Chapter 4

Monitoring Physiological Responses in Giant Toad (*Rhinella marina*) from Coatzacoalcos, Mexico: A Comparative Study after 8 Years

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Abstract

The objective of this chapter is to present the results of a monitoring study carried out with physiological responses (biomarkers) in *Rhinella marina* (giant toad) for two different years, inhabiting the low basin of Coatzacoalcos river, one of the most contaminated regions in Mexico. A decrease in delta aminolevulinic acid dehydratase (δ-ALAD) (considered inhibition) and in the condition factor (1.2–1.5 times) found in toads of the industrial zone compared with reference organisms, each year. As for the hematological parameters, the mean corpuscular hemoglobin concentrations (MCHC), in the amphibians of industrial zone of the first sampling year show a decrease, while for the second sampling year, show an increment of 1.5 times than organisms of reference site. These effects could be associated with exposure to pollutants such as heavy metals (mainly Pb), which have been registered in different studies. This study demonstrates the usefulness of giant toads as biomonitors of contaminated sites.

Keywords: monitoring, physiological biomarkers, giant toads, pollution

1. Introduction

Amphibians are vertebrates that represent the link between life in the aquatic environment and adaptation to terrestrial life. They have important characteristics; such as its ectothermic physiology, metabolism, and highly permeable skin, which makes them sensitive to disturbances in the ecosystem such as changes in water conditions, as well as the presence of pollutants and certain diseases [1–3]. This complex life cycle makes them susceptible to different routes of exposure to environmental pollutants, which is why they have been considered as bioindicators of environmental quality [4]. Currently, environmental pollution is considered one of the main factors in the worldwide decline of amphibians that has occurred since 1990 [5]. Some species of amphibians have all the characteristics of a bioindicator or biomonitor, other species can satisfy only some of the criteria and are less suitable as study animals [2, 6].

The giant toad or cane toad (*R. marina*), **Figure 1** is a species of anuran, omnivorous, and opportunistic amphibian [7], with high somatic and hepatosomatic index [8], high biomass, and a voracious appetite [9], which makes it susceptible to the bioaccumulation of organic and inorganic pollutants and their toxicological effects [10, 11]. This amphibian is native to South America, Central America, and Mexico [12]. In Mexico, it is not listed in the species with special protection or in danger of extinction [13]. It has been used as a bioindicator in the evaluation of air pollution [14], infectious diseases [15], organochlorine pesticides [16], endocrine disrupters [17], pollution by lead [11], and other persistent organic pollutants (POPs) [10].

In some of the previous research, different physiological responses have been used, from general to specific responses of pollutants from the study sites. These responses are called biomarkers, where all biochemical, physiological, histological, morphological, and behavioral measurements are quantifiable in tissue or biological fluids from different organisms, including amphibians, like the *R. marina* [4, 18, 19]. An advantage of biomarkers is that variations that arise can be related by the influence of stressors and can serve as an early indication of major effects on the organism or the population of these [20]. Also, with this, it is possible to compare values of biomarkers of a resident population to contaminated sites with another reference population, as well as monitoring by seasons or years, to indicate the influence of exposure to pollutants in the general health status of organisms [21].

Moreover, in Mexico, there are several types of environmental pollution scenarios that have not been assessed from the point of view of the effects to the biota living these sites. In addition to this, there are very little environmental regulations as to some types of pollutants, such as: persistent organic compounds (POPs), polycyclic aromatic hydrocarbons (PAHs), and

Figure 1. Giant or cane toad (*R. marina*).

heavy metals. One of the sites has a higher record environmental pollution is the region of the lower Coatzacoalcos River Basin (Veracruz); region highly impacted due to industrial activities and urbanization that have contributed to the deterioration of ecosystems since the 1960s. Currently, Coatzacoalcos is considered one of the most polluted regions in Mexico [22, 23], to such a degree that, in environmental matrices such as water, soil, air, or sediment, and even in fauna, the presence of POPs, volatile organic compounds (VOCs), dioxins, heavy metals, among others, has been detected [11, 24–27]. Some of these pollutants have been associated with genotoxic or enzymatic effects in aquatic and terrestrial organisms at this site [10, 11, 28, 29]. In this context, the objective of this chapter is to present and compare the results of a monitoring of physiological responses (biomarkers) in *R. marina* carried out in the lower basin of the Coatzacoalcos river in different years as a follow-up of the environmental quality of the region.

2. Materials and methods

2.1. Study site

The lower basin of the Coatzacoalcos river is in the southeastern state of Veracruz, Mexico (18° 08′56″ N; 94°24′41″ W). It comprises 21 municipalities, which house petrochemical, industrial, urban complexes, and agricultural land areas. Pollution has been historic since oil exploration and refining works began at the beginning of the twentieth century up to industrialization, agricultural development, and urban growth at present, causing a rapid deterioration of the ecosystems found there.

The sampling stations were established along the basin according to the degree of contamination found. Thus, they were grouped by two zones: industrial and rural. In May 2008, the first sampling of monitoring study was carried out, while the second was carried out in February 2016 (**Figure 2**).

The industrial zone (I) was formed by the following sampling stations (red oval, **Figure 2**):

- Ejido Cangrejera: site adjacent to the petrochemical complexes of Pajaritos and Cangrejera, where various derivatives of chlorine and ethylene compounds are produced. In addition, the presence of POPs in both environmental and biological matrices has already been evidenced in this area, as well as the effects that these could be causing in terrestrial organisms [24, 28, 29].
- Estero del Pantano: site located on the banks of the Calzadas River. According to [11] the river receives discharges of sewage and industrial waters. Also, the presence of POPs and lead (Pb) has been demonstrated, as well as the effects of these compounds on terrestrial and aquatic organisms [10, 11, 30].

While the rural area (R) (yellow oval, **Figure 2**) was formed by:

- Ejido Limonta: Located upstream of the Coatzacoalcos river, in the municipality of Hidalgotitlán. It presents scarce urbanization and ecosystems still well preserved. At present, concentrations of pollutants have not been reported in this sampling station.

- San Carlos: Located next to the Uxpana river, upstream and that ends at the Coatzacoalcos river. It has well-preserved ecosystems. Like the previous one, no contaminant concentrations have been reported in this site.

The sampling stations of the rural area selected are characterized by semi-preserved ecosystems and minor impact by agricultural production, being susceptible areas where organophosphorus, organochlorine, or carbamate pesticides can be used to control pests or vectors.

As a reference, giant toads (seven organisms) kept in the laboratory for 1 year under conditions of feeding, humidity, and controlled temperature were selected, collected in a site outside the study area (Huasteca Potosina, San Luis Potosí) and free of exposure to pollutants.

2.2. Biological sampling

Adult male giant toads were collected per site by night transects and hand capture. In the first sampling (May 2008), 40 toads were collected, while in the second sampling (February, 2016), 30 toads were collected. The organisms were transported in containers to the laboratory (Facultad de Química-Universidad Veracruzana-Campus Coatzacoalcos). The body weight and snout-venth length (SVL) were taken. Subsequently, a blood sample (3–5 mL) was taken with heparinized syringes (following the guidelines established for amphibians and reptiles [31]). An aliquot

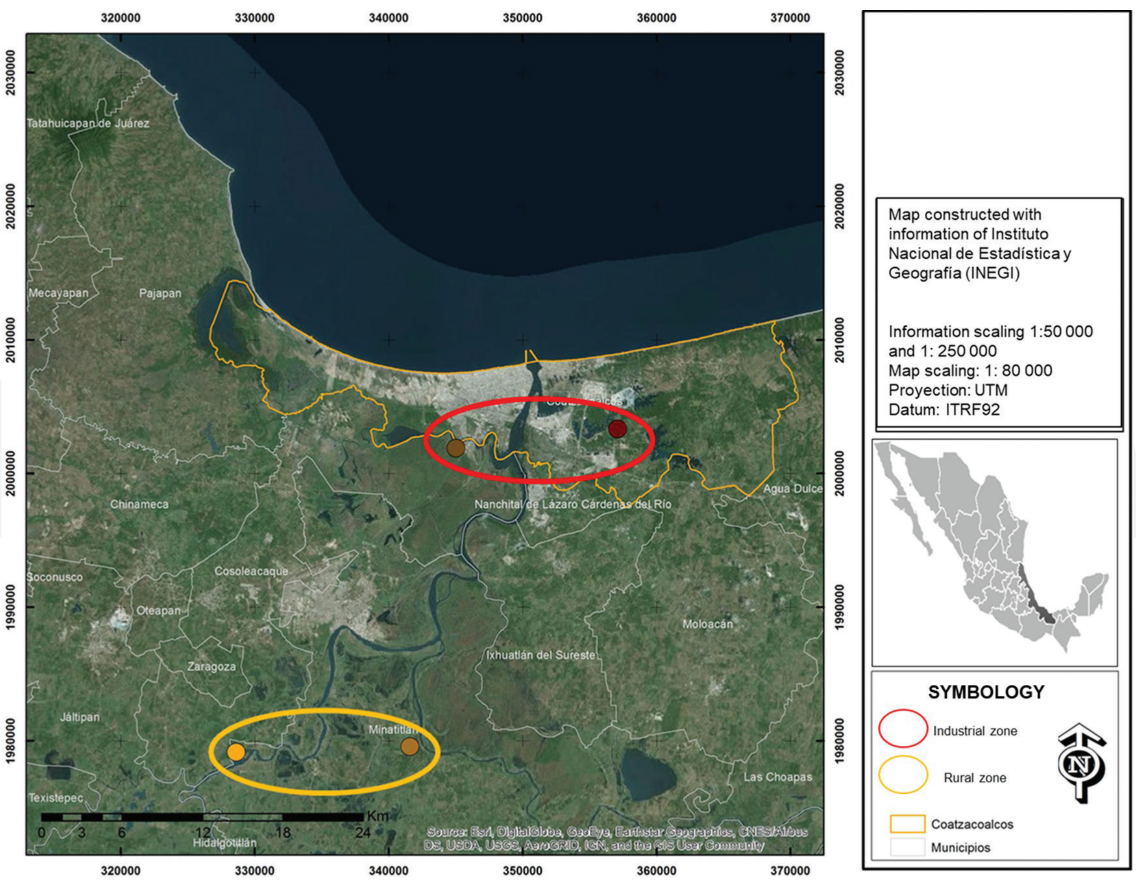

Figure 2. Zones of sampling in low basin of Coatzacoalcos (source: Google Earth, 2016).

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of whole blood was stored in liquid nitrogen (−186°C) for enzymatic analysis. While with the rest the hematological parameters were analyzed. The toads were released in their respective habitat. The toads were collected under a scientific collection permit (SGPA/DGVS/09731/15) issued by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT).

2.3. Evaluation of physiological responses (biomarkers)

2.3.1. Delta aminolevulinic acid dehydratase

The inhibition of the activity of the enzyme δ-ALAD is a biomarker that has shown in several studies a response to a specific contaminant. This enzyme is very sensitive to the effects of increased blood lead, even at very low levels [32]. In the biosynthesis of the heme group, the prosthetic group of hemoglobin (Hb) is selectively damaged by the binding of lead, thus affecting important steps of biosynthesis, inhibiting the activity of δ-ALAD (necessary for the synthesis of the heme group), and affecting the synthesis of protoporphyrin IX and hemoglobin [33, 34].

The determination of δ-ALAD was based on the method of [35] following modifications [11]. The whole blood samples (0.05 mL) were diluted with deionized water (1:35). Then 1 mL of 10 mM aminolevulinic acid (in phosphate buffer pH 6.4) was added. The samples were incubated at 38°C for 60 min in a water bath in complete darkness. The reaction was stopped by placing 1 mL of trichloroacetic acid (10%), then the samples were centrifuged for 10 min at 2000 rpm (Thermo Scientific® Sorvall Legend X1R). The supernatant was mixed with 1 mL of the Erich's solution. After 10 min, the absorbance was measured at 555 nm in a UV–Visible spectrophotometer (Thermo Scientific® Genesys 10S). Units of enzymatic activity were expressed as micromole per min per liter of red blood cells (RBC), which were calculated using the following formula (Eq. 1):

$$\delta\text{-ALAD} = \frac{\text{Abs} \times 100 \times D \times F}{\text{HT} \times 60 \times 0.062} \qquad (1)$$

where Abs = absorbance of the sample, F = 2 (porphobilinogen conversion factor to δ-ALA), D = 35 (dilution factor), HT = hematocrit (%), 60 = incubation time (min), and 0.062 = molar extinction coefficient (L/μmol × cm).

2.3.2. Hematological parameters

The chemical and morphological parameters of the blood can provide a wide range of biomarkers; its use has increased because the sampling can be very fast and non-destructive [36]. The hematological parameters can provide evidence of pathology including anemia, dehydration, infectious processes, parasitism, or poisoning [37]. These parameters in turn may be related to pollution and its effects [38]. The volume of the cell pack or hematocrit (HT) is the percentage of the total volume of blood composed of red blood cells. The mean corpuscular hemoglobin concentration (MCHC) is the range of the weight of hemoglobin by the volume of the erythrocyte. An iron deficiency, increased immature erythrocytes (reticulocytosis) and methemoglobin can result in a decrease in MCHC values [32]. Some toxic compounds can alter the functioning of the hematological system through interference with cellular production in the bone marrow or in the synthesis of the heme group, by direct cytotoxicity to the cells, or by injuries in other tissues resulting in a loss of blood cells [32, 39].

Therefore, the hemoglobin (Hb), hematocrit (HT), and the mean corpuscular hemoglobin concentration (MCHC) were determined. The hemoglobin content (g/dL) was measured using the kit HemoCue Hb 201$^+$ (microcuvettes and HemoCueHb 201$^+$ Analyzer). The determination was made following the protocol of the commercial distributor [40]. The hematocrit was determined with the globular sedimentation method with the aid of the hematocrit chart (Critocaps™ tube reader). The MCHC was calculated integrating the two previous parameters following equation.

$$MCHC = \frac{Hb\ (g/dL) \times 100}{HT\ (\%)} \tag{2}$$

2.3.3. Condition factor (CF)

Condition indices are potentially attractive biomarkers because they are simple to implement and provide information on the use of energy as well as the general health status of the organism [41]. The morphometric index most used is the condition factor (CF) expressed as the weight (g)/length (cm). Pollutants can produce rapid and marked changes in condition indices.

To calculate the CF, the snout-vent length (SVL) of the toads was taken with a vernier caliper (mm) and its body weight (BW) (g) with an electronic scale. Subsequently, these two parameters were integrated and used the following formula (Eq. (3)) to calculate the CF (%).

$$CF = \frac{BW(g) \times 100}{SVL\ (mm)} \tag{3}$$

2.4. Statistical analysis

The statistical analysis was carried out with the GrapPhad Prism 6.0 software (for Windows, La Jolla California USA, www.graphpad.com). The results are reported in media ± standard error. A comparison analysis of means (Mann-Whitney test) was carried out to evaluate the differences between the biomarkers per years, with a level of significance of 1 and 5%. To evaluate the difference in δ-ALAD between years, zones, and the laboratory, the Kruskall-Wallis test was used. A correlation between δ-ALAD and hematologic parameters (Log-transformed) was realized with Spearman's test.

3. Results and discussion

3.1. δ-ALAD activity

The results of the δ-ALAD activity in blood of *R. marina* per year and per sampling area are shown in **Figure 3A**. When the results were compared per sampling area for each year, it was obtained that in 2008 the toads of the industrial zone (70.2 ± 3.3 μM × min × mL RBC) had lower activity of δ-ALAD compared with the organisms residing in the rural area (15.4 ± 2.3 μM ×

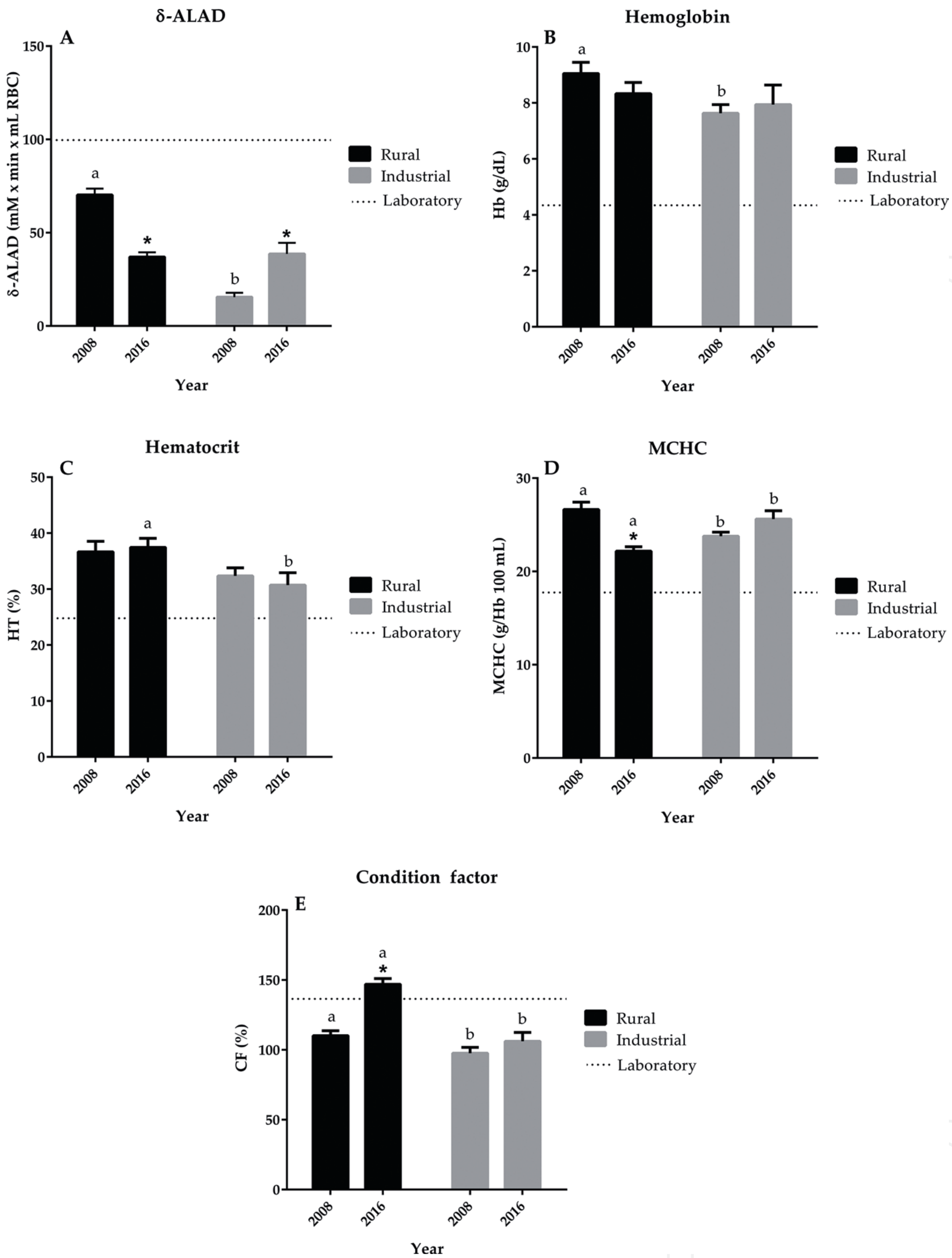

Figure 3. Comparison of biological responses (biomarkers) in blood of giant toad (*R. marina*) per year and per zone). *denotes a statistical differences between years per zone (MW-U test, $p < 0.001$), letters denotes a statistical differences between zones per each year (MW-U, $p < 0.05$, $p < 0.001$), 2008: R(n = 20), I(n = 20); 2016: R(n = 19), I(n = 11).

min × mL RBC) ($MW\text{-}U_{20,20} = 0.0$, $p < 0.001$). But, for 2016, the levels of this enzyme were found similar for the toads of both zones (36.9 ± 2.5 and 38.6 ± 5.9 μM × min × mL RBC, respectively). When comparing areas by year, for 2016, in the toads of the rural area, there was a statistically significant decrease in the δ-ALAD activity, compared with those of 2008 ($MW\text{-}U_{20,19} = 8.0$, $p < 0.001$). In contrast, with the organisms of the industrial zone, a significant increase was observed for the year 2016 ($MW\text{-}U_{20,11} = 26.0$, $p < 0.001$) (**Figure 3A**). On the other hand, the levels of total δ-ALAD, per year and per sampling area in *R. marina* of Coatzacoalcos were 35–60% lower than in the laboratory (99.6 ± 5.6 μM × min × mL RBC) ($KW\text{-}H_{2,77} = 16.5$, $p < 0.05$), which were not exposed to any pollutants.

The δ-ALAD activity has been shown to be a specific biomarker for evidence of lead exposure in organisms such as birds, amphibians, and mammals, both in the field and in the laboratory [42–44]. Our results show that, in 2008, toads from the industrial zone showed an inhibition of the δ-ALAD enzyme compared to those from the rural zone, which can be consistent and expected if this area is taken as a reference. This inhibition can be attributed to concentrations of lead found in the blood of giant toads, where residents of the industrial zone contained higher concentrations of this metal [11]. However, for 2016, the activity of δ-ALAD was similar for both areas. Refs. [33, 43] demonstrated in laboratory studies that exposure to lead in adult toads (*R. arenarum*) inhibited the δ-ALAD enzymatic activity, as concentrations of this metal increased. Likewise, it has been demonstrated the inhibition of δ-ALAD in birds, reptiles, and mammals resident in mining or industrial areas, which are associated with exposure to Pb [45, 46]. In this sense, intense industrialization in the lower basin of the Coatzacoalcos river is associated with the increase of heavy metals such as lead [47, 48]. Different studies in this region have reported the presence of lead in organisms such as fish, turtles, toads, and even humans, associated with neighboring industrial complexes [11, 30, 49]. Therefore, these could be factors that led to exposure to lead in the toads of the industrial zone in both 2008 and 2016. However, as mentioned above, in 2016, the levels of δ-ALAD decreased in the toads of the rural area and were found to be similar to those of the industrial zone. This could be due to two factors. The first is that the rural area is close to Minatitlán, municipality of Veracruz where industries are found, especially refining and petrochemical, which could be sources of non-point pollution of lead, and in recent years can also be considered to have been increased emissions (or possible sources), and therefore increased exposure to lead in organisms. On the other hand, a second factor that could be causing this exposure would be the extraordinary meteorological phenomena that have occurred in this area, for example, recent floods. It has been documented that some heavy metals, such as lead, and organic compounds can be mobilized and affect their bioavailability due to the removal of sediments when extreme weather events such as floods occur [27], so possibly an event like this has increased the exposure to lead in the organisms in the area.

On the other hand, the δ-ALAD activity in the laboratory toads was greater for those of Coatzacoalcos; this would also confirm an enzymatic inhibition in the organisms of this region, increasing in recent years. Finally, it could be said that the δ-ALAD activity could reflect a chronic exposure to lead, because the erythrocytes that carry out the transport of hemoglobin have a half-life of between 700 and 1400 days in *R. marina* [50].

3.2. Hematological parameters

The concentrations of hemoglobin and the percentage of hematocrit are presented in **Figure 3**. No statistical differences were found per year between zones (MW-U, $p > 0.05$). The levels of both parameters were similar in the toads resident in the rural area in 2008 (Hb = 9.05 ± 0.4 g/dL, HT = 34.6 ± 1.9%) and 2016 (Hb = 8.3 ± 0.4 g/dL, HT = 37.4 ± 1.6%) as well as those of the industrial zone in both years (Hb = 7.6 ± 0.3, HT = 32.3 ± 1.4; Hb = 7.9 ± 0.7 g/dL, HT = 30.7 ± 2.2%, respectively) (**Figure 3B** and **C**). However, when comparing by area in each year, a statistically significant decrease in the hemoglobin concentration of *R. marina* of the industrial zone was obtained in the sampling of 2008 ($MW\text{-}U_{20,20} = 86.5$, $p < 0.05$). While for 2016, Hb levels were similar ($p > 0.05$) (**Figure 3B**). Otherwise, with the percentage of HT, the levels in 2008 were similar in both areas. However, for 2016, a significant decrease was observed in the toads of the industrial zone ($MW\text{-}U_{19,11} = 54.0$, $p < 0.05$) (**Figure 3C**). When both parameters were integrated in MCHC (**Figure 3D**), a statistically significant decrease was observed in the toads of the rural area in 2016 (22.1 ± 0.4 g/Hb 100 mL)($MW\text{-}U_{20,19} = 50.5$, $p < 0.001$). When comparing areas for each year, a statistically significant decrease was obtained for organisms in the industrial zone (23.7 ± 0.4 g/Hb 100 mL) compared to those in the rural area (26.6 ± 0.7 g/Hb 100 mL) in the 2008 sampling ($MW\text{-}U_{20,20} = 118.0$, $p < 0.05$). While in the 2016 sampling, toads from the rural area showed a significant decrease compared to those in the industrial zone (25.6 ± 0.9 g/Hb 100 mL)($MW\text{-}U_{19,11} = 37.5$, $p < 0.05$). In turn, it is also observed that Coatzacoalcos toads showed higher levels (1.2–2.1 times) in the hematological parameters compared to those maintained in the laboratory (17.7 ± 1.1 g/Hb 100 mL).

Very few studies, in relation to pollutants and hematological parameters, have been carried out with amphibians. [51] used these parameters to evaluate the effects of agroecosystems on the health of amphibians; however, no statistically significant differences were found between the reference site and the agroecosystems. The results obtained in our study are contrary to those obtained in [52] where they showed a decrease in hematological parameters according to the presence of lead in tissues of the Egyptian toad *Amietophrynus regularis*.

We consider that the hematological parameters of Coatzacoalcos toads are altered (increases or decreases) when comparing areas in both years. But not so, if they are compared per year between zones, because similar values are obtained in Hb and HT, only one alteration was found in the MCHC in the organisms of the rural area in 2016. The increase of some parameters, such as Hb, could be related to a response of organisms to a decrease in the transport of oxygen in the blood (hypoxia), derived from anemia (anemic hypoxia) [53]. Hypoxia in some vertebrate organisms (fish, amphibians) has been related to the natural and anthropogenic increase of ammonium, sulfides, or organic matter, as well as the presence of pollutants (organic or heavy metals—lead) and excess nutrients [54, 55]. In the case of MCHC, the decrease in organisms residing in industrial zones, in the sampling of 2008, can be attributed to contamination in these places, being associated with a microcytic anemia, which could have been caused by exposure to pollutants such as heavy metals (e.g. lead) [11]. But for 2016, MCHC concentrations in *R. marina* decreased in the rural area and increased in the industrial zone. The above could support the hypothesis of the increase of pollutant concentrations in the rural area which is affecting the resident organisms of this area. On the other hand, the fact of finding parameters of greater hematological parameters in the Coatzacoalcos organisms compared with the laboratory ones could support the hypothesis proposed by [11], where they point out that one of the mechanisms of response of vertebrates to an exposure could be the increase in the number of erythrocytes (polycythemia) increasing the capacity of oxygen transport, and therefore, some hematological parameters. Suggesting this phenomenon as a compensation mechanism against anemic hypoxia.

3.3. Condition factor (CF)

The results of the condition factor of *R. marina* are shown in **Figure 3**. As observed, only a significant increase in CF of the toads of the rural area was found in 2016 (146.9 ± 4.0%) ($MW\text{-}U_{19,11} = 21.0$, $p < 0.001$). When comparing between areas for both years, a significant decrease of 11.3 and 27% in CF of the industrial zone organisms was observed, compared with those of rural areas in both years (2008 = $p < 0.05$, 2016 = $p < 0.001$) (**Figure 3E**). When the CF of the Coatzacoalcos toads are compared with the ones of the laboratory (136.5 ± 8.8%), it is observed, only similar in the toads of the rural area sampled in 2016, the others show the pattern of decrease (11–27%).

The estimation of the condition factor in amphibians is important to evaluate if they are under environmental stress [56]. This biomarker is commonly used to assess the general health status of aquatic and terrestrial organisms, because it is considered a non-destructive biomarker of energy reserves [57]. This is very important given that energy reserves can be used for the maintenance, development, or reproduction of amphibians. Commonly, a greater reserve of energy in organisms gives them greater resistance without food, greater survival, and better reproductive performance compared to individuals with lower reserves [58].

There is very little information on the use of the condition factor in amphibians in contaminated sites. Some researchers [58] reported a decrease in the condition factor and enzymatic alterations in semi-aquatic and terrestrial frogs resident in agricultural sites, associating it with a possible activation of compensatory or detoxification systems in amphibians in the face of environmental stress or a decrease in their prey (mosquitoes) by the application of insecticides. In other organisms, such as fish and birds, the decrease in the condition factor after exposure to heavy metals and organochlorine compounds, respectively, has been reported [59, 60]. Exposure of organisms to pollutants can increase energy requirements, decrease the metabolic or nutrient assimilation rate, and even alter digestion enzymes [41, 54, 61]. This could explain the decrease in the condition factor of the resident toads of the industrial zone in our studies (both samplings), given that, as already mentioned, a greater presence of pollutants has been demonstrated in this area. Regarding the similarity found between the toads resident in the rural area of 2016 and the laboratory ones and their increase in comparison of the organisms of the rural (2008) and industrial sites (2016), another factor that could be influencing these differences could be the scarce availability of food, caused either by natural conditions or by anthropogenic conditions.

3.4. Relationship between δ-ALAD and MCHC

The results of the correlations between the δ-ALAD enzymatic activity and the MCHC are shown in **Figure 4**. As observed in this figure, for the organisms of the 2008 sampling, in the rural area, there is an association between these physiological responses. This same pattern is observed considering both (total) sites in this sampling year (**Figure 4A** and **C**—2008). As previously mentioned, the relationship of the activity of this biomarker and the hematological parameters is found in the fact that the former is part of the metabolic pathway of hemoglobin, which is congruent with these results. However, when making the correlations for the toads of the 2016 sampling, only a statistically significant correlation ($p < 0.05$) between both biomarkers is observed for the industrial zone (**Figure 4**B-2016). It is important to mention that for the laboratory toads, no correlations were found between the physiological responses, besides that the values of δ-ALAD in these were found above those of Coatzacoalcos, and the hematological

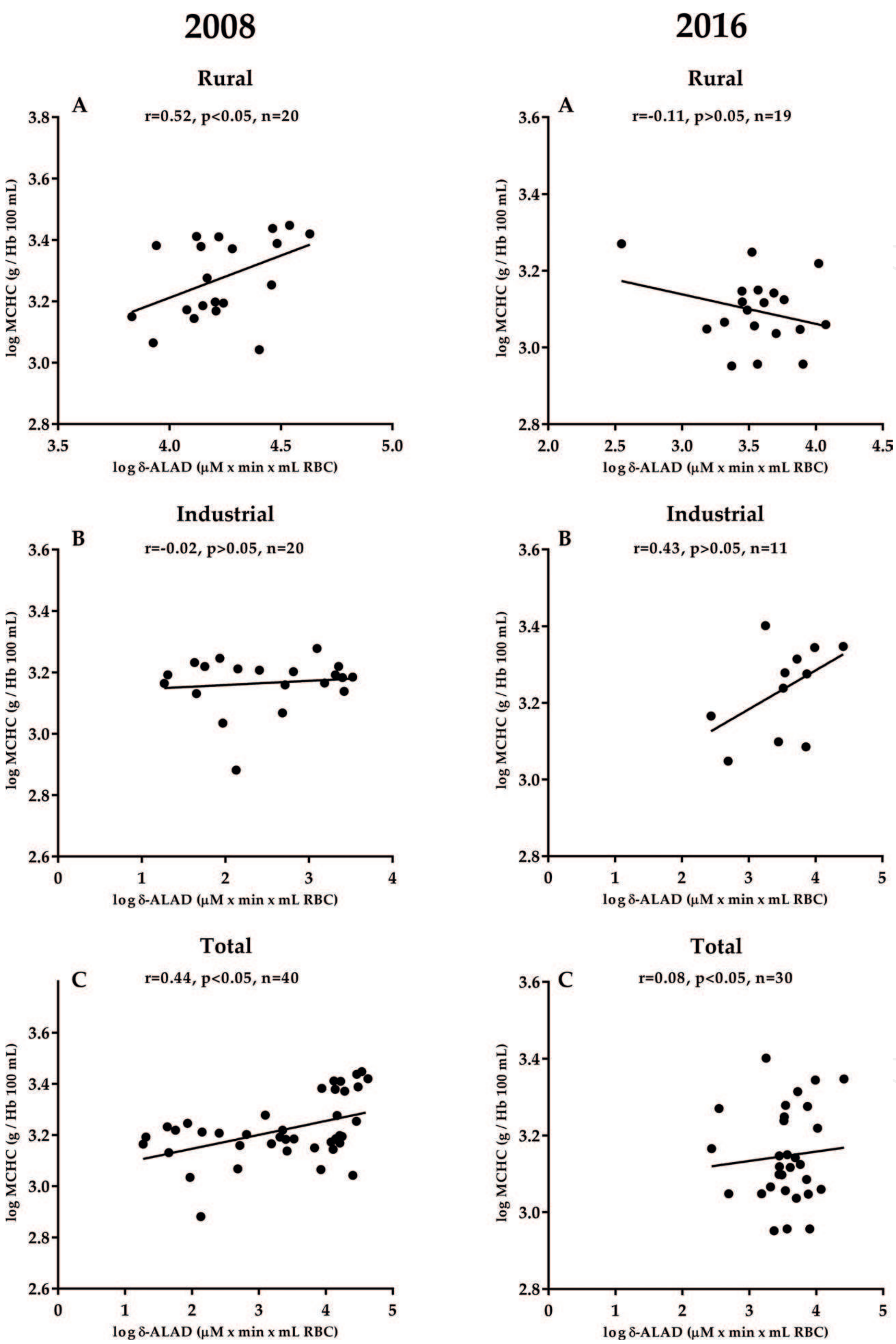

Figure 4. Correlations between δ-ALAD and hematologic parameters per site and per year (total) in blood of *R. marina* (Spearman test); A= Rural, B= Industrial, C= Total.

parameters in smaller quantity. This could support the hypothesis that organisms from this site could be producing more Hb and HT (MCHC) to defend against the effects caused by pollutants (heavy metals such as lead) found in the area, which is evident in the organisms of the rural area of the 2008 sampling. However, for the 2016 sampling, the rural area shows a decrease in the MCHC. Therefore, the hypothesis could be supported that the concentrations of lead have increased in this site and consequently the exposure, thus affecting the compensation system of the organisms in this area. In addition to confirming a chronic exposure to this pollutant.

It is important to mention that several routes and sources of exposure to lead can be found in the toads of Coatzacoalcos due to their complex life cycle. One of these can occur when the tongue of this organism catches its prey, because this organ can be in contact with the soil or sediments (which can be exposed by dredging that takes place in the area), and, as a consequence, ingest particles in which lead may be present [62]. Also, the exposure in this case could be increased by consuming preys that have been exposed to this metal and that contain it; in this case, the presence of lead has been demonstrated in insects that inhabited industrial sites [63] that could be part of the diet of the giant toad in Coatzacoalcos due to their voracious appetite [9]. Likewise, it has been demonstrated that the consumption of prey in some organisms can increase the concentrations of lead and manifest the toxic effects [64]. Therefore, this would complement the possible explanation of the decrease or increase in some physiological responses in organisms of the 2016 sampling.

Finally, it should be mentioned that the physiological responses quantified in *R. marina* are considered non-destructive biomarkers, because they are evaluated in the organism without sacrificing it. The above turns out to be important, because in this way, organisms could be monitored by seasons, years, or by places. Moreover, these studies would be relevant in *R. marina* because this amphibian has a life expectancy of 10–15 years, which could be complemented with a tagging of these. It should be noted that in this study no labeling of organisms was used.

4. Conclusion

The δ-ALAD activity, the hematological parameters, and the condition factor can be considered as biomarkers of exposure and/or effect, non-destructive, in giant toads in monitoring studies in sites contaminated by heavy metals or other pollutants. Especially δ-ALAD, which could reflect a chronic exposure in organisms. On the other hand, the results found with the organisms of the lower basin of the Coatzacoalcos river could lead to the need to make a new monitoring with emphasis on the rural regions to affirm or discard out if there is an increase in the concentration of pollutants, especially Pb, its possible causes and, if this may be affecting other organisms and even the human settlements that are there. New studies carried out in this region should take into account this type of physiological responses in a battery of biomarkers that reflect the response to other pollutants already registered on the site.

Once again, it was confirmed that the giant toad (*R. marina*) is a good biomonitor of contaminated sites and can be useful for the evaluation of exposure and effects by pollutants in different scenarios in Mexico due to its ease of capture, wide distribution, and, as already demonstrated, to its susceptibility to xenobiotics. Likewise, it is recommended to create new lines of research with this amphibian to be able to elucidate the effects or compensation mechanisms that may arise from natural or anthropogenic activities.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this chapter.

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