Toxin Reviews

ISSN: 1556-9543 (Print) 1556-9551 (Online) Journal homepage: http://www.tandfonline.com/loi/itxr20

Acute toxicity of apple snail Pomacea canaliculata's eggs on Rhinella arenarum tadpoles

Rafael Carlos Lajmanovich, Andrés Maximiliano Attademo, Paola Mariela Peltzer, Celina María Junges & Candela Soledad Martinuzzi

To cite this article: Rafael Carlos Lajmanovich, Andrés Maximiliano Attademo, Paola Mariela Peltzer, Celina María Junges & Candela Soledad Martinuzzi (2016): Acute toxicity of apple snail Pomacea canaliculata's eggs on Rhinella arenarum tadpoles, Toxin Reviews, DOI: 10.1080/15569543.2016.1243561

To link to this article: http://dx.doi.org/10.1080/15569543.2016.1243561

Published online: 19 Oct 2016.

http://informahealthcare.com/txr
ISSN: 1556-9543 (print), 1556-9551 (electronic)

Toxin Reviews

Toxin Rev, Early Online: 1–7
 DOI: 10.1080/15569543.2016.1243561

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Acute toxicity of apple snail *Pomacea canaliculata*'s eggs on *Rhinella arenarum* tadpoles

Rafael Carlos Lajmanovich[1,2], Andrés Maximiliano Attademo[1,2], Paola Mariela Peltzer[1,2], Celina María Junges[1,2], and Candela Soledad Martinuzzi[2]

[1]*CONICET-FBCB-UNL Pje, Santa Fe, Argentina and* [2]*Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (FBCB-UNL-CONICET), Santa Fe, Argentina*

Abstract

The effect of the crude homogenate of snail *Pomacea canaliculata*'s eggs (CHSE) exposition on *Rhinella arenarum* tadpoles was investigated. Exposure to 10 g CHSE/L treatment produced 95% tadpole mortality, whereas the median lethal concentration (48-h LC_{50}) was 4.35 g/L. Moreover, CHSE inhibited significantly the activities of acetylcholinesterase (AChE) and glutathione-S-transferase (GST) with respect to control tadpoles, and modified tadpoles swimming behavior. Together all these evidences indicate that eggs of snail *P. canaliculata* have a neurotoxic effect on tadpoles limiting survival at low concentrations and short time exposition.

Keywords

Amphibians, crude homogenate of snail eggs, enzymes, swimming behavior, natural toxin

History

Received 5 July 2016
Accepted 28 September 2016
Published online 17 October 2016

Introduction

Numerous studies on the effects of natural neurotoxins over a wide range of aquatic organisms (invertebrates and vertebrates), have reported acute effects (e.g. mortality, diminution in survivorship rate, feeding inhibition, paralysis), chronic effects (e.g. decrease in growth and fecundity), biochemical alterations (e.g. activity changes of phosphatases, glutathione-S-transferase (GST), acetylcholinesterase (AChE), proteases), and behavioral alterations (e.g. alterations in swimming) (e.g. Ownby & Odell, 1988).

The susceptibility of living organisms to systemic toxicity by chemical defense agents (i.e. neurotoxin) attacking their nervous system is an important factor affecting their survival (Derby & Aggio, 2011). Natural inhibitors of the acetylcholine hydrolyzing enzyme AChE carry a significant share of such poisons (Shapira et al., 1998). Due to its essential physiological function in terminating neurotransmission, AChE is a target for numerous cholinergic toxins. Indeed, when AChEs are inhibited, an excess of the neurotransmitter acetylcholine (ACH) builds up, that initially results in hyperactivity and then leads to uncontrolled muscular spasms, decreased activity and paralysis, thus causing respiratory failure and finally death (Walker et al., 2001). Moreover, selective and nonselective cholinesterase inhibitors have been used in the treatment of human diseases and in the control of insects (Becker et al., 1996; Giacobini, 1996). On the other hand, the neuroecology attempts to unify principles from diverse disciplines, by integrating knowledge gained from biophysical properties of nerve and muscle cells to community-wide impacts of trophic interactions (Ferrer & Zimmer, 2009). Some behavioral testing has been performed in the field under natural conditions using predators sympatric with the prey of interest, but more often these trials have been based on laboratory assays, usually, but not always, using natural predators (Parker et al., 2007). In this sense, in the last decade, video-tracking technologies offer the possibility of accurately quantifying locomotor behaviors in aquatic organisms (e.g. Egea-Serrano & Tejedo, 2014; Tooming et al., 2013).

Rhinella arenarum (Hensel, 1867), the common South American toad, is a species with extensive Neotropical distribution (IUCN, 2010), and it is frequently found in forest, wetlands, agricultural land, and urban territories (Bionda et al., 2015; Peltzer et al., 2006). Population tendency's studies predict extinctions in site domains by crops in the central region of the Córdoba Province, Argentina (Bionda et al., 2013).

Apple snails (*Ampullariidae*) are among the largest and most ecologically important freshwater snails of the world. In this sense, the great majority of the work to date concerns a single species, *Pomacea canaliculata* (Lamarck), which we see as having the potential to become a model organism in a wide range of fields (Hayes et al., 2015). Recent discoveries have revealed that the eggs of *P. canaliculata* contain a unique natural poison that includes two storage-proteins (ovorubin) that provide an apparent (probably warning) coloration and has antinutritive and antidigestive property, and *perivitelline*

Address for correspondence: Dr. Rafael C. Lajmanovich, Faculty of Biochemistry and Biological Sciences, National University of Littoral (FBCB-UNL), Paraje el Pozo s/n, 3000, Santa Fe, Argentina. Fax: +54 342 4750394. E-mail: lajmanovich@hotmail.com

(PV2) a novel neurotoxin with lethal effects on rodents (Dreon et al., 2013, 2014; Heras et al., 2008). Most probably, the aposematic color of *Pomacea* eggs is due to pigmented proteins that advertise the existence of neurotoxins, antinutritive proteins and digestive inhibitors at least in *P. canaliculata* (i.e. Dreon et al., 2010, 2013; Heras et al., 2008). Also, one important application of egg and gonads of *P. canaliculata* is the presence of a large amount of substances with high anti-oxidative activity, namely astaxanthin (e.g. Chen et al., 2015; Dreon et al., 2004, 2007). On the other hand, this egg neurotoxin interacts with intestinal epithelium to penetrate into the blood circulatory system of predators (Dreon et al., 2013).

Taking into account all the information available, the objective of this work was to investigate some biological properties of eggs of the apple snail *P. canaliculata* to estimate the acute toxicity on *R. arenarum* tadpoles, and analyze responses at metabolic (AChE and GST activities), and physiological (swimming behavior) levels.

Materials and methods

Tadpoles and snail eggs

Tadpoles of *R. arenarum* were selected as model test organisms, because their larvae are easy to handle and acclimate to laboratory conditions (Ferrari et al., 2011). Premetamorphic larvae ($n = 180$) were collected in November 2015 from temporary ponds (31°39'53.90"S–60°42'51.20"W, *South Park* Lake, Santa Fe Province, Argentina); these sites had not been treated with chemical pesticides, as prohibited by the laws to protect human and wildlife health. The average size (snout–tail tip) was 17 ± 0.5 mm and weight was 0.055 ± 0.009 g; Gosner stages (GS) 29–32 (Gosner, 1960). The tadpoles were acclimated for 48 h at 12-h light/dark cycle with dechlorinated tap water (DTW), pH 7.2 ± 0.05; conductivity, 165 ± 12.6 μmhos/cm; dissolved oxygen concentration, 6.5 ± 1.5 mg/L hardness, 50.6 mg/L of $CaCO_3$ at 22 ± 2 °C, and feed on boiled lettuce (*Lactuca sativa*) at the beginning of the experiment. Tadpoles used in this research have been treated according to ASIH (2011) criteria and according to approval from the animal ethics committee of the Facultad de Bioquímica y Ciencias Biológicas, FBCB Res. CD N°: 388/06.

Egg masses from *P. canaliculata* were collected in the same sites previously described and transported immediately to the laboratory. Egg clutches weighted around 3 g and had approximately 3–4 cm length (Figure 1). All egg masses used had developed embryos to no greater than the morula stage (Heras et al., 2008). Eggs (a pool of twenty clutches for each treatment) are lysed in a ceramic mortar and pestle to obtain a crude homogenate of snail eggs (CHSE). A pool of snails was deposited on the biological collection of the Laboratorio de Ecotoxicología (FBCB-UNL).

Experimental design

Short-term (48 h) static toxicity tests were run to determine the median lethal concentration (LC_{50}) of CHSE. Tests were conducted in 1 L glass aquaria (12.5 cm in diameter and 13.5 cm in height), CHSE was dissolved in DTW and the following concentrations were used, 0 (control group), 0.625, 1.25, 2.5, 5.0 and 10.0 g/L. Photoperiod, water temperature, hardness, and pH were maintained as described for the acclimation period. Both control and test solutions were carried out in triplicate with 10 tadpoles per aquarium ($n = 30$). Larval mortality was monitored and dead larvae were removed from the test vessels each 24 h. A subsample of control and treated animals ($n = 10$; respectively) of each concentrations that had a survival rate $\geq 50\%$ at 48 h were used to test the effects of CHSE on AChE and GST activities, and behavioral alterations.

Figure 1. Adult female of aquatic snail *P. canaliculata* by depositing the aerial reddish egg clutches in the sampling site. See the detail of egg laying in the picture.

Enzymatic responses

Each tadpole was homogenized (on ice) in 0.1% t-octylphenoxypolyethoxy-ethanol (triton X-100) in 25 mM tris (hydroxyl methyl) aminomethane hydrochloride (pH 8.0) using a polytron. Suspensions were centrifuged at 10 000 rpm for 15 min at 4 ± 1 °C and the supernatant (crude extract) was extracted. The Biuret method was used to determine protein concentration in the supernatants (Kingsley, 1942). When sample volume was enough, enzyme kinetics assays were carried out in triplicate or duplicate. AChE activities were measured according to Ellman et al. (1961). The reaction mixture consisted of 0.01 ml of extract, 2 mM dithiobis 2-nitrobenzoic acid (DTNB), 20 mM acetylthiocholine iodide (AcSCh), 25 mM Tris-HCl, and 1 mM $CaCl_2$ (pH 7.6). Assays were conducted at 25 °C. The variation in optical density was recorded at 410 nm for 1 min at 25 °C using a JENWAY 6405 UV-VIS spectrophotometer. AChE activities were expressed as nmol/min/mg protein using a molar extinction coefficient of 13.6×10^3/M cm. GST activity was determined spectrophotometrically by the method described by Habig et al. (1974) as adapted by Habdous et al. (2002) for mammal serum GST activity. Enzyme assay was performed at 340 nm

in 100 mM Na-phosphate buffer (pH = 6.5), 2 mM CDNB and 5 mM GSH. Enzyme kinetics assays were performed at 25 °C, and whole GST activity was expressed as μmol/min/mg protein using a molar extinction coefficient of 9.6×10^3/M cm.

Behavioral endpoints

Behavioral modifications can be measured as endpoints for sub-lethal toxicity. At the end of the CHSE exposure (48 h) one larva was released at the center of a Petri dish (15 cm diameter, 2 cm height) filled with 200 ml of DTW. After of 30 seconds of acclimation, behavioral endpoints were recorded during 5 min using a digital video-camera (Motic®, 10.0 mega pixel) placed just above the dishes. Preliminary assays of behavior with tadpoles were performed and, from these data, it was determined that video lengths of 5-min were appropriate. Each larva was treated as an independent experimental unit (Van Buskirk & McCollum, 2000), and ten replicates were conducted for each concentration of CHSE, including the control. Behavioral endpoints quantified in tadpoles were: total distance moved (cm), mean speed (cm/s), maximum speed (cm/s), resting time (s) and global activity (cm^2) defined as the accumulated activity during the assay. Video data were automatically processed by video-tracking software (Smart 3.0.02, Panlab Harvard Apparatus®).

Statistical analysis

Lethal concentration (LC_{50}) values and their respective 95% confidence limits (CL) were calculated using the Trimmed Spearman–Karber method (Hamilton et al., 1977). All biomarkers data were expressed as the mean ± SEM. AChE and GST enzyme activities were analyzed with Kruskal–Wallis test and Dunn's test for *post hoc* comparisons (Lajmanovich et al., 2013). Correlations between CHSE concentrations and specific enzyme activities were tested using the Spearman's correlation test. An analysis of variance (ANOVA) was applied to assess the effects of CHSE treatments on behavioral endpoints followed by the Dunnett's test for pairwise comparisons. These statistical methods were performed using BioEstat software 5.0 (Ayres et al., 2008). A value of $p < 0.05$ was considered significant.

Results

Acute toxicity and enzymatic activity

The calculated 48 h acute LC_{50} value (95% confidence limits) of CHSE to *R. arenarum* tadpoles was 4.35 g/L (3.46–5.47). No mortality was observed in the control treatment. NOEC value was 0.625 g CHSE/L and LOEC was 2.5 g CHSE/L. The highest concentration (10 g/L) killed 95% of tadpoles exposed to CHSE.

The mean value of the AChE activity in control tadpoles was 20.12 ± 4.85 nmol/min/mg protein at 48 h. Concentrations of CHSE assayed affected significantly activity of AChE in all cases respect to the control AChE activity (Dunnett's *post hoc* test $p < 0.05$; $p < 0.01$, Figure 2). The maximum percentage of inhibition of AChE activity in treated tadpoles after 48 h of exposure was 55.18% to 0.625 g CHSE/L (NOEC value).

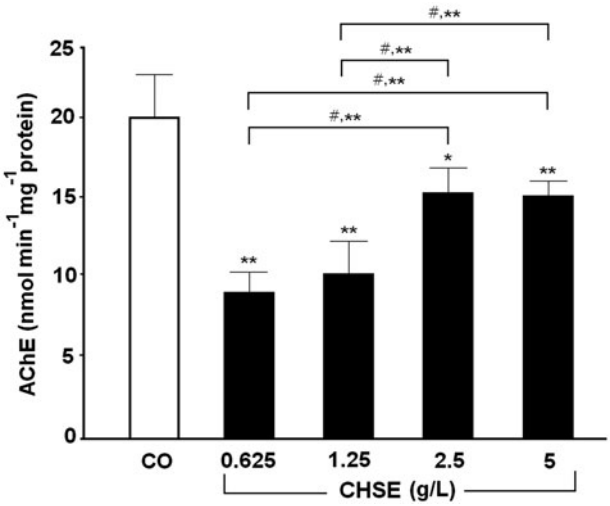

Figure 2. Acetylcholinesterase (AChE) activity in *Rhinella arenarum* tadpoles exposed (48 h) to crude homogenate of snail eggs (CHSE). Data are expressed as mean ± SEM. Significant differences were $*p < 0.05$ and $**p < 0.01$ with respect to the control; #, $p < 0.05$ and $**p < 0.01$ between different concentrations (Dunnett's *post hoc* test). $n = 10$. *Note*: Spearman's correlation coefficients between AChE activity and CHSE concentrations: $r = 0.581$, $p < 0.05$.

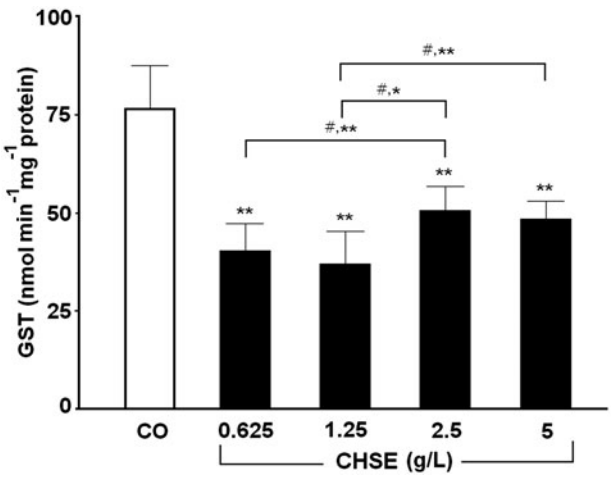

Figure 3. Glutathione S-transferases (GST) activity in *Rhinella arenarum* tadpoles exposed (48 h) to crude homogenate of snail eggs (CHSE). Data are expressed as mean ± SEM. Significant differences were $*p < 0.01$with respect to the control; $\#p < 0.05$ and $**p < 0.01$ between different concentrations (Dunnett's *post hoc* test). $n = 10$. *Note*: Spearman's correlation coefficients between GST activity and CHSE concentrations: $r = 0.24$, $p =$ NS.

The mean value of the GST activity in control tadpoles was 76.71 ± 12.5 nmol/min/mg protein at 48 h. CHSE affected significantly activities of the GST ($p < 0.01$; Figure 3) at all quantities tested, with a percentage of inhibition from 49.73% (1.25 g/L) to 27.10% (2.5 g/L).

Behavioral endpoints

The exposure of tadpoles to CHSE concentrations caused alterations of swimming endpoints (Figure 4). Tadpoles of *R. arenarum* exhibited significant effects on the following behavioral patterns: distance moved ($F = 5.32$; $p = 0.0007$), global activity ($F = 2.88$; $p = 0.03$), resting time ($F = 2.60$;

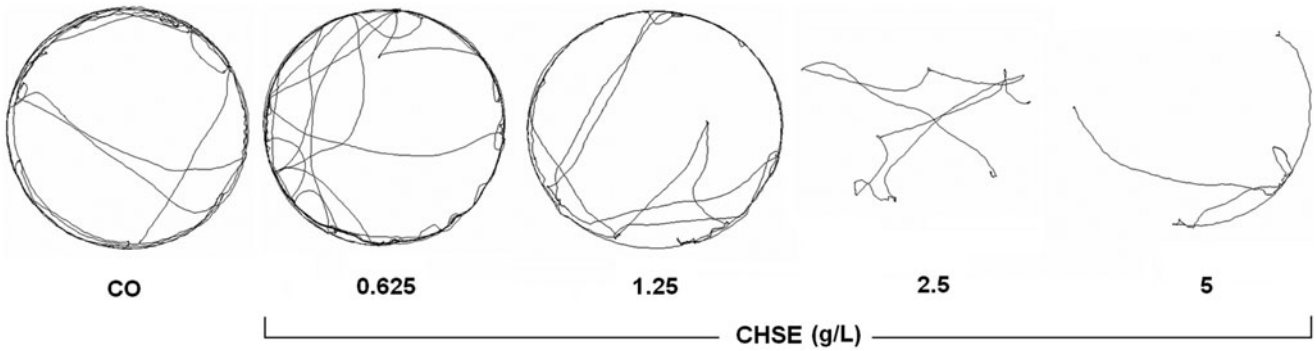

Figure 4. Representative video tracks of tadpoles after exposition (48 h) to crude homogenate of snail eggs (CHSE). Co = Control (dechlorinated tap water).

Table 1. Summary of swimming parameters (mean ± SEM) evaluated in *Rhinella arenarum* tadpoles exposed to crude homogenate of snail eggs (CHSE).

	Treatments of CHSE exposure				
Behavioral parameters	Control	0.625 g/L	1.25 g/L	2.5 g/L	5 g/L
Distance moved (cm)	80.53 ± 27.60	73.37 ± 14.98	68.54 ± 23.32	16.09 ± 6.80**	34.47 ± 17.33*
Mean speed (cm/s)	0.37 ± 0.13	0.42 ± 0.08	0.38 ± 0.13	0.08 ± 0.03	0.19 ± 0.09
Maximumspeed (cm/s)	4.84 ± 0.85	7.10 ± 0.67*	4.88 ± 0.86	3.60 ± 0.97	2.87 ± 0.78
Global activity (cm^2)	141.46 ± 42.41	102.26 ± 16.90	84.51 ± 22.79	35.26 ± 5.84*	56.19 ± 17.50
Immobility (s)	169.41 ± 10.85	153.07 ± 6.67	160.46 ± 5.77	174.64 ± 1.90	167.10 ± 5.50
Resting time (s)	175.65 ± 10.60	150.53 ± 6.51**	150.86 ± 8.92**	172.42 ± 3.80	168.21 ± 5.30

Asterisks denoted significant differences with the control (Dunnett's *post hoc* test):
*$p < 0.05$;
**$p < 0.01$. $n = 10$.

$p = 0.04$) and maximum speed ($F = 3.68$; $p = 0.01$). Post-hoc tests showed tadpoles exposed to 2.5 g/L of CHSE moved less and had less global activity than controls (Table 1). At 5 g/L of CHSE tadpoles also moved less than the control group (Table 1). The tadpoles exposed to 0.625 and 1.25 g/L spent less time at rest than those in the control group. The maximum speed was reached at 0.625 g/L of CHSE (Table 1). However, no significant effects of CHSE treatments were found on the mean speed (Table 1).

Discussion

The presence of toxins in aquatic animals is an important strategy that enhances their survival in a highly competitive ecosystem (Abirami et al., 2014). One of the primary mechanisms for avoiding predation is antipredation coloration, that either reduces the probability that the predator will detect the prey or reduces the probability of predation after detection, because the predator expects the prey to be unprofitable (Mappes et al., 2005; Ruxton et al., 2004). Eggs of *P. canaliculata* are the first description of a defense system employed by snail against predators in an animal (Dreon et al., 2013). According to its discoverers, unforeseen similarities between poisonous seeds and poisonous eggs exist, indicating that protection mechanisms thought to be confined to plants are also part of an animal's defensive repertoire. On the other hand, the study of biologically active molecules, including peptides and proteins, present in tissues of invertebrates and vertebrates, has been a core research focus in the life and health sciences for many years (Erspamer, 1994). Such study has produced many important leads in drug discovery (e.g. Bailon & Won, 2009; Galloway et al., 2010).

To our knowledge no studies exist regarding the toxicity or bioactivity of eggs of the apple snail on amphibian's tadpoles. If the results are categorized using the scoring system Chemical hazard identification and exposure (O'Bryan & Ross, 1988), this study showed that CHSE toxicity (48 h-CL_{50} = 4.35 g/L) are relatively harmless (> 1000 mg/L). However, toxins of eggs of apple snails (i.e. PV2) can be classified as extremely toxic, considering that the LD_{50} 96 h on mice with a single dose of ingestion was around 0.25 mg/kg (Heras et al., 2008). Possibly, effect on tadpoles was not stronger because the route of exposure is different. Animal venoms and toxins are recognized as major sources of bioactive molecules that may be tomorrow's new drug leads (Omar, 2013). This secretion must contain molecules that disrupt normal physiological processes (Fry et al., 2006).

On the other hand, several natural products or bioactive substances in fish, snakes, crustaceans, scorpions, and spiders are inhibitors of the enzyme AChE, (e.g. Barbosa-Filho et al., 2006; Cervenansky et al., 1991; Li, 1965; Sang-Bo et al., 2014). The analysis of AChE revealed significant variations in tadpoles exposed to CHSE compared to the control group, suggesting the influence of the specific treatment. Dreon et al. (2004, 2006) described that eggs of *P. canaliculata* contain some chemical defenses that include the carotene protein ovorubin with protease inhibitor activity and photoprotective actions, agglutinins, and strong antioxidants such as astaxanthin. Thus, the carotenoid contents may explain the

inhibition of AChE; for example, the extracts of Pandalid Shrimps (Decapoda Caridea:Pandalidae) (rich in carotenoid) exhibited the highest antioxidant and anti-cholinesterase activities (Sang-Bo et al., 2014). Another possible explanation for these effects could be given because the *P. canaliculata* egg neurotoxin resembles bacterial botulinum toxin (Dreon et al., 2013), known for its ability to inhibit the action of AChE (Marshall & Quinn, 1967; Ray, 1993).

The great response of an organism to the low dose of a toxin is considered an adaptive compensatory process following an initial disruption in homeostasis (Calabrese & Blain, 2005). Moreover, some living substances, although toxic at higher doses, can be stimulatory or even beneficial at low doses (Calabrese, 2008; Calabrese & Baldwin, 2002). For instance, a similar situation is observed with pharmaceuticals that are used for their beneficial effects, as well as compounds such as certain pesticides that are normally used as toxicants (Calabrese & Baldwin, 2003). Moreover, natural products have already proven to be sources of useful AChE inhibitors a promising therapeutic strategy (e.g. in Alzheimer's disease; Mehta et al., 2012). It is interesting to note that AChE was inhibited 55.18% at 0.625 g/L in tadpoles exposed to CHSE, whereas its inhibition was only 19.62% at 2.5 g/L. The results from the AChE activity measurements reveal that the effects of CHSE are dose-dependent ($r = 0.58$, $p = 0.05$). Our data apparently indicated that this enzyme displayed symptoms of hormetic-like responses, even though a decrease in AChE activity at low doses is a subject for further research because they are not accompanied with mortality.

Oxidative stress is produced when the balance between oxidants and antioxidants is interrupted either by reduction of antioxidant defenses or by excessive increase of reactive oxygen species (ROS) (Valavanidis et al., 2006). Eggs of ampullariid species are provided with carotenoproteins that play several roles ranging from photoprotection, and antioxidant or antitrypsin actions to nutrient provision for development (Heras et al., 2007). In the same sense, marine carotenoids may have properties of reducing oxidative stress markers and potential applications in preventing and treating inflammatory diseases in human health (Gammone et al., 2015). Short exposure of *R. arenarum* tadpoles to CHSE not only produced mortality but also some sub-lethal effects such as significant variations in GST activity compared with the control group. In diverse natural toxins evidently one the producer mechanisms of detoxification pathways is related with the GST activity (e.g. Chen et al., 2005; Sang-Bo et al., 2014). GST enzyme activity of *R. arenarum's* tadpole exposed to CHSE decreased, and this may be caused by *P. canaliculata* eggs astaxanthin as a very potent antioxidant (Dreon et al., 2004). However, further studies are necessary to elucidate the role of oxidative stress with bioactive substances contained in apple snail eggs.

In amphibian tadpoles, the use of swimming activity as a behavioral endpoint is a well-established biomeasure of sub-lethal toxicity studies because they are rapid and appear sensitive to a broad range of toxicant (Melvin & Wilson, 2013), with implications for higher-level biological processes (Denoël et al., 2013; Groh et al., 2015). In the present study, swimming performance of larvae exposed to CHSE was significantly influenced. Swimming speed was the most sensitive endpoint which caused hyperactivity (i.e. maximum speed and resting time) at a NOEC of 0.625 g CHSE/L. Nevertheless, responses observed in tadpoles indicate possible physiological effects, since increased swimming activities and burst swimming may be linked to increased physiological stress levels in exposed animals. Also, at the other concentrations of CHSE, exposed tadpoles showed effects such as resting time, distance moved and decrease of the global activity. The alteration of the normal nervous system functions by inhibition of AChE could affect muscular function of tadpoles, and therefore locomotor performance (e.g. Peltzer et al., 2013; Preud' homme et al., 2015). While this greater loss of enzyme activity occurs at lower concentration toxins, all concentrations tested resulted in significant percentage inhibitions; in appearance sufficient to produce observed behavioral alterations. Likewise, in mouse tests the egg protein toxin of *P. canaliculata* produced neuronal apoptosis in spinal cord (Heras et al., 2008). In tadpoles, further experiments are needed to assess if CHSE causes only inhibition of AChE or other neurological damage on the central nervous system.

In conclusion, the crude extract of eggs of *P. canaliculata* is toxic for amphibian tadpoles at low doses and a relatively short time period. Acute and sub-lethal exposures (among 0.625–10 g/L of CHSE) inhibited AChE *and GST* activities, and altered behavioral responses of *R. arenarum* larvae.

Acknowledgements

The authors are grateful to three anonymous reviewers for critical comments and valuable suggestions that greatly improved the manuscript. We thank to Oscar Scremin for critical reading of the MS and correct the English language, we also thanks to their valuable and disinterested collaborations with our laboratory.

Declaration of interest

The authors declare that there are no conflicts of interest. This project is supported in part by Secretaría de Ciencia y Tecnología (SECYT), Curso de Acción para la Investigación y Desarrollo (Programa de I + D Orientado a Problemas Sociales y Productivos, UNL), and Agencia Santafesina de Ciencia, Tecnología e Innovación (ASaCTeI).

References

Abirami P, Arumugam M, Giji S, et al. (2014). Bio-prospecting of catfish sting venom *Arius maculatus* available along south east coast of India. Int J Pharm Pharm Sci 6:110–15.

ASIH, HL, SSAR. (2011). Guidelines for use of live amphibians and reptiles in field and laboratory research, Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. Washington DC, USA.

Ayres Jr M, Ayres D, Santos A. (2008). BioEstat. Versão5.0. Belém, Pará, Brazil: Sociedade Civil Mamirauá, MCT-CNPq.

Bailon P, Won CY. (2009). PEG-modified biopharmaceuticals. Expert Opin Drug Deliv 6:1–16.

Barbosa-Filho JM, Medeiros KCP, Diniz MFFM, et al. (2006). Natural products inhibitors of the enzyme acetylcholinesterase. Brazilian J Pharmac 16:258–85.

Becker RE, Moriearty P, Unni L, et al. (1996). Cholinesterase inhibitors as therapy in Alzheimer disease: benefit to risk considerations in clinical application In: Becker R, Giacobini E, eds. Alzheimer disease from molecular biology to therapy. Boston, USA: Birkhäuser, 257–66.

Bionda CL, Kost S, Salas NE, et al. (2015). Age structure, growth and longevity in the common toad, *Rhinella arenarum*, from Argentina. Acta Herp 10:55–62.

Bionda C, Lajmanovich R, Salas N, et al. (2013). Demografia poblacional de *Rhinella arenarum* (Anura: Bufonidae) *Physalaemus biligonigerus* (Anura: Leiuperidae) en agroecosistemas de la provincia de Córdoba, Argentina. Ver Biol Trop 61:1389–400.

Calabrese EJ. (2008). Hormesis: why it is important to toxicology and toxicologists. Environ Toxicol Chem 27:1451–74.

Calabrese EJ, Baldwin LA. (2002). Applications of hormesis in toxicology, risk assessment and chemotherapeutics. Trends Pharmacol Sci 23:331–7.

Calabrese EJ, Baldwin LA. (2003). Hormesis: the dose-response revolution. Annu Rev Pharmacol Toxicol 43:175–97.

Calabrese EJ, Blain R. (2005). The occurrence of hormetic dose responses in the toxicological literature, the hormesis data base: an overview. Toxicol Appl Pharmacol 202:289–301.

Cervenansky C, Dajas F, Harvey AL, et al. (1991). Fasciculins, anticholinesterase toxins from mamba venoms: biochemistry and pharmacology. In: Harvey AL, ed. Snake toxins. New York: Pergamon Press, 303–21.

Chen YY, Lee PC, Wu YL, et al. (2015). In vivo effects of free form astaxanthin powder on anti-oxidation and lipid metabolism with high-cholesterol diet. PLoS One 10:e0134733.

Chen W, Song L, Ou D, et al. (2005). Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. Environ Toxicol 20:323–30.

Denoël M, Libon S, Kestemont P, et al. (2013). Effects of a sublethal pesticide exposure on locomotor behavior: a video-tracking analysis in larval amphibians. Chemosphere 90:945–51.

Derby CD, Aggio JF. (2011). The neuroecology of chemical defenses. Integr Comp Biol 51:771–80.

Dreon MS, Schinella G, Heras H, et al. (2004). Antioxidant defense system in the apple snail eggs, the role of ovorubin. Arch Biochem Biophys 422:1–8.

Dreon MS, Ceolín M, Heras H. (2007). Astaxanthin binding and structural stability of the apple snail carotene protein ovorubin. Arch Biochem Biophys 460:107–12.

Dreon MS, Frassa MV, Ceolín M, et al. (2013). Novel animal defenses against predation: a snail egg neurotoxin combining lectin and pore-forming chains that resembles plant defense and bacteria attack toxins. PLoS One 8:e63782.

Dreon MS, Fernández PE, Gimeno EJ, et al. (2014). Insights into embryo defences of the invasive apple snail *Pomacea canaliculata*: egg mass ingestion affects rat intestine morphology and growth. PLoS Negl Trop Dis 8:e2961.

Dreon MS, Heras H, Pollero RJ. (2006). Biochemical composition, tissue origin and functional properties of egg perivitellins from *Pomacea canaliculata*. Biocell 30:359–65.

Dreon MS, Ituarte S, Heras H. (2010). The role of the proteinase inhibitor ovorubin in apple snail eggs resembles plant embryo defense against predation. PLoS One 5:e15059.

Egea-Serrano A, Tejedo M. (2014). Contrasting effects of nitrogenous pollution on fitness and swimming performance of Iberian waterfrog, *Pelophylaxperezi* (Seoane, 1885), larvae in mesocosms and field enclosures. Aquat Toxicol 146:144–53.

Ellman L, Courtey KD, Andreas Jr V, et al. (1961). A new rapid colorimetric determination of cholinesterase activity. Biochem Pharmacol 7:88–95.

Erspamer V. (1994). Bioactive secretions of the integument. Amphibian biology, vol. 1. Chipping Norton: Surrey Beatty and Sons.

Ferrari A, Lascano C, Pechen de D'Angelo AM, et al. (2011). Effects of azinphos methyl and carbaryl on *Rhinella arenarum* larvae esterases and antioxidant enzymes. Comp Biochem Physiol C Toxicol Pharmacol 153:34–9.

Ferrer RP, Zimmer RK. (2009). Chemical neuroecology and community dynamics. Ann NY Acad Sci 1170:450–5.

Fry BG, Vidal N, Norman JA, et al. (2006). Early evolution of the venom system in lizards and snakes. Nature 439:584–8.

Galloway WR, Isidro-Llobet A, Spring DR. (2010). Diversity-oriented synthesis as a tool for the discovery of novel biologically active small molecules. Nat Commun 1:80–93.

Gammone MA, Riccioni G, D'Orazio N. (2015). Marine carotenoids against oxidative stress: effects on human health. Mar Drugs 13: 6226–46.

Giacobini E. (1996). Cholinesterase inhibitors do more than inhibit cholinesterase In: Becker R, Giacobini E, eds. Alzheimer disease from molecular biology to therapy. Boston, USA: Birkhäuser, 187–204.

Gosner KL. (1960). A simplified table for staging anuran embryos and larvae, with notes on identification. Herpetologica 16: 183–90.

Groh KJ, Carvalho RN, Chipman JK, et al. (2015). Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: II. A focus on growth impairment in fish. Chemosphere 120:778–92.

Habdous M, Vincent-Viry M, Visvikis S, et al. (2002). Rapid spectrophotometric method for serum glutathione S-transferases activity. Clin Chem Acta 326:131–42.

Habig WH, Pabst MJ, Jakoby WB. (1974). Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–9.

Hamilton MA, Russo RC, Thurston RV. (1977). Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–9.

Hayes KA, Burks RL, Castro-Vazquez A, et al. (2015). Insights from an integrated view of the biology of apple snails (Caenogastropoda: Ampullariidae). Malacologia 58:245–302.

Heras H, Dreon MS, Ituarte S, et al. (2007). Egg carotenoproteins in neotropical Ampullariidae (Gastropoda: Arquitaenioglossa). Comp Biochem Physiol C Toxicol Pharmacol 146:158–67.

Heras H, Frassa MV, Fernández PE, et al. (2008). First egg protein with a neurotoxic effect on mice. Toxicon 52:481–8.

IUCN: The International Union for Conservation of Nature and Natural Resources. An Analysis of Amphibians on the 2010, IUCN Red List, 2010. Available at: www.iucnredlist.org/amphibians. Accessed on July 22, 2014.

Kingsley GR. (1942). The direct Biuret method for the determination of serum proteins as applied to photoelectric and visual calorimetry. J Lab Clin Med 27:840–5.

Lajmanovich RC, Junges CM, Attademo AM, et al. (2013). Individual and mixture toxicity of commercial formulations containing glyphosate, metsulfuron-methyl, bispyribac-sodium, and picloram on *Rhinella arenarum* tadpoles. Wat Air Soil Poll 224:1404.

Li KM. (1965). Ciguatera fish poison: a cholinesterase inhibitor. Science 147:1580–1.

Mappes J, Marples N, Endler JA. (2005). The complex business of survival by aposematism. Trends Ecol Evol 20:598–603.

Marshall R, Quinn LY. (1967). In vitro acetylcholinesterase inhibition by type A botulinum toxin. J Bacteriol 94:812–4.

Mehta M, Ademand A, Sabbagh M. (2012). New acetylcholinesterase inhibitors for Alzheimer disease. Int J Alzheimers Dis 2012:1–9.

Melvin SD, Wilson SP. (2013). The utility of behavioral studies for aquatic toxicology testing: a meta-analysis. Chemosphere 93: 2217–23.

O'Bryan TR, Ross RH. (1988). Chemical scoring system for hazard and exposure identification. J Toxicol Environ Health 1988:119–34.

Omar HEDM. (2013). The biological and medical significance of poisonous animals. J Biol Earth Sci 3:25–41.

Ownby CL, Odell GV. (1988). Natural toxins. Characterization, pharmacology and therapeutics. New York: Pergamon Press.

Parker JD, Burkepile DE, Collins DO, et al. (2007). Stream mosses as chemically-defended refugia for freshwater macroinvertebrates. Oikos 116:302–12.

Peltzer PM, Junges CM, Attademo AM, et al. (2013). Cholinesterase activities and behavioral changes in *Hypsiboas pulchellus* (Anura: Hylidae) tadpoles exposed to glufosinate ammonium herbicide. Ecotoxicology 22:1165–73.

Peltzer PM, Lajmanovich RC, Attademo AM, et al. (2006). Diversity of anurans across agricultural ponds in Argentina. Biodiv Conser 15: 3499–513.

Preud' homme V, Milla S, Gillardin V, et al. (2015). Effects of low dose endosulfan exposure on brain neurotransmitter levels in the African clawed frog *Xenopus laevis*. Chemosphere 120:357–64.

Ray P. (1993). Botulinum toxin A inhibits acetylcholine release from cultured neurons in vitro. In Vitro Cell Dev Biol 29:A:456–60.

Ruxton GD, Sherratt TN, Speed MP. (2004). Avoiding attack: the evolutionary ecology of crypsis, warning signals, and mimicry. Oxford: Oxford University Press.

Sang-Bo K, Na YY, Kil BS, et al. (2014). Antioxidant and cholinesterase inhibitory activities of the by-products of three Pandalid Shrimps. Fish Aquat Sci 17:421–5.

Shapira M, Seidman S, Livni N, et al. (1998). In vivo and in vitro resistance to multiple anticholinesterases in *Xenopus laevis* tadpoles. Toxicol Lett 102–103:205–9.

Tooming E, Merivee E, Must A, et al. (2013). Sub-lethal effects of the neurotoxic pyrethroid insecticide Fastac® 50EC on the general motor and locomotor activities of the non-targeted beneficial carabid beetle *Platynusassimilis* (Coleoptera: Carabidae). Pest Manag Sci 70: 959–66.

Valavanidis A, Vlahogianni T, Dassenakis M, et al. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 64:178–89.

Van Buskirk J, McCollum SA. (2000). Influence of tail shape on tadpole swimming performance. J Exp Biol 203:2149–58.

Walker CH, Hopkin SP, Sibly RM, et al. (2001). Principles of ecotoxicology. 2nd edn. New York: Taylor and Francis.