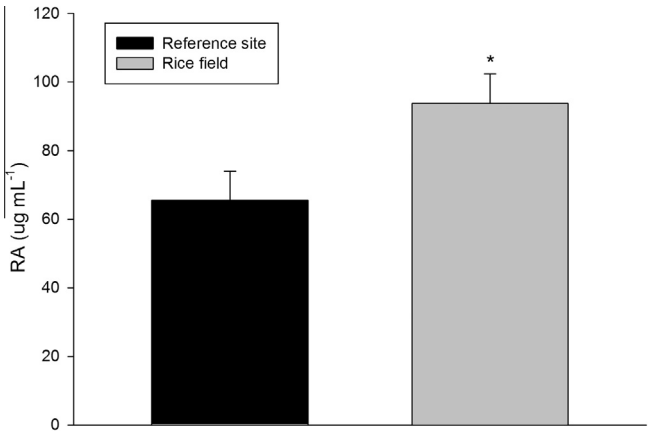


**Fig. 4.** Comparative values of retinoic acid (RA) in individuals of *L. chaquensis*. Bars represent the mean ± SEM. *$p < 0.05$ compared with RS.

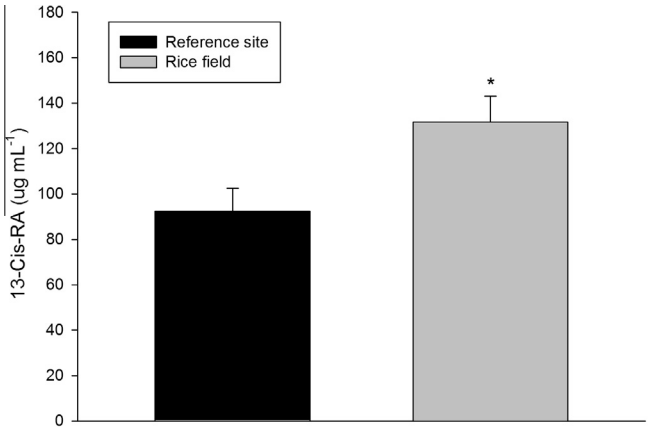


**Fig. 5.** Comparative values of 13-Cis-retinoic acid (13-Cis-RA) in individuals of *L. chaquensis*. Bars represent the mean ± SEM. *$p < 0.05$ compared with RS.

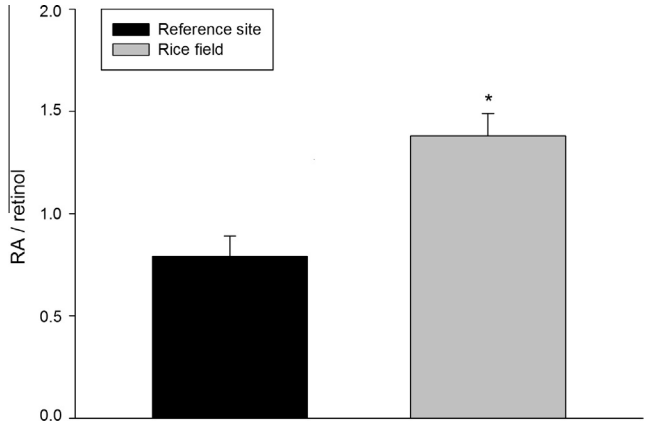


**Fig. 6.** Comparative values of retinoic acid (RA) over retinol in individuals of *L. chaquensis* (dimensionless units: μg $mL^{-1}$/μg $mL^{-1}$). Bars represent the mean ± SEM. *$p < 0.05$ compared with RS.

## 4. Discussion

Retinoids have been studied in mammals, birds, and fish, in which their imbalances were associated with growth inhibition, decreased embryo survival, and morphological abnormalities,

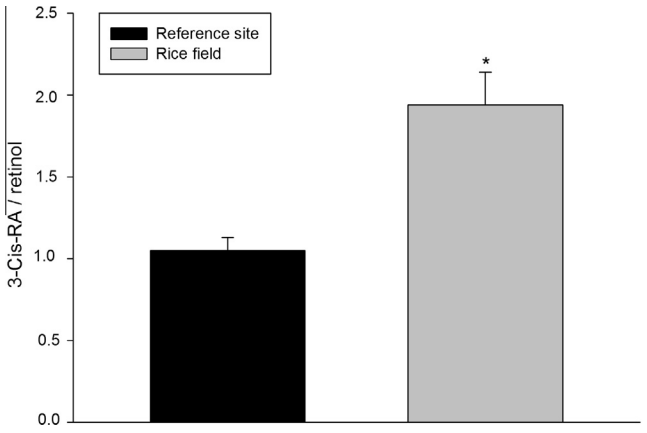


**Fig. 7.** Comparative values of 13-Cis-retinoic acid (13-Cis-RA) over retinol in individuals of *L. chaquensis* (dimensionless units: $\mu g\ mL^{-1}/\mu g\ mL^{-1}$). Bars represent the mean ± SEM. $^{*}p < 0.05$ compared with RS.

along with several other effects on reproduction and physiological functions in adults (Blomhoff and Wake, 1991; Rommert et al., 1998; Rolland, 2000); however few studies have been conducted in amphibians (Boily et al., 2009; Yu et al., 2011). .

In the present study, ROH concentration in plasma samples of *L. chaquensis* adult from both reference site (RS) and rice fields (RF) are similar; that may be due to these values are in the normal range of ROH of this species (Clugston and Blaner, 2014). However, Bérubé et al. (2005) found that the higher concentration of ROH is related with sites under soybean cultivation, suggesting a direct relation with potential ROH disruptive pesticides (e.g. atrazine). In contrast, Boily et al. (2003) suggested that organochlorines such as polychlorinated biphenyl (PCB) would inhibit liver-plasma passage of ROH and that the binding of retinol-binding protein with ROH would be favored; overall, these two processes would cause a decrease in the concentration of endogenous plasma ROH. In this sense, more studies are necessary to elucidate the relation of alteration ROH concentration with environmental contaminants on *in situ* field studies.

Plasma RA concentrations in *L. chaquensis* were significantly higher in frogs from RF than in RS. These ROH metabolites are part of the signaling pathways in embryos, but an increase in their concentrations are involved in teratogenic processes (Thibodeau et al., 2012). At this point, several evidences reinforced the hypothesis that environmental pollutions in both aquatic and terrestrial habitats may interfere with the retinoic signaling pathway (Gardiner et al., 2003; Bérubé et al., 2005; Novák et al. 2007). For instance, Lemaire et al. (2005) conclude that endosulfan, dieldrin, endrin, chlordane, and aldrin were able to bind to retinoic acid receptors (RARs) and induce gene transcription. Recently, Paganelli et al. (2010) demonstrated that exposure to glyphosate and glyphosate-based herbicide formulation in tadpoles *Xenopus laevis* increased endogenous RA activity and had related to teratogenic and carcinogen effects. Indeed, experiments with exposure of the European common frog (*Rana temporaria*) to *p,p*'-DDE, have shown a dose-dependent increase of hepatic RA (Leiva-Presa and Jenssen, 2006). Triadimefon, a systemic fungicide, was found to produce craniofacial malformations in *X. laevis* by altering endogenous RA signaling (Papis et al., 2007). In addition, atrazine produces teratogenic effects and decreases the concentration of cyp26 transcripts in *Xenopus* tadpoles, suggesting that this herbicide also disrupts the RA signaling pathway (Lenkowski et al., 2008, 2010). Although many of these studies carried out under laboratory condition, they highlight the relation RA concentration at different contaminant exposition; consequently field studies are necessary because they could indicate ROH metabolites variation at realistic scenarios.

Furthermore, higher concentration of 13-Cis-RA in frogs was found in RF than in RS. The physiological significance of the isomerization all-trans-retinoic acid (RA) to Cis-RA is yet to be ascertained (Sass et al., 1999; Lee et al., 2004; Das et al., 2014). The RA isomers are presumably intermediates in the activation or degradation of retinol. Mainly, increases in these isomers are linked to inhibition of visual cycle function, producing nigh blindness in mammals (Gollapalli et al., 2004; Blomhoff and Blomhoff, 2006). Few studies revealed effects of Cis-RA increase in amphibians, and the results revealed acute toxic effects, high teratogenicity to amphibian embryos, and induction of different phenotypes of malformations by disrupting RA signal (Yu et al., 2011). It is important to note that AR isomers is circulated at higher concentration in plasma of embryos stages than in adults, as stated in zebrafish grows pattern of retinoid metabolism with growth (Costaridis et al., 1996). For this reason, we suggest that the concentrations of the RA isoforms should be included in ROH, and RA acids and their metabolites not only in embryos and larvae but also in amphibian adults.

Moreover, the relation between RA or 13-Cis-RA and ROH are important because the existence of RA or its 13-Cis-RA in frog plasma may be explained in term of an impairment in their metabolization, lack of binding to specific RA receptors (RARs), and consequently alteration in signalling pathways, mainly due to pesticides exposition (Paganelli et al., 2010). This observation was reinforced with the differences in RA/ROH and 13-Cis-RA/ROH ratios that were observed in *L. chaquensis* frogs from RF.

Although the sample size is relative small, but similar number to those for ecotoxicological *in situ* studies, our results demonstrate at first time that frogs that inhabit in rice fields have alteration in endogenous retinoic acid and isoforms circulating in plasma, and one explanations may be due to some pesticides, such as glyphosate that is usually used in rice cultivation could resemble retinoid acid in vertebrates (Paganelli et al., 2010). These field evidences highlight the need of exhaustive monitoring of potential pesticides that disrupt AR signaling pathway in water and soil/sediment (not searched here for cost, and expensive methods for different matrices), that also vary in their half-lives.

In conclusion, our results demonstrate that plasma RA and 13-Cis-RA varied between *L. chaquensis* individuals from natural forest (RS) and agricultural site dominated by rice cultivation (RF). Furthermore the ratios RA/ROH and 13-Cis-RA/ROH were significantly higher in individuals collected from RF than in those from RS. In these sense, the importance of analyze RA and its ratios could be linked with the mechanisms of teratogen processes in anuran wild fauna and human health (Paganelli et al., 2010), but long-field term studies are necessary. Future studies should be focused on differences on ontogenetic variation in ROH, RA, and its isomers to detect which is the range of retinoid vulnerability to environmental contaminants.

## Acknowledgments

We thank the field rice owner for allowing us to conduct the study. Jorgelina Brasca assisted us with English style. All frogs were collected with authorization of the Ministerio de Aguas, Servicios Públicos y Medio Ambiente, Santa Fe province, Argentina. This study was supported in part by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANCyT) and Curso de Acción para la Investigación y Desarrollo (CAI + D-UNL).

## References


Alvisio, A., 1998. Arroz. Modelos zonales de producción en el movimiento CREA: región litoral Norte. Cuad. Act. Tech. 61, 141–145.

Attademo, A.M., Peltzer, P.M., Lajmanovich, R.C., Cabagna, M., Fiorenza, G., 2007. Plasma B-esterase and glutathione S-transferase activity in the toad *Chaunus schneideri* (Amphibia, Anura) inhabiting rice agroecosystems of Argentina. Ecotoxicology 16, 533–539.

Attademo, A.M., Cabagna-Zenklusen, M., Lajmanovich, R.C., Peltzer, P.M., Junges, C., Basso, A., 2011. B-esterase activities and blood cell morphology in the frog *Leptodactylus chaquensis* (Amphibia: Leptodactylidae) on rice agroecosystems from Santa Fe Province (Argentina). Ecotoxicology 20, 274–282.

Attademo, A.M., Peltzer, P.M., Lajmanovich, R.C., Basso, A., Junges, C., 2014. Tissue-specific variations of esterase activities in the tadpoles and adults of *Pseudis paradoxa* (Anura: Hylidae). Wat. Air Soil Poll. 225, 1903.

Bagenal, T.B., Tesch, F.W., 1978. Methods for assessment of a fish production in fresh 376 waters. In: Bagenal, B.T. (Ed.), Age and growth. Blackwell Scientific Publications, Oxford, pp. 101–136.

Bérubé, V.E., Boily, M.H., DeBlois, C., Dassylva, N., Spear, P.A., 2005. Plasma retinoid profile in bullfrogs, *Rana catesbeiana*, in relation to agricultural intensity of sub-watersheds in the Yamaska River drainage basin, Québec, Canada. Aquat. Toxicol. 71, 109–120.

Blomhoff, R., Blomhoff, H.K., 2006. Overview of retinoid metabolism and function. J. Neurobiol. 66, 606–630.

Blomhoff, R., Wake, K., 1991. Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J. 5, 271–277.

Boily, M.H., Ndayibagira, A., Spear, P.A., 2003. Retinoid metabolism (LRAT, REH) in the yolk-sac membrane of Japanese quail eggs and effects of mono-ortho-PCBs. Comp. Biochem. Physiol. Part C 134, 11–23.

Boily, M.H., Bérubé, V.E., Spear, P.A., Deblois, C., Dassylva, N., 2005. Hepatic retinoids of bullfrogs in relation to agricultural pesticides. Environ. Toxicol. Chem. 24, 1099–1106.

Boily, M., Thibodeau, J., Bisson, M., 2009. Retinoid metabolism (LRAT, REH) in the liver and plasma retinoids of bullfrog, *Rana catesbeiana*, in relation to agricultural contamination. Aquat. Toxicol. 91, 118–125.

Boone, M.D., Semlitsch, R.D., Little, E.E., Doyle, M.C., 2007. Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. Ecol. Appl. 17, 291–301.

Burkart, R., Barbaro, N.O., Sánchez, R.O., Gómez, D.A., 1999. Eco-regiones de la Argentina. PRODIA, Buenos Aires, Argentina.

Busk, M., Jensen, F.B., Wang, T., 2000. Effects of feeding on metabolism, gas transport, and acid-base balance in the bullfrog *Rana catesbeiana*. Am. J. Physiol. Reg. Integr. Comp. Physiol. 278, 185–195.

Cei, J.M., 1980. Amphibians of Argentina. Monitore Zool, Ital, p. 609.

Champoux, L., 2000. Évaluation d'indicateurs biochimiques chez le grand héron, *Ardea herodias*, et le bihoreau gris, *Nycticorax nycticorax*, en relation avec la contamination du fleuve Saint-Laurent. Environment Canada, Ottawa.

Clugston, R.D., Blaner, W.S., 2014. Vitamin A (retinoid) metabolism and actions: what we know and what we need to know about amphibians. Zoo Biol. 33, 527–535.

Costaridis, P., Horton, C., Zeitlinger, J., Holder, N., Maden, M., 1996. Endogenous retinoids in the zebrafish embryo and adult. Dev. Dyn. 205, 41–51.

Das, B.C., Thapa, P., Karki, R., Das, S., Mahapatra, S., Liu, Ting.-Chun., Torregroza, I., Wallace, D.P., Kambhampati, S., Van Veldhuizen, P., Verma, A., Ray, S.K., Evans, T., 2014. Retinoic acid signaling pathways in development and diseases. Bioorg. Med. Chem. 22, 673–683.

Dollé, P., Niederreither, K., 2015. The Retinoids: Biology. Biochemistry, and Disease. Wiley-Blackwell.

Duré, M.I., Kehr, A.I., Schaefer, E.F., Marangoni, F., 2008. Diversity of amphibians in rice fields from northeastern Argentina. Interciencia 33, 528–531.

Frost, D.R., 2007. Amphibian Species of the World: an Online Reference. New York: American Museum of Natural History. Captured on January 2008.

García-Muñoz, E., Guerrero, F., Parra, G., 2011. Effects of previous sublethal pulse to ammonium nitrate on mortality and total length on *Epidalea calamita* larvae. Chemosphere 84, 671–675.

Gardiner, D.N.A., Grün, F., Blumberg, B., 2003. Deformed frogs and environmental retinoids. Pure Appl. Chem 75, 2263–2273.

Gislaine, G., Gomes, W.L., Tavechio, R., Massato, T., Pavanelli, G.C., 2011. Relative condition factor and parasitism in anostomid fishes from the floodplain of the Upper Paraná River Brazil. Veterinary Parasitology 177 (1-2), 98.

Gollapalli, D.R., Maiti, P., Rando, R.R., 2004. RPE65 operates in the vertebrate visual cycle by stereospecifically binding all-trans-retinyl esters. Biochemistry 43, 7226.

Horst, R.L., Reinhardt, T.A., Goff, J.P., Nonnecke, B.J., Gambhir, V.K., Fiorella, P.D., Napoli, J.L., 1995. Identification of 9-cis, 13-cis-retinoic acid as a major circulating retinoid in plasma. Biochemistry 34, 1203–1209.

Kosian, P.A., Makynen, E.A., Ankley, G.T., Degitz, S.J., 2003. Uptake and metabolism of all-trans retinoic acid by three native north American ranids. Toxicol. Sci. 74, 147–156.

Lajmanovich, R.C., Sánchez-Hernández, J.C., Stringhini, G., Peltzer, P.M., 2004. Levels of serum cholinesterase activity in the rococo toad (*Bufo paracnemis*) in agrosystems of Argentina. Bull. Environ. Contam. Toxicol. 72, 586–591.

Lajmanovích, R.C., Peltzer, P., Attademo, A.M., Cabagna, M.C., Junges, C.M., Basso, A., 2012. Amphibia, Anura, Hylidae, *Argenteohyla siemersi pederseni* (Williams and Bosso, 1994): first record and some hematological data in Santa Fe Province, Argentina. Check List 8, 790–791.

Lee, G.S., Kochhar, D.M., Collins, M.D., 2004. Retinoid-induced limb malformations. Curr. Pharm. Design 10, 2657–2699.

Lee, Y., Grill, S., Sanchez, A., Murphy-Ryan, M., Poss, K.D., 2005. Fgf signaling instructs position-dependent growth rate during zebrafish fin regeneration. Development 132, 5173–5183.

Leiva-Presa, À., Jenssen, B.M., 2006. Effects of p,p′-DDE on retinoid homeostasis and sex hormones of adult male European common frogs (*Rana temporaria*). J. Toxicol. Environ. Health 69, 2051–2062.

Lemaire, G., Balaguer, P., Michel, S., Rahmani, R., 2005. Activation of retinoic acid receptor-dependent transcription by organochlorine pesticides. Toxicol. Appl. Pharm. 202, 38–49.

Lenkowski, J.R., Reed, J.M., Deininger, L., McLaughlin, K.A., 2008. Perturbation of organogenesis by the herbicide atrazine in the amphibian *Xenopus laevis*. Environ. Health Perspect. 116, 223–230.

Lenkowski, J.R., Sanchez-Bravo, G., McLaughlin, K.A., 2010. Low concentrations of atrazine, glyphosate, 2, 4-dichlorophenoxyacetic acid, and triadimefon exposures have diverse effects on *Xenopus laevis* organ morphogenesis. J. Environ. Sci. 22, 1305–1308.

López, S.L., Aiassa, D., Benítez-Leitec, S., Lajmanovich, R.C., Mañas, F., Poletta, G., Sánchez, N., Simoniello, M.F., Carrasco, A.E., 2012. Pesticides used in South American GMO-based agriculture: a review of their effects on humans and animal models. Adv. Mol. Toxicol. 6, 41–75.

Mann, R.M., Hyne, R.V., Choung, C.B., Wilson, S.P., 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. Environ. Pollut. 157, 2903–2927.

Nau, H., 1993. Teratogenesis, transplacental pharmacokinetics, and metabolism of some retinoids in the mouse, monkey, and human. In: Livrea, M.A., Packer, L. (Eds.), Retinoids: Progress in Research and Clinical Applications. Marcel Dekker, New York, pp. 599–616.

Nau, H., Chahoud, I., Dencker, L., Lammer, E.J., Scott, W.J., 1994. Teratogenicity of vitamin A and retinoids. In: Blomhoff, R. (Ed.), Vitamin A in Health and Disease. Marcel Dekker Inc, New York, pp. 615–664.

Novák, J.í., Beníšek, M., Pacherník, J.í., Janošek, J., Šidlová, T., Kiviranta, H., Verta, M., Giesy, J.P., Bláha, L., Hilscherová, K., 2007. Interference of contaminated sediment extracts and environmental pollutants with retinoid signaling. Environ. Toxicol. Chem. 26, 1591–1599.

Paganelli, A., Gnazzo, V., Acosta, H., López, S.L., Carrasco, A.E., 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem. Res. Toxicol. 23, 1586–1595.

Papis, E., Bernardini, G., Gornati, R., Menegola, E., Prati, M., 2007. Gene expression in *Xenopus laevis* embryos after Triadimefon exposure. Gene Express. Patt. 7, 137–142.

Peltzer, P.M., Lajmanovich, R.C., Sánchez- Hernández, J.C., Cabagna, M.C., Attademo, A.M., Bassó, A., 2008. Assessment of agricultural pond eutrophication on survival and health status of the *Scinax nasicus* tadpoles. Environ. Ecotoxicol. Saf. 70, 185–197.

Peltzer, P.M., Lajmanovich, R.C., Sánchez, L.C., Attademo, A.M., Junges, C.M., Bionda, C., Martino, L., Bassó, A., 2011. Morphological abnormalities in amphibian populations from the mid-eastern of Argentina. Herpet. Conserv. Biol. 6, 432–442.

Pijnappel, W.W.M., Hendrlks, H.F.J., Folkers, G.E., Van Den Brink, C.E., Dekker, E.J., Edelenbosch, C., Van Der Saag, P.T., Durston, A.J., 1993. The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specification. Nature 366, 340–344.

Rolland, R.M., 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. J. Wildl. Dis. 36, 615–635.

Rommert, A., Tuvendal, P., Simon, A., Dencker, L., Eriksson, U., 1998. The identification of a 9-cis retinol dehydrogenase in the mouse embryo reveals a pathway for synthesis of 9-cis retinoic acid. P. Natl. Acad. Sci. USA 95, 4404–4409.

Sass, J.O., Tzimas, G., Elmazar, M.M., Nau, H., 1999. Metabolism of retinaldehyde isomers in pregnant rats: 13-cis- and all-trans-retinaldehyde, but not 9-cis-retinaldehyde, yield very similar patterns of retinoid metabolites. Drug Metab. Dispos. 27, 317–321.

Shaw, S.D., 1998. Organochlorines and biomarkers of immune and endrocrine effects in Pacific Harbour seals and Northern elephant seal pups. New York State University, Albany.

Teglia, C.M., Gil García, M.D., Galera Martínez, M., Goicoechea, H.C., 2014. Enhanced high-performance liquid chromatography method for the determination of retinoic acid in plasma. Development, optimization and validation. J. Chromatogr. A 1353, 40–48.

Thibodeau, J., Filion, S., Spear, P., Paquin, J., Boily, M., 2012. Oxidation of retinoic acids in hepatic microsomes of wild bullfrogs *Lithobates catesbeianus* environmentally-exposed to a gradient of agricultural contamination. Ecotoxicology 21, 1358–1370.

Tyler, M.J., 1999. Frogs and toads as experimental animals. ANZCCART News 12 (1), 1–4.

Vaira, M., Akmentin, M., Attademo, M., Baldo, D., Barrasso, D., Barrionuevo, S., Basso, N., Blotto, B., Cairo, S., Cajade, R., Céspedez, J., Corbalán, V., Chilote, P., Duré, M., Falcione, C., Ferraro, D., Gutierrez, F.R., Ingaramo, M.R., Junges, C., Lajmanovich, R., Lescano, J.N., Marangoni, F., Martinazzo, L., Marti, R., Moreno, L., Natale, G.S., Pérez Iglesias, J.M., Peltzer, P., Quiroga, L., Rosset, S., Sanabria, E., Sanchez, L., Schaefer, E., Úbeda, C., Zaracho, V., 2012. Categorización del estado de conservación de los anfbios de la República Argentina. Cuad. Herpetol. 26, 131–159.

Vernadakis, A., Rutledge, C.O., 1973. Effects of ether and pentobarbital anaesthesia on the activities of brain acetylcholinesterase and butyrylcholinesterase in young adult rats. J. Neurochem. 20, 1503–1504.

Yu, L., Zhang, X., Yuan, J., Cao, Q., Liu, J., Zhu, P., Shi, H., 2011. Teratogenic effects of triphenyltin on embryos of amphibian (*Xenopus tropicalis*): A phenotypic comparison with the retinoid X and retinoic acid receptor ligands. J. Hazard. Mat. 192, 1860–1868.