Ecotoxicology (2019) 28:48–61
https://doi.org/10.1007/s10646-018-1998-1

Toxicity of wine effluents and assessment of a depuration system for their control: assay with tadpoles of *Rhinella arenarum* (BUFONIDAE)

Ana Laura Navas Romero[1] · **Mario Andrés Herrera Moratta**[1] · **María Rosa Rodríguez**[2] · **Lorena Beatriz Quiroga**[3] · **Marcelo Echegaray**[2] · **Eduardo Alfredo Sanabria**[3,4]

Accepted: 26 October 2018

Abstract

We evaluated the toxicity of the winery effluent and the efficiency of a symbiotic depuration system by means an experiment with *Rhinella arenarum* tadpoles. The studied effluent was taken from warehouses during the cleaning season. These effluents subsequently subjected to the purification treatment under evaluation. The effluent samples differentiated into two treatment levels: "raw" where the effluent was evaluated with field conditions and "treated" where the effluent was previously filtered with the symbiotic depuration system. The results of the bioassays compared with the physicochemical parameters determined in the effluent samples. The lethal response had a clear-cut correspondence with the effluent quality assessed utilizing physicochemical parameters. In all cases, dilution of the samples resulted in a significant reduction of their toxicity. It concluded that (a) winery effluents could be harmful to tadpoles of *R. arenarum*, (b) the symbiotic purification system used to treat wine effluents it would produce a significant reduction in the contaminant levels of the effluent. However, this reduction in contaminant levels does not provide sufficient safety for the release of the effluents into the environment.

Keywords Bioassays · Contaminants · Winery · Symbiotic

Introduction

Viticulture is one of the basic economic activities of many regions of Argentina and the world (Fernández Portela 2013). Effluents are generated during the processing of the grape (Bustamante et al. 2005), with around 1.5 l of industrial effluents for each liter of wine made (Crites and Tchobanoglous 2000). On many occasions, reuse of these discharges for irrigation has considered; however, their high content of organic matter makes prior treatment indispensable (González et al. 2003).

Ignorance of the impact that these viticulture effluents can have when released to the environment has led to studying alternative technologies such as "symbiotic depuration" (García et al. 2008). This system distinguished by the existence of two zones, one for purification and another one for cultivation. The purification zone consists of a bed of gravel, which is isolated from the ground by a waterproof base. The residual water is applied for irrigation through a network of underground drippers, placed directly on the gravels, to cause its percolation through them. Once the impermeable base reached, the residual water flows, by gravity, to the pouring points, for reuse on other areas (Rodríguez et al. 2013).

Assessing exposure through determination of environmental concentrations of chemical agents, by itself, does not ensure the protection of aquatic life since it does not allow predicting their potential toxic consequences when synergistic or additive effects occur between the chemical elements in the mixture (Vighi et al. 2003). On the other hand, the toxicity of chemical agents is affected by other variables such as pH, organic matter, hardness, which determine their

✉ Ana Laura Navas Romero
anavas@mendoza-conicet.gob.ar

[1] Instituto Argentino de Investigaciones en Zonas Áridas – CCT Mendoza – CONICET, Mendoza, Argentina

[2] Facultad de Ingeniería, Universidad Nacional de San Juan, San Juan, Argentina

[3] Instituto de Ciencias Básicas, Universidad Nacional de San Juan – CONICET, San Juan, Argentina

[4] Facultad de Ciencias Exactas, Universidad Nacional de Cuyo, Mendoza, Argentina

bioavailability and their potential for toxicity (Baker et al. 2003). To assess the effect of chemical agents, biological tools known as toxicity tests have developed, which use organisms that can represent the different trophic levels of an aquatic ecosystem (Sponza 2003).

Amphibians have a big part of vertebrate biomass and are key elements in the food chain (Blaustein and Wake 1990). Their dependence on water and moisture, their complex life cycle, and physiological sensitivity to environmental conditions through their extremely permeable skin make them true bioindicators and therefore are valuable for toxicity tests (Wake 1991).

Many of the wetland habitats that are crucial for amphibian reproduction and survival have altered by human activities including viticulture (Babini et al. 2016; Bishop et al. 1999). Amphibians that breed in ponds immersed or surrounded by industrial effluent are probably exposed to high levels of chemicals and can suffer serious consequences at the population level.

Thus, the potential effects of winery effluent on amphibian are of particular concern due to the lack of pristine habitats available, particularly for the reproduction and development of eggs and tadpoles. In general, the eggs and newly metamorphosed tadpoles are the most sensitive life stages to environmental contaminants (Power et al. 1989).

Although wine production does not have a reputation as a polluting industry, the winery wastewater has an acidic pH, a high organic charge and micronutrient and heavy metal contents all of which indicate that the wastewater has the potential to pose an environmental threat (Bustamante et al. 2005; Malandra et al. 2003; Mosse et al. 2011). An excess of organic charge could lead to eutrophication with a drastic decrease in dissolved oxygen and subsequent mortality of aerobic aquatic organisms (Mitsch and Gosselink 2015, 2000). A high heavy metal contents, an elevate nitrate level and low pH, could be reduce survivorship, alter the epidermis, both feeding and swimming activity, and could be generate malformations, decreasing the growth and development of amphibian tadpoles (Berger 1989; Brand et al. 2010; Gross et al. 2009; Smith et al. 2006).

Acute and short-term chronic toxicity tests have been widely applied in fish, macroinvertebrates and tadpoles for decades (Bélanger-Deschênes et al. 2013; Ji et al. 2008; Lajmanovich et al. 2018; Nimmo and Boraas 1982; Scott and Crunkilton 2000). Nonetheless, there are few studies examining the effects of chemical complex mixture on amphibian populations (Bishop and Pettit 1992). This is the first study that evaluates the influence of winery effluents in anurans and the effectiveness of an effluent treatment system using bioassays.

In this study, we tested the hypothesis that viticulture effluents still treated with symbiotic depuration system, are not safe for the environment, affecting aquatic and terrestrial life. Winery effluents generates sublethal and lethal effects on anuran tadpoles. In this way, the objective of this work was to assess the toxicity of effluents from wineries and the performance of a symbiotic filtration system using acute bioassays with tadpoles of *Rhinella arenarum* as a sentinel organism.

Materials and methods

The tadpoles of *R. arenarum* were selected to carry out the present study. This species has an extensive neo-tropical distribution and it frequently found both natural and agricultural land. Frequent reproduction, large numbers of eggs in nests (Sanabria et al. 2007), and the easy maintenance under laboratory conditions make this organism an interesting candidate for toxicity testing. Egg masses used for this study collected from temporary and unpolluted ponds located at 25 km west of the city of San Juan, Department Zonda (31° 55’ S, 68° 70’ W). Ponds are within Monte phytogeography province (Cabrera 1971).

Eggs used in the exposure experiments were cultured in the laboratory until hatching. The hatching of the eggs occurred on the 3rd day of their arrival in the laboratory. The tadpoles acclimatized in glass tanks that contained dechlorinated tap water at 22 ± 2 °C for four days, artificial aeration, and 12 –12 h light–dark cycle. The tests according to USEPA Standard Methods (2002). Tadpoles were fed daily ad libitum with a mixture of boiled lettuce and gelatin up to reaching Gosner stage 26–30 (Gosner 1960) at which point they were deposited randomly in the trial recipients according to the experimental design.

Exposure agent: effluents from “raw” and “treated” warehouses

Toxicity tests were carried out using as a test substance 30 L of effluents from three wineries distributed in the province (“toxic agent”).

Samples of winery effluents were taken from three different points in the winery industries (output, middle and contact of winery effluents with surrounding). The samples were taken during the process of washing and cleaning the winery industries, stored in polyethylene containers previously washed with 10% of nitric acid (HNO_3) and rinsed thoroughly with distilled water, recording their temperature, pH, and conductivity.

The samples were transported to the laboratory while maintained at 4 °C and within 5 h of sampling. Samples were kept refrigerated until their analysis. An experimental reactor was used to evaluate the effluent treatment system.

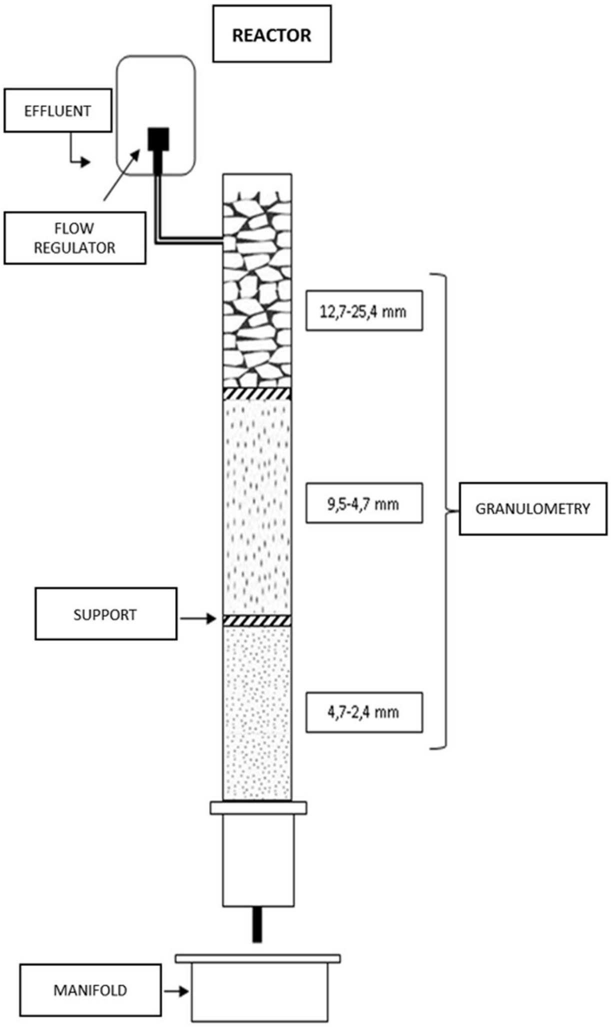


Fig. 1 Reactor used in the treatment of winery effluents (schematic draw), follow the Rodríguez et al. (2013)

Description and use of the reactor

The study conducted in a laboratory scale reactor that was constructed to mimic symbiotic depuration systems used by wineries. This consists of a vertical transparent acrylic tube, 11 cm in diameter and 100 cm in bed height. The treatable effluent has placed in a tank and through a conduit, and the effluent was evenly distributed in the upper part of the bed. Below the distributor is the purification zone, consisting of a bed of gravels 100 cm high and with a certain granulometry. In this way, the effluent descended through the gravel bed and collected from the bottom of the reactor for further analysis. In the upper part of the distributor was placed a bed of sand of 10 cm whose function was to allow the diffusion of oxygen as the effluent descended through the bed. The flow velocity of the effluent in the reactor with which it worked was 0.5 L/h, and identified as the most efficient in the treatment (Fig. 1).

Physicochemical parameters of the winery effluent

The physicochemical profile of the samples determined by evaluating the following parameters: pH, conductivity, dissolved solids, turbidity, nitrates, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), and dissolved heavy metals (Cd, Cr, and Pb). All the analyzes were carried out in triplicate, following the normalizing methods of Clesceri et al. (1992) and the American Public Health Association (1992).

Water quality was also characterized by means of the application of a physicochemical index: WQI contamination water index (Brown et al. 1970). It is determined considering temperature, DO, pH, BOD, nitrates, turbidity, and dissolved solids. It varies between 0-100, the scale being: very bad 0–25 (1), bad 26–50 (2), regular 51–70 (3), good 71–90 (4) and excellent > 90 (5). A score of 10 corresponds with a pollution status equivalent to that of a sewer effluent.

Exposure to the test substance

The test was conducted in 1 L polyethene containers with five organisms per containers and 500 ml of test solution. The tadpoles were acclimated for 48 h in hard water according to the United States Environmental Protection Agency (2002).

Tadpoles were placed in polyethylene recipients containing the following solutions: (A) hard artificial water (control); (B) raw wine effluents. (B1) without dilution (100%), (B2) diluted (10, 20, 30, 40, 50, 60, 70, 80, 90%); (C) treated vitiviniculture effluent, (C1) without dilution (100%), (C2) diluted (10, 20, 30, 40, 50, 60, 70, 80, 90%). After its preparation, it was allowed to stand for one hour, to achieve uniformity and homogeneity in the solution.

The assays were semi-static with ten replicates of each condition. The dilution water was dechlorinated by aeration for 48 h. The length of the test was 96 h according to USEPA Standard Methods (2002). At the end of the experiment was labeled and fixed in 10% v/v formaldehyde for further evaluation of morphological abnormalities. The experiment was carried out under controlled conditions of temperature 22 ± 1 °C and a photoperiod of 12 light-dark hours with artificial aeration. Tadpoles not fed throughout the experiment.

Survival

Survival was quantified every 24 h during the exposure time. Each individual considered dead if no movement detected after gentle prodding. The dead individuals removed from containers. Dead individuals have taken at each observation time, were labeled and fixed in 10% v/v formaldehyde for further evaluation of morphological abnormalities.

Table 1 Physicochemical parameters of the winery effluents samples used with the *R. arenarum* tadpoles assay

		Raw effluent		Treated effluent		Control		CMP		
		Mean	SD	Mean	SD	Mean	SD	FAO	DH	LVA
pH		6.41	0.11	7.25	0.23	7.66	0.20	6.90	43379	43349
Conductivity (µS/cm)		1202	1.00	1307	6.08	26.00	5.40	>1200	>1400	
DO (mg O_2/l)		3.00	0.10	6.00	0.00	10.00	0.00			1000.00
Nitrates (mg N-NO3/l)		16.57	4.13	16.80	0.46	0.00	0.00	17.00	20.00	
BOD (mg O_2/l)		3480.00	207.85	1026.66	11.55	0.80	0.02	100.00–200.00	10.000–200.00	
COD (mg O_2/l)		1945.00	207.86	1053.33	5.77	0.00	0.00	250.00–500.00	25.000–500.00	
Dissolved solids		806.67	0.58	403.66	0.63	0.00	0.00			
Turbidity (UTN)		80.00	1.66	30.00	1.44	0.00	0.00	100.00		
Heavy metals (µg/l)	Cd	0.50	0.37	0.43	0.29	0.00	0.00	0.01	0.01	0.20
	Cr	0.22	0.02	0.18	0.03	0.00	0.00	0.10	0.10	2.00
	Pb	0.00	0.00	0.00	0.00	0.00	0.00	0.00		1.00

The mean, the standard deviation (SD) and maximum permitted quantity (CMP) for each parameter according to Hydraulics (DH, Annex I of Decree No. 0638/87, Regulatory of Law No. 5824/87, 2006), United Nations Organization for Agriculture and Food (FAO, 1985), and the protection of the aquatic life of Argentina (LVA, Law 24,051, Decree 831/93 Argentina, 1993)

Sub-lethal effects

Registration of behavior every 6 h done after gently swirling the water five times with a glass rod and observing for 1 min the swimming activity of each organism.

Observed behaviors were identified previously and categorized as (A) ES: Erratic swimming (swimming in circles); (B) LOR: Loss of reflex (delayed response after stimulation); (C) LOB: Loss of balance (zigzag swimming); (D) RS: Regular swimming (Reyes et al. 2003; Agostini et al. 2010). The observer was blind with respect to the treatment combination the boxes belonged to.

Development of tadpoles

Growth assessed by measuring body length and weight of the tadpoles. The length of the body (LB) measured with a digital caliper (0.01 mm precision) after 24 h exposure with four measurements in total. The average weight measured in the wet weight of the tadpoles, determined with an analytical balance (OHAUS / PAD14 of 0.0001 g precision). The measurements have taken on living organisms. To minimize stress, tadpoles were submerged in water during staging and measurement, except to take the weight mass.

Anatomical anomalies

After fixation, the tadpole's external morphology examined with a binocular stereoscopic microscope (Arcano, China, Magnification 2×–4×). Tadpoles were stained with Alcian Blue for cartilage visualization and cleared according to Wassersug (1976). Their branchial skeletons were then examined with a binocular microscope and photographed with a digital camera (Nikon D80, Japan and normal lens 50 mm Sigma, Japan).

Data analyses

The results were recorded as cumulative mortality and expressed as the surviving proportion. The normality of the distribution of the data was checked by means of the Kolmogorov-Smirnov test and the homogeneity of variance by the Levene median test (Zar 1999).The LC_{50} value determined by the PROBIT Analysis (Finney 1952). Biological data (survival, body length, body weight) were statistically analyzed using the Mann–Whitney test. A level of probability below 0.05 was considered to be significant.

The water quality index (WQI) determined following Dinius (1972). Finally, the level of acute effects not observed (LAENO) was determined (highest concentration for which the recorded mortality is 10% or less).

Analyses were performed using the SPSS software (Version 12.0) and package in 'R′ version 3.0 (Team 2015).

Results

The water quality index revealed a "bad" type of pollution (1) for the raw winery effluent, WQI = 30.6, and "regular" for the treated effluent WQI = 51.2. The value of WQI obtained for raw effluent indicated that its quality is not adequate for irrigation without first performing a treatment, unacceptable for fishing with aquatic life limited and danger for contact. Contrary, the values of WQI for treated effluent is much encouraging, indicate that its quality is adequate for irrigation and all industry without before treatment and only uncertain for fishing. According to the results at 24 h, the toxicity of the diluted raw effluent was higher than that of the treated effluent. The physicochemical parameters of the winery effluent samples used in the present work summarized in Table 1.

Table 2 Acute toxicity (LC_{50}) of the winery effluent (raw and treated) to *R. arenarum* larvae

Exposure time (h)	Raw effluent			Treated effluent		
	LC_{50} (%)	LL–UL	df	LC_{50}(%)	LL–UL	df
24	54.90	50.20-59.40	98	90.00	76.20-98.70	98
48	39.20	27.40-50.70	98	86.10	70.90-95.30	98
72	36.10	25.40-46.30	98	86.10	71.50-90.80	98
96	35.50	25.90-44.70	98	86.00	70.90-89.90	98

gl degrees of freedom, *LL-UL* lower limit-upper limit with 95% confidence intervals

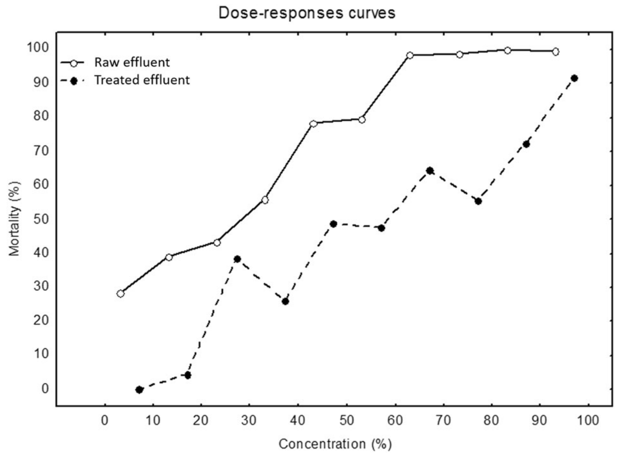


Fig. 2 Dose-responses curves of tadpole *R. arenarum* for raw and treated effluent concentrations

Survival

The results of the PROBIT analysis for raw and treated effluents are presented in Table 2; showing a harmful effect to the effluent even after having undergone symbiotic treatment. The highest LC_{50} was obtained for the raw effluent with a value of 54.5% at 24 h whereas the treated effluent had a value of 90% and remained relatively constant.

The longest survival after the experimental phase (96 h) obtained in the control (100 %), followed by the tadpoles in the treated effluent ($\bar{X} = 2.38 \pm 2.03$), and finally the raw effluent ($\bar{X} = 1 \pm 1.56$). Survival decreased with the increase in concentration and with the passing of hours, reaching zero survivors between 6 and 12 h at concentrations of 70, 80, 90 and 100% of the raw effluent (Fig. 2). Survival decreased exponentially from the start of the bioassay until the 30 h, after this period, it remained relatively constant until completely stabilized 40 h after the start of the experiment (Fig. 3).

Significant differences were found in larval mortality between raw effluent vs control ($U = 11760$, $p = 0.0001$, $n = 110$), treated effluent vs control ($U = 50318$, $p = 0.0001$, $n = 110$) and raw effluent vs treated effluent ($U = 33294$, $p = 0.012$, $n = 100$). LC_{50} was markedly lower for all hours, reaching a value of 35.5% for the raw effluent at 96 h (Table 2).

Growth

The tadpoles exposed to the winery effluents (raw and treated) presented a reduction in average body length concerning the control treatment. The average length of the tadpoles was greater in all cases for the control. Significant differences were found between length of tadpoles in raw effluent ($\bar{X} = 12.21 \pm 0.86$) vs control ($\bar{X} = 13.35 \pm 1.04$) ($U = 1639.5$, $p = 0.01$, $n = 55$), and between treated effluent ($\bar{X} = 12.95 \pm 1.67$) vs control ($U = 2832$, $p = 0.006$, $n = 55$). Regarding the weight of the tadpoles subjected to the treatments (raw effluent-treated effluent), they showed a reduction compared to the control. The highest average weight values for all hours obtained in the control ($\bar{X} = 2.5 \pm 0.5$), followed by the treated effluent ($\bar{X} = 1.95 \pm 0.6$), with the lowest average weights corresponding to the raw effluent ($\bar{X} = 1.79 \pm 0.75$) (Fig. 4, Table 3). However, no significant differences in weight were found between tadpoles in raw effluent vs treated effluent, and vs control.

Behavior

All effects on larval behavior resulted in a higher degree of injury with an increase in concentration. The three LOB, LOR and ES behaviors were observed in the two treatments, raw and treated effluents, in different percentages. The most observed behavior was LOR, followed by ES. At 96 h of testing, 97% of the surviving individuals in the raw effluent and 95% in the treated effluent had LOR (Table 4).

Abnormalities

The alterations detected after 96 h of exposure to the effluent can be seen in Fig. 5. These included anomalies in the axial axis in different degrees and epithelial tissue destruction.

Tadpoles exposed to different effluent concentrations showed varying degrees of notochord collapses compared

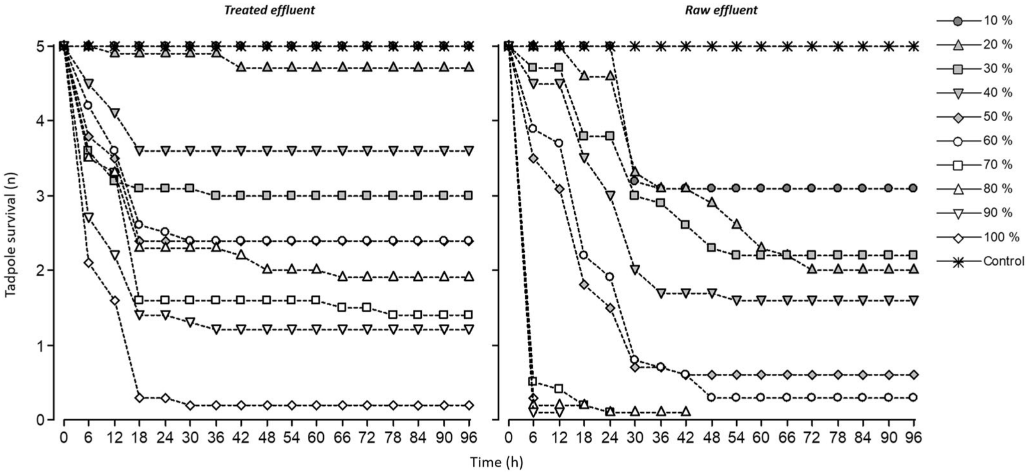


Fig. 3 Concentration–response relationship for *R. arenarum* tadpoles continuously exposed to raw effluent **a** and treated effluent **b** describing the rate of survival from the beginning of the exposure up the finish. * Significantly different with respect to control group ($P < 0.05$)

to those of the control, which had a uniform shape. The number of individuals with scoliosis (collapse of the notochord) increased with an increase in concentration of the effluent. Of the total tadpoles exposed, 23.4% had malformations in the raw effluent and only 8.4% in the treated effluent. Of the total malformed tadpoles corresponding to the raw effluent, 25% have exposed to concentrations of 100%. In the group exposed to the treated effluent, 24% have exposed to 100% concentrations (Fig. 6). In all cases, the malformation observed is a ventro-dorsal curvature, known as kyphosis (Yaman and Dalbayrak 2014).

Discussion

The continuous growth of industrial activity and lacks of control for environmental protection, contribute to a progressive alteration of the environment (Omer 2008). Unlike isolated toxins, changes in water quality have received little attention regarding their adverse effects on amphibian tadpoles, despite being a constant element in cities and towns (Hatch and Blaustein 2000; Ferrari et al. 2005; Peltzer et al. 2008). Our results show that the survival and health status of tadpoles of *R. arenarum* would be affected by the physicochemical characteristics of viticulture effluents.

Survival

The tadpoles of *R. arenarum* showed little resistance to raw or treated effluents in high concentrations. At the lowest concentrations, survived individuals showed signs of sublethal toxicity. Similar results on mortality were found by Goswami et al. (2013) when evaluating the effect of urban effluents on tadpoles of *D. melanostictus*. The LC_{50} values obtained were very low and were directly related to time; the highest mortality has found during the first hours of exposure to the effluent. The values obtained for survival and LC_{50} are comparable to those obtained by Ferrari et al. (2005) on the effect on *R. arenarum* of receiving water from urban effluents, having a clear correspondence with the concentration of the effluent, since its dilution resulted in a significant toxicity reduction in the samples.

The low content of DO, high values of BOD and COD, and high nitrate concentrations found in wine effluents could be among the main causes of the high mortality of tadpoles of *R. arenarum*. The impact is remarkable on anurans given their high oxygen consumption throughout metamorphosis (Smith 1997). States of hypoxia or anoxia result in a slowing of the physiological and metamorphic changes necessary for the development of tadpoles (Burggren and Mwalukoma 1983). Smith (1997) found that they cause decreased activity, vigor, and deformities in bullfrog tadpoles. Costa (1967) found that swimming increases in an excessive way, a behavior associated with improving water oxygenation.

The concentrations of heavy metals were higher for the raw effluent. In our case, we found two heavy metals, Cd and Cr. The concentrations of Cd and Cr in treated and raw effluents exceed the regulatory values for irrigation and drainage systems established by Decree 2 107/2006 for the province of San Juan. The values obtained are similar to those reported by Vivas Agrafojo et al. (2008), and lower than those obtained for effluents from food industries by Pellón et al. (2003). Heavy metals cause, in most cases, mortality of organisms. Their presence can lead to biochemical imbalances and glandular damage, producing an abnormal development (Goswami et al. 2013). In the aqueous medium, chromium can be found as Cr (III) and Cr

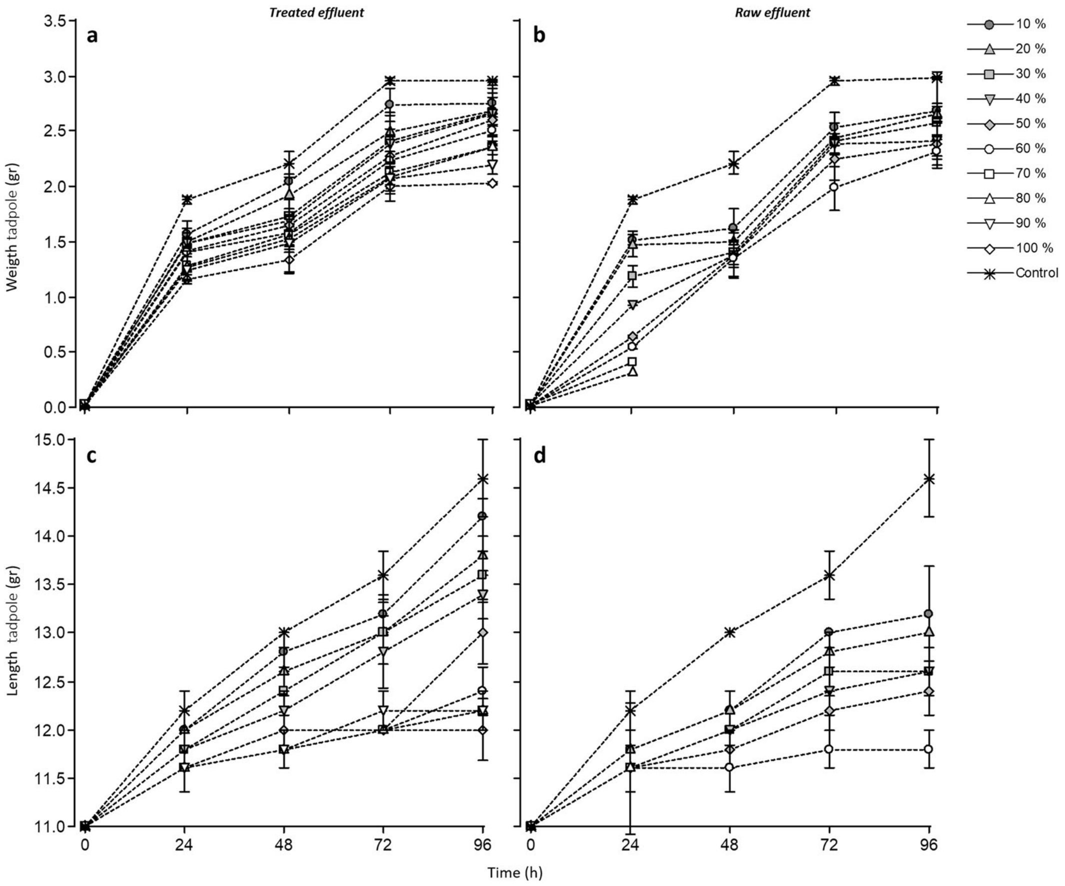


Fig. 4 Effluents concentration response curves of body weight and length of the tadpoles during acute exposure. **a** Body weight treated effluent, **b** body weight raw effluent, **c** body length treated effluent, and **d** body length raw effluent

Table 3 Gravimetric data of *Rhinella arenarum* tadpoles exposed to the winery effluents (raw and treated) and control every 24 h

Treatment	Hours	Weight larvae (g)	SD
Raw	24	0.5146	0.4120
	48	0.9439	0.3123
	72	1.7893	0.4039
	96	1.9773	0.4823
Treated	24	0.8789	0.2445
	48	1.1520	0.3452
	72	1.7926	0.4033
	96	2.0040	0.3654
Control	24	1.3740	0.0705
	48	1.7100	0.2288
	72	2.4520	0.0690
	96	2.4820	0.0204

All data are expressed as the mean and standard deviation (SD)

Table 4 Behaviors dateof *Rhinella arenarum* tadpoles exposed to the winery effluent (raw and treated) every 24 h

Exposure time (h)	Behaviors		
	LOB (%)	LOR (%)	ES(%)
Raw effluent			
24	02	80	18
48	00	94	06
72	00	97	03
96	00	97	03
Treated effluent			
24	14	65	21
48	08	81	11
72	06	86	08
96	00	95	05

LOB loss of balance, *LOR* loss of reflex, *ES* erratic swimming

(VI). Cr(III) and, in low concentrations, it is essential to maintain fundamental processes of life (metabolism of glucose, lipids and proteins) (Alvarado-Gámez et al. 2002). However, it is toxic in high concentrations (Pawlisz et al. 1997; Natale et al. 2000). On the other hand, Cr (VI) is not known for exerting beneficial actions and is defined as toxic, being attributed mutagenic and carcinogenic properties (Rai and Mehrotra 2008; Wan Ngah et al. 2006). As for

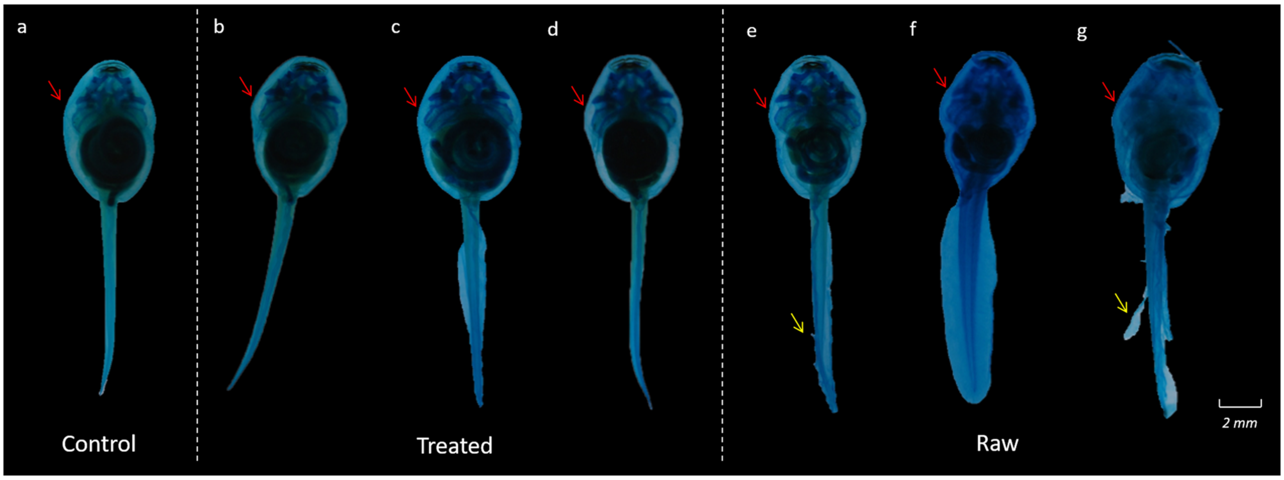
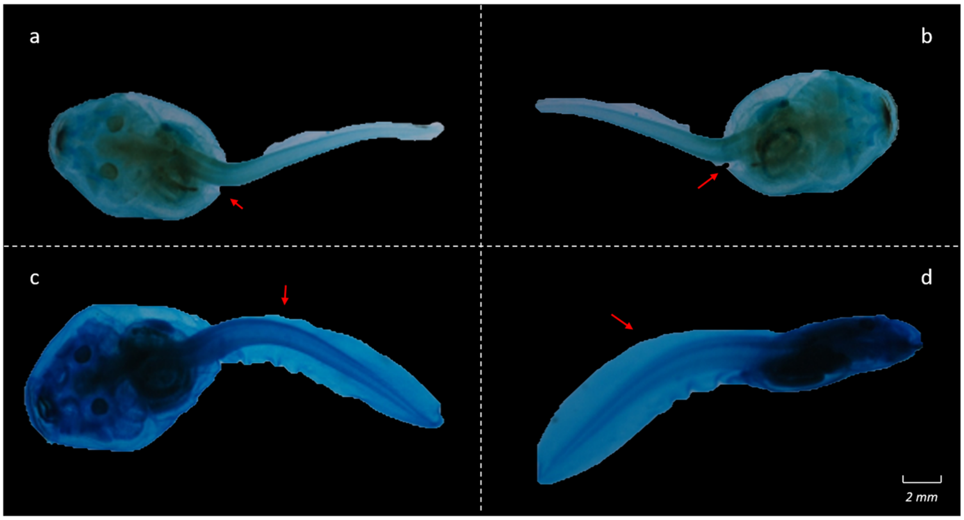


Fig. 5 Lateral full-body views of tadpoles *R. arenarum* exposed to the winery effluent, diaphanized and stained with Alcian blue. Control tadpoles **a**, treated effluent at 10% **b**, 50% **c** and 100% **d**; and 10% raw effluent **e**, 50% **f**, and 100% **g**. Red arrows indicate branchial apparatus; yellow arrows indicate destruction of tissues. Scale bar 2 mm

Fig. 6 Tadpoles with different degrees of notochordal curvature (Kiphosis) after exposed 24 h to 100 % of raw effluent **a**, **b** and 48 h to 50 % **c**, **d** to raw effluents. Scale bar 2 mm



Cd, it is characterized by being very toxic in small concentrations (James and Little 2003; Gross et al. 2009). Gross et al. (2009) found that Cd concentrations of 0.00025 mg/l increased larval growth, but concentrations of 0.2 mg/l increased their mortality. James and Little (2003) also observed its lethal effect on *B. americanus* tadpoles at concentrations of 0.54 mg/l. Cd has shown to have drastic effects on the growth and development of tadpoles (Read and Tyler 1994). The cumulative effect of Cd on tissues and organs is the main problem caused by this heavy metal (Pérez-Coll and Herkovits 1996).

Changes in the pH of water can be generated by various agricultural, industrial and domestic substances (Muñoz-Escobar and Palacio-Baena 2010). The pH values found for two effluents (raw and treated) were within the regulatory values for irrigation water set by the Hydraulics Department; and within the pH range of 4.6-8 established for winery effluents by Monge and Gutiérrez-Barquín (2001); pH 3.9-7.9 by Prodanov and Cobo Reuters (2004); and pH 4.2-7.8 by Oliva (2007). However, Henao Muñoz and Bernal Bautista (2011) observed a delay in the development of embryos and erratic nests in tadpoles exposed to pH lower than regulated for irrigation ($7.0 < pH > 7.5$), and detected detachment of their external membranes and sudden death. For his part, Rios-López (2008) found that conductivity values of 800 ms/cm, less than do those established by the current regulations (1200–2000 ms/cm), decreased the survival of tadpoles of *B. marinus* and *L. arbritaris*.

Growth

This study provided evidence that *R. arenarum* tadpoles exposed to raw and treated effluents are affected in their development, experiencing a reduction in their body size. Their reduction in weight and length could be associated with an increase in metabolic cost due to cellular detoxification and depuration processes, to the reduced activity of some enzymes (Vallee and Ulmer 1972), and to a greater demand for the synthesis of amino acids (Nishisaka and Kishimoto 1994). This reduction in weight and length has a potentially negative effect on anurans versus predation since large tadpoles will have a greater chance of surviving and reaching full adulthood than small ones (Collins 1979; Smith 1987). During stress, the major amount of energy is used for defensive physiological mechanisms and maintenance of homeostasis that is an energetically expensive process, and less energy is left for investment in life history traits (mass, growth) (Costantini 2014).

On the other hand, those surviving tadpoles will be able to undergo an early metamorphosis and to prolong it before reaching their threshold size, resulting in individuals with reduced body sizes and/or deformities (Harris et al. 2000; Boone and Semlitsch 2002). Long periods of metamorphosis or small sized adults can have many consequences in nature. Small larval sizes on reaching metamorphosis affect individual reproduction, survival, immunocompetence and the ability to escape predators and defend territory (Carey et al. 1999; Hayes et al. 2006; Shenoy et al. 2009). In this way, the population would be negatively affected by a low recruitment of juvenile individuals and/or by the presence of adults affected in their performance (Werner 1986; Bridges and Boone 2003).

The presence of nitrates and heavy metals (Cd-Cr) in values above the levels regulated for viticulture effluents with high conductivity values could also explain the reductions in size observed in *R. arenarum* tadpoles. Even at very low concentrations, all of them negatively affect growth and development time (Gallo-Delgado et al. 2006; Muñoz-Escobar and Palacio-Baena 2010; Smith et al. 2006). In our study, nitrate concentrations in the two treatments were within the range established by Prodanov and Cobo Reuters (2004) for effluents from cellars (13–220 mg/l), lower than those recorded by Monge and Gutiérrez-Barquín (2001) (61 mg/l), and did not exceed those established by the Hydraulics Department. However, Rouse et al. (1999) found that lakes with nitrate concentrations of 16.8 mg/l had sublethal and lethal effects on amphibians. Nitrate alters the growth and development of amphibian tadpoles (Berger 1989; Jofre and Karasov 1999). Among the most common adverse effects are reduced feeding and mobility, doubled tails, body swelling and deformities (Berger 1989). It can also lower pH and oxygen levels (Tattersall and Boutilier 1999).

Sublethal effects of acidity on embryos and tadpoles of amphibians have also been reported to affect their embryonic development, growth, foraging ability and avoidance of predators (Pierce 1985). Regarding conductivity, the values in wine effluents did not exceed those established by the current regulations to be turned over for irrigation, natural courses and drains. These values are within the range reported by Oliva (2007) (600–2000 ms/cm). However, Chinathamby et al. (2006) found growth retardation and abnormal behaviors when exposing tadpoles of *L. ewingi* to concentrations of 500–800–1000 ms/cm.

Behavior

Knowledge about alterations in the behavior of tadpoles of *R. arenarum* against toxic components is scarce, the observed behaviors are important as sublethal signs because they give us more rapid information about the effect of a toxic substance, allowing us to prevent the spread of the toxic agent to organisms where its effect may still be masked.

Studies on sublethal effects on larval behavior are scarce and, in the works referred to, they are part of the final observations (Reyes et al. 2003; Álvarez-Colombo et al. 2011). In our study, in both treatments, we found three types of behavior: erratic swimming, loss of reflexes and loss of balance. The "LOR" behavior increased with the increase in exposure time and with increased concentrations, however, "LOB" and "ES" decreased with these increases. These behaviors had already been observed by exposing tadpoles of the Cuban frog *O. septentrionalis* to the herbicide glyphosate (Reyes et al. 2003). Similar behaviors observed in bullfrog tadpoles, *L. catesbeianus*, exposed to potassium permanganate (Álvarez-Colombo et al. 2011) and in tadpoles of *D. bogerti* exposed to mercury chloride (Muñoz-Escobar and Palacio-Baena 2010).

Abel and Skidmore (1975), and Jonsson and Toledo (1993), showed similar alterations of swimming behavior in fish. Some of these alterations can be explained by acclimatization to the toxic agent or by energy expenditure, which involves metabolism (Rondón-Barragán et al. 2007).

All of the components found in viticulture effluents have been shown to have some effect on the behavior of tadpoles and could explain the ethological characteristics observed in the common toad *R. arenarum*.

All alterations in the observed behaviors are significant because of their importance to the conservation of the ability to escape, which determines a great part of the survival of this species in the natural environment (Rondón-Barragán et al. 2007). Thus, tadpoles with behaviors such as LOB, LOR, and ES show a reduction in their ability to escape, to search for food or move to more suitable areas in the face of adverse conditions affecting survival of the population.

Phenotypic abnormalities

Skin

Although information on the effects of toxic elements on the tissue of amphibians is limited, some studies have shown their high susceptibility to foreign substances (Rondón-Barragán et al. 2007; Álvarez-Colombo et al. 2011). Anurans exposed in a direct way to the aquatic environment; their skin is a complex organ and the main route of exchange of gases for respiration, water, and ions, thus being particularly exposed to the action of toxins (Duellman and Trueb 1994; Natale 2006; Prokić et al. 2016)

The tadpoles exposed to raw and treated effluents showed the destruction of the epidermis in different degrees, depending on the concentration to which they were exposed and the treatment they underwent. Tadpoles exposed to raw and treated effluents at the highest concentrations experienced the most serious effects on their epidermis. Similar effects on skin destruction were observed both by Álvarez and Nicieza (2002) as Prieto et al. (1986), where the damage occurred externally and with irreversible characteristics.

Alterations in the epidermis or the destruction thereof are usually associated with an inflammatory reaction to an irritant (Rondón-Barragán et al. 2007). In those cases in which skin detachment was observed and individuals have resisted the lethal effect of the toxic agent, the damage turned out to be irreversible (Álvarez and Nicieza 2002).

In most cases, toxic agents such as heavy metals, detergents and even pH can be the cause of skin destruction and capillarity. The presence of these compounds in viticulture effluents and of a pH of 6 (raw effluent) and 7 (treated effluent) could explain the damage observed. This damage often leads to a reduction in oxygen diffusion capacity and subsequent collateral effects (Lajmanovich et al. 1998).

Malformations

In this study, the malformation observed in *R. arenarum* tadpoles, due to the effect of the effluent, was scoliosis, and was observed in samples of raw and treated effluents, although in a greater percentage in the former. This malformation coincides with that reported for tadpoles of the crab *C. granulata* exposed to sublethal concentrations (Keselman 2002), and with that of tadpoles of *P. biligonigerus* found by Sandoval (2008), with different degrees of curvature in the notochord. It has also been documented in investigations with heavy metals such as Cd and Cr (Unrine et al. 2004). Scoliosis has been reported in *P. cruciger* tadpoles exposed to agrochemicals such as glyphosate, chlorpyriphos, etc. (Jayawardena et al. 2010) and in tadpoles of anurans exposed to nitrate values of 16.8 mg/l.

The concentrations of nitrates and heavy metals in both effluents (raw and treated) above the regulation could explain the results obtained. Brand et al. (2010) observed malformations in tadpoles exposed to Cd.

Caudal scoliosis adversely affects the swimming of tadpoles (Burke and Michel 2008). Scoliotic tadpoles show slower swim and escape velocities, being more susceptible to predation than tadpoles exhibiting a normal phenotype (Burke and Michel 2008).

It is important, however, to distinguish between acute toxicity from specific pesticides and biological results based on synergistic interactions between mixtures of contaminants (Bridges and Boone 2003; Fagotti et al. 2005; Relyea 2005). In this sense, a clear cause-effect relationship cannot be found for physicochemical characteristic or substance in particular, given that certain compounds are capable of interacting chemically when combined. Klaassen (1996), hypothesized that exposure to mixtures of pesticides can result in synergistic or antagonistic effects for health because the metabolism of one pesticide can affect the metabolism of another one.

Although the toxic effects observed in tadpoles of *R. arenarum* exposed to viticulture effluents could attributed to the synergy between chemical compounds and environmental factors, the effects of cumulative exposure to contaminants and their synergistic interactions with environmental stress factors, natural or anthropic, are uncertain (Fagotti et al. 2005).

Conclusions

Contamination by effluents from wineries could affect the aquatic life of the tadpoles studied, exerting a harmful effect on their behavior, morphology, growth and survival. The symbiotic filtering system used causes a reduction in the effluent pollutant levels; however, it is not sufficient for the effluent to be released into the environment.

Finally, although the physicochemical characteristics are central when analyzing an effluent, they do not by themselves allow characterizing the impact of its release. It is considered that an integrated approach is required for an adequate characterization of the quality of an effluent or a treatment system, using physical-chemical analysis and bioassays in a complementary manner.

Acknowledgements We thank G. Herrera and H. Merenda for their collaboration in the field work. To F. Jofre Barud for her help with the statistical analyzes. Special thanks to D. Barrasso for help in the diaphanized of tadpoles. We thank the provincial fauna office of San Juan for permission to conduct our research (SA y DS n° 1300-4736-2011). To all the technical staff of the Institute of Chemistry of the FI-UNSJ for their collaboration in the laboratory. Special thanks to Anna Murakozy for the correction of the English and suggestions that improved this work. Finally, to the anonymous reviewers for helping us greatly improve this effort.

Funding This work was funded by a CICITCA UNSJ project (I1010 Director: Miguel A. Navas).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. All members at the time of the investigation belonged to the Faculty of Exact, Physical, and Natural Sciences of the National University of San Juan. The corresponding author was the beneficiary of the CICITCA Scholarship.

Ethical approval Although at the time of the investigation there was no ethical committee at the National University of San Juan, all international, national and institutional guidelines applicable to the care and use of animals have followed.

Informed consent All authors declare to be participants in the research and their respective future consequences.

References

Abel P, Skidmore J (1975) Toxic effects of an anionic detergent on the gills of rainbow trout. Water Res 9(8):759–765. https://doi.org/10.1016/0043-1354(75)90068-8

Agostini M, Natale G, Ronco A (2010) Lethal and sublethal effects of cypermethrin to *Hypsiboas pulchellus* tadpoles. Ecotoxicol 19 (8):1545–1550. https://doi.org/10.1007/s10646-010-0539-3

Alvarado-Gámez A, Blanco-Sáenz R, Mora-Morales E (2002) El cromo como elemento esencial en los humanos. Rev Costa Cienc Med 23(1–2):55–68. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0253-29482002000100006&lng=en&nrm=iso&tlng=es

Álvarez D, Nicieza A (2002) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecol 131(2):186–195. https://doi.org/10.1007/s00442-002-0876-x

Álvarez-Colombo G, Dato C, Macchi G et al. (2011) Distribución y comportamiento de las larvas de la merluza argentina: evidencias de un mecanismo biofísico de autorreclutamiento en aguas de la plataforma continental norte de la Patagonia. Cienc Mar 37(4 B):633–657. https://doi.org/10.7773/cm.v37i4B.1777

American Public Health Association (1992) Standard methods for the examination of water and wastewater. In: Greenberg A, Clesceri L, Eaton A (Eds), Standard Methods. United States of America: American Public Health Association, American Water Works Association, W*ater Environ. Fed.*

Babini M, Bionda C, Salas N et al. (2016) Adverse effect of agroecosystem pond water on biological endpoints of common toad (*Rhinella arenarum*) tadpoles. *Environ Monit Assess* 188(8). https://doi.org/10.1007/s10661-016-5473-2

Baker S, Herrchen M, Hund-Rinke K et al. (2003) Underlying issues including approaches and information needs in risk assessment. Ecotoxicol Environ Saf 56(1):6–19. https://doi.org/10.1016/S0147-6513(03)00046-0

Bélanger-Deschênes S, Couture P, Campbell P et al. (2013) Evolutionary change driven by metal exposure as revealed by coding SNP genome scan in wild yellow perch (*Perca flavescens*). Ecotoxicol 22(5):938–957. https://doi.org/10.1007/s10646-013-1083-8

Berger L (1989) Disappearance of amphibian larvae in the agricultural landscape. Ecol Int Bull 17:65–73

Bishop C, Mahony N, Struger J et al. (1999) Anuran development, density and diversity in relation to agricultural activity in the Holland River Watershed, Ontario, Canada (1990–1992). Environ Monit Assess 57:21–43. https://doi.org/10.1023/A:1005988611661

Bishop C, Pettit K (1992) Declines in Canadian amphibian populations. Canadian Wildlife Service Occasional Papers, Minnesota, Canada

Blaustein A, Wake D (1990) Declining amphibian populations: A global phenomenon? Trends Ecol Evol 5(7):203–204. https://doi.org/10.1016/0169-5347(90)90129-2

Boone M, Semlitsch R (2002) Interactions of an insecticide with competition and pond drying in amphibian communities. Ecol Appl 12(1):307–316. https://doi.org/10.1890/1051-0761(2002)012[0307:IOAIWC]2.0.CO;2

Brand A, Snodgrass J, Gallagher M et al. (2010) Lethal and sublethal effects of embryonic and larval exposure of *Hyla versicolor* to stormwater pond sediments. Arch Environ Contam Toxicol 58 (2):325–331. https://doi.org/10.1007/s00244-009-9373-0

Bridges C, Boone M (2003) The interactive effects of UV-B and insecticide exposure on tadpole survival, growth and development. Biol Conser 113(1):49–54. https://doi.org/10.1016/S0006-3207(02)00348-8

Brown R, McClelland N, Deininger R et al. (1970) A water quality index- do we dare? Water Sew Works 117(10):339–343

Burggren W, Mwalukoma A (1983) Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs (*Rana catesbeiana*) I. Morphological responses of lungs, skin and gills. J Exp Biol 105(1):191–203

Burke S, Michel M (2008) The effects of caudal scoliosis on swimming potential and survivability of wood frog (*Rana sylvatica*) tadpoles. BIOS 35502: Practicum in Field Biology

Bustamante M, Paredes C, Moral R et al. (2005) Uses of winery and distillery effluents in agriculture: characterization of nutrient and hazardous components. Water Sci Technol 51(1):145–151. https://doi.org/10.2166/wst.2005.0018

Cabrera A (1971) Fitogeografía de la república Argentina. Bol Soc Argen Bot XIV(1–2):1–50

Carey C, Cohen N, Rollins-Smith L (1999) Amphibian declines: An immunological perspective. Dev Comp Immunol 23(6):459–472. https://doi.org/10.1016/S0145-305X(99)00028-2

Chinathamby K, Reina R, Bailey P et al. (2006) Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog. Litoria ewingii Aust J Zool 54:97–105. https://doi.org/10.1071/ZO06006

Clesceri L, Greenberg A, Trussell R (1992) Métodos normalizados para el análisis de aguas potables y residuales. (Díaz de Santos, Ed.) (10° en esp). Madrid. https://doi.org/10.1007/s13398-014-0173-7.2

Collins J (1979) Intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog. Rana Catesbeiana Ecol 60(4):738–749. https://doi.org/10.2307/1936611

Costa H (1967) Avoidance of anoxic water by tadpoles of *Rana temporaria*. Hydrobiol 30(3–4):374–384. https://doi.org/10.1007/BF00964023

Costantini D (2014) Oxidative stress and hormesis in evolutionary ecology and physiology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-54663-1

Crites R, Tchobanoglous G (2000). Sistemas de manejo de aguas residuales: para núcleos pequeños y descentralizados. MCGRAW-HILL, Ed., Colombia

Dinius S (1972) Social accounting system for evaluating water resources. Water Resour Res 8(5):1159–1177. https://doi.org/10.1029/WR008i005p01159

Duellman W, Trueb L (1994). Amphibians: biology of amphibians. J. H. U. Press, Ed., Vol. xxi, Baltimore

Fagotti A, Morosi L, Di Rosa I et al. (2005) Bioaccumulation of organochlorine pesticides in frogs of the *Rana esculenta* complex in central Italy. Amphib -Reptil 26(1):93–104. https://doi.org/10.1163/1568538053693297

Fernández Portela J (2013) La evolución reciente del sector vitivinícola internacional. GeoGraphos. Rev Dig Estud Geog Cienc Soc 4:173–194. https://doi.org/10.14198/GEOGRA2013.4.39

Ferrari L, De La Torre F, Demichelis S et al. (2005) Ecotoxicological assessment for receiving waters with the premetamorphic tadpoles acute assay. Chemosphere 59(4):567–575. https://doi.org/10.1016/j.chemosphere.2005.01.045

Finney D (1952) Probit analysis: A statistical treatment of the sigmoid response curve (Second Edn). Camb. Univ. Press, Great Britain

Gallo-Delgado S, Palacio-Baena J, Gutiérrez C (2006) Efectos del insecticida clorpirifos sobre la tasa de crecimiento y la metamorfosis de *Smilisca phaeota* (Cope, 1862) (Anura: Hylidae) Actual Biol 28(84):51–58.http://scienti.colciencias.gov.co:8084/publindex/docs/articulos/0304-3584/3/87.pdf

García L, Pérez J, Riquelme M et al. (2008) Depuración simbiótica: una nueva tecnología biológica para la depuración de aguas residuales del sector de conservas vegetales. Tecnol Agua 303 (28):48–54. Retrieved from 0211-8173

González G, Navarro A, Borbón L et al. (2003) Caracterización química de efluentes de bodegas, Mendoza (Argentina). Rev Fac Cienc Agrar 1(35):99–105

Gosner K (1960) A simplified table for staging anuran embryos larvae with notes on identification. Herpetol 16(3):183–190. https://doi.org/10.2307/3890061

Goswami G, Sengupta S, Baruah B (2013) Effect of pollution on growth and development of some of the larval stages of *Duttaphrynus melanostictus* (Schneider 1799). Clar Int Multidiscip J 2(2):38–41

Gross J, Johnson P, Prahl L et al. (2009) Critical period of sensitivity for effects of cadmium on frog growth and development. Environ Toxicol Chem 28(6):1227–1232. https://doi.org/10.1897/08-205.1

Harris M, Chora L, Bishop C et al. (2000) Species- and age-related differences in susceptibility to pesticide exposure for two amphibians, *Rana pipiens*, and *Bufo americanus*. Bull Environ Contam Toxicol 64(2):263–270. https://doi.org/10.1007/s001289910039

Hatch A, Blaustein A (2000) Combined effects of UV-B, nitrate, and low pH reduce the survival and activity level of larval cascades frogs (*Rana cascadae*). Arch Environ Contam Toxicol 39 (4):494–499. https://doi.org/10.1007/s002440010132

Hayes T, Case P, Chui S et al. (2006) Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? Environ Health Perspect, 114(S- 1:40–50. https://doi.org/10.1289/ehp.8051

Henao Muñoz L, Bernal Bautista M (2011) Tolerancia al pH en embriones y renacuajos de cuatro especies de anuros colombianos. Rev Acad Colomb Cienc 35(134):105–110

James S, Little E (2003) The effects of chronic cadmium exposure on American toad (*Bufo americanus*) tadpoles. Environ Toxicol Chem 22(2):377–380. https://doi.org/10.1897/1551-5028(2003)022<0377:TEOCCE>2.0.CO;2

Jayawardena U, Rajakaruna R, Navaratne A (2010) Toxicity of agrochemicals to common hourglass tree frog (*Polypedates cruciger*) in acute and chronic exposure International J Agr Biol 12 (5):641–648

Ji K, Kim Y, Oh S et al. (2008) Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (*Daphnia magna* and *Moina macrocopa*) and fish (O*ryzias latipes*). Environ Toxicol Chem 27(10):2159–2168. https://doi.org/10.1897/07-523.1

Jofre M, Karasov W (1999) Direct effect of ammonia on three species of north American anuran amphibians. Environ Toxicol Chem 18 (8):1806–1812. https://doi.org/10.1897/1551-5028(1999)018<1806:DEOAOT>2.3.CO;2

Jonsson C, Toledo M (1993) Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Arch Environ Contam Toxicol 24(2):151–155. https://doi.org/10.1007/BF01141341

Keselman D (2002) Thesis. Efectos del plomo sobre la embriogénesis y la eclosión larval de *Chasmagnatus granulata* (Decapoda, Brachyura). Universidad de Concepción

Klaassen C (1996). Toxicology: the basic science of poisons. In: Klaassen CD, (ed) NewYork: McGraw-Hil

Lajmanovich R, Izaguirre M, Casco V (1998) Environmental contamination and toxicology alteration of leopard frog (*Rana pipiens*) metamorphosis by the herbicide acetochlor. Arch Environ Contam Toxicol 34(4):364–369. https://doi.org/10.1007/s002449900331

Lajmanovich R, Peltzer P, Martinuzzi C et al. (2018) Acute toxicity of colloidal silicon dioxide nanoparticles on amphibian larvae: emerging environmental concern. Int J Environ Res 12 (3):269–278. https://doi.org/10.1007/s41742-018-0089-8

Malandra L, Wolfaardt G, Zietsman A et al. (2003) Microbiology of a biological contactor for winery wastewater treatment. Water Res 37(17):4125–4134. https://doi.org/10.1016/S0043-1354(03)00339-7

Mitsch W, Gosselink J (2015). Wetlands. Aging (Fifth Edn). John Wiley & Sons, ltd. https://doi.org/10.1017/CBO9781107415324.004

Mitsch W, Gosselink J (2000) The value of wetlands: Importance of scale and landscape setting. Ecol Econ 35(1):25–33. https://doi.org/10.1016/S0921-8009(00)00165-8

Monge A, Gutiérrez-Barquín M (2001) Estrategias de minimización de vertidos en el sector agroalimentario. Aliment Equipo Tecnol 20 (161):95–102

Mosse K, Patti A, Christen E et al. (2011) Review: Winery wastewater quality and treatment options in. Aust Aust J Grape Wine Res 17 (2):111–122. https://doi.org/10.1111/j.1755-0238.2011.00132.x

Muñoz-Escobar E, Palacio-Baena J (2010) Efectos del cloruro de mercurio ($HgCl_2$) sobre la sobrevivencia y crecimiento de renacuajos de *Dendrosophus bogerti* Actual Biol 32(93):189–197. https://aprendeenlinea.udea.edu.co/revistas/index.php/actbio/article/viewFile/13814/12258

Natale G (2006) Thesis: Análisis ecotoxicológico de una comunidad de anuros de la región pampeana: efecto del Cr [VI] sobre embriones y larvas de distintas especies de una taxocomunidad. Universidad Nacional de la Plata

Natale G, Basso N, Ronco A (2000) Effect of Cr(VI) on early life stages of three species of hylid frogs (Amphibia, Anura) from South America. Environ Toxicol 15(5):509–512. https://doi.org/10.1002/1522-7278(2000)15:5<509::AID-TOX21>3.0.CO;2-S

Nimmo D, Boraas M (1982) Mysids in toxicity testing -a review. In: Morgan M(ed.) Ecology of Mysidacea (Development. Dr W. Junk Publishers, Netherlands pp 171–178. https://doi.org/10.1007/978-94-009-8012-9

Nishisaka N, Kishimoto T (1994) Sensitivity of immature regenerating proximal tubular cells in rabbit kidney to mercuric chloride--a light and electron microscopic analysis. Nihon Jinzo Gakkai Shi 36(4):298–306. https://doi.org/10.14842/jpnjnephrol1959.36.298

Oliva J (2007) Tratamientos de residuos en bodegas. *Virtual Pro Proc Ind*, 1–9. https://www.revistavirtualpro.com/biblioteca/tratamientos-de-residuos-de-bodegas-

Omer A (2008) Energy, environment and sustainable development. *Renew Sustain Energy Rev*. https://doi.org/10.1016/j.rser.2007.05.001

Pawlisz A, Kent R, Schneider U et al. (1997) Canadian water quality guidelines for chromium. Environ Toxicol Water Qual 12 (2):123–183. https://doi.org/10.1002/(SICI)1098-2256(1997)12:2<123::AID-TOX4>3.0.CO;2-A

Pellón A, Benítez F, Frades J et al. (2003) Empleo de microalga *Scenedesmus obliquas* en la eliminación de cromo presente en aguas residuales galvánicas. Rev De Metal 39(1):9–16. https://doi.org/10.3989/revmetalm.2003.v39.i1.312

Peltzer P, Lajmanovich R, Sanchez-Hernandez J et al. (2008) Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Ecotoxicol Environ Saf 70 (1):185–197. https://doi.org/10.1016/j.ecoenv.2007.06.005

Pérez-Coll C, Herkovits J (1996) Stage-dependent uptake of cadmium by *Bufo arenarum* embryos. Bull Environ Contam Toxicol 56 (4):663–669. https://doi.org/10.1007/s001289900097

Pierce B (1985) Acid tolerance in amphibians. BioSci 35(4):239–243. https://doi.org/10.2307/1310132

Power T, Clark K, Harfenist A et al. (1989) A Review and evaluation of the amphibian toxicological literature. Technical Report Series, Canadian Wildlife Service, Canadá

Prieto A, Fajer E, Vinjoy M et al. (1986) Monogeneos parásitos de las especies de peces exóticas. Ministerio de la Industria Pesquera (MIP). Vol. 11 (26). Cuba

Prodanov M, Cobo Reuters R (2004) Impacto ambiental de la industria vinícola (I). Aliment Equipos Tecnol 186:97–100

Prokić M, Borković-Mitić S, Krizmanić I et al. (2016) Bioaccumulation and effects of metals on oxidative stress and neurotoxicity parameters in the frogs from the *Pelophylax esculentus* complex. Ecotoxicol 25(8):1531–1542. https://doi.org/10.1007/s10646-016-1707-x

Rai V, Mehrotra S (2008) Chromium-induced changes in ultramorphology and secondary metabolites of *Phyllanthus amarus* Schum & Thonn. - An hepatoprotective plant. Environ Monit Assess 147(1–3):307–315. https://doi.org/10.1007/s10661-007-0122-4

Read J, Tyler M (1994) Natural levels of abnormalities in the trilling frog (*Neobactrachus centralis*) at the Olympic Dam mine. Bull Environ Contam Toxicol 53(1):25–31. https://doi.org/10.1007/BF00205134

Relyea R (2005) The lethal impacts of Roundup on aquatic and terrestrial amphibians. Ecol Appl 15(4):1118–1124. https://doi.org/10.1890/04-1291

Reyes M, Hondal O, Hernández J (2003) Toxicidad aguda del herbicida químico Glifosan en larvas de la rana cubana: *Osteopilus septentrionalis* Rev Toxicol En Línea 16:34–45. https://www.sertox.com.ar/modules.php?name=Content&pa=showpage&pid=523

Rios-López N (2008) Effects of increased salinity on tadpoles of two anurans from a Caribbean coastal wetland in relation to their natural abundance. Amphib –Reptilia 29(1):7–18. https://doi.org/10.1163/156853808783431451

Rodríguez R, Echegaray M, Palacios C et al (2013) Alternativa innovativa de un sistema de tratamiento de efluentes vinícolas: modelado matemático. Rev Cient Ing Des 31(1):105–119.http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=91534815&lang=es&site=ehost-live

Rondón-Barragán I, Ramírez-Duarte W, Eslava-Mocha P (2007) Evaluación de los efectos tóxicos y concentración letal 50 del surfactante Cosmoflux® 411F sobre juveniles de cachama blanca (*Piaractus brachypomus*) Rev Colomb Cienc Pecu 20 (4):431–446. http://dialnet.unirioja.es/descarga/articulo/2544475.pdf

Rouse J, Bishop C, Struger J (1999) Nitrogen pollution: an assessment of its threat to amphibian survival. Environ Health Perspect 107 (10):799–803. https://doi.org/10.1289/ehp.99107799

Sanabria E, Quiroga L, Acosta J (2007) Sitios de ovoposición y esfuerzo reproductivo en *Chaunus arenarum* (Anura: Bufonidae) en el desierto del Monte, Argentina Rev Esp Herpet 21:49–53. https://dialnet.unirioja.es/servlet/articulo?codigo=2877435

Sandoval M (2008) Efectos producidos por el biocida Carbofurán® sobre larvas de *Physalaemus biligonigerus*. Resultados preliminares. *Rev Anat Comp* 3(41):1–3

Scott G, Crunkilton R (2000) Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*, *Ceriodaphnia dubia*, and *Daphnia magna*). Environ Toxicol Chem 19(12):2918–2922. https://doi.org/10.1002/etc.5620191211

Shenoy K, Cunningham B, Renfroe J et al. (2009) Growth and survival of northern leopard frog (*Rana pipiens*) tadpoles exposed to two common pesticides. Environ Toxicol Chem 28 (7):1469–1474. https://doi.org/10.1897/08-306.1

Smith D (1987) Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecol 68(2):344–350. https://doi.org/10.2307/1939265

Smith G (1997) The effects of aeration on amphibian larval growth: an experiment with bullfrog tadpoles. Transactions of the Nebraska Academy of Sciences, 24, 63–66. http://digitalcommons.unl.edu/tnas%5Cn or http://digitalcommons.unl.edu/tnas/74

Smith G, Temple K, Dingfelder H et al. (2006) Effects of nitrate on the interactions of the tadpoles of two ranids (*Rana clamitans* and *R. catesbeiana*). Aquat Ecol 40(1):125–130. https://doi.org/10.1007/s10452-005-9015-1

Sponza D (2003) Application of toxicity tests into discharges of the pulp-paper industry in Turkey. Ecotoxicol Environ Saf 54 (1):74–86. https://doi.org/10.1016/S0147-6513(02)00024-6

Tattersall G, Boutilier R (1999) Behavioral oxy-regulation by cold-submerged frogs in heterogeneous oxygen environments. Can J Zool 77(6):843–850. https://doi.org/10.1139/z99-049

Team R Core (2015). R: A language and environment for statistical computing. R Foundation for StatisticalComputing, Vienna, Austria. URL http://www.R-project.org/

United States Environmental Protection Agency (2002) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4 edition. United States Environmental Protection Agency, United States of America. https://doi.org/http://www.dep.state.fl.us/water/wastewater/docs/ctf.pdf

Unrine J, Jagoe C, Hopkins W et al. (2004) Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environ Toxicol Chem 23 (12):2964–2970. https://doi.org/10.1897/03-696.1

Vallee B, Ulmer D (1972) Biochemical effects of mercury, cadmium, and lead. Annu Rev Biochem 41(1):91–128. https://doi.org/10.1146/annurev.bi.41.070172.000515

Vighi M, Altenburger R, Arrhenius A et al. (2003) Water quality objectives for mixtures of toxic chemicals: problems and perspectives. Ecotoxicol Environ Saf 54:139–150. https://doi.org/10.1016/S0147-6513(02)00047-7

Vivas Agrafojo D, Fernández Sánchez M, Hernández Pacheco M et al. (2008) Estudio sobre la contaminación real producida por bodegas y almazaras en diez municipios de Extremadura (Fundación Biodiversidad). Extremadura

Wake D (1991) Declining amphibian populations. Sci 253 (5022):860–860. https://doi.org/10.1126/science.253.5022.860

Wan Ngah W, Kamari A, Fatinathan S et al. (2006) Adsorption of chromium from aqueous solution using chitosan beads. Adsorpt 12(4):249–257. https://doi.org/10.1007/s10450-006-0501-0

Wassersug R (1976) A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. Biotechn

Histochem 51(2):131–134. https://doi.org/10.3109/10520297609116684

Werner E (1986) Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. Am Nat 128 (3):319–341. https://doi.org/10.1086/284565

Yaman O, Dalbayrak S (2014) Kyphosis and review of the literature. Türk Neurosurg 24(4):455–465. https://doi.org/10.5137/1019-5149.JTN.8940-13.0

Zar J (1999) Biostatistical analysis. Pearson Education India, Indian