#### **RESEARCH ARTICLE**



# Associations between trace elements and clinical health parameters in the North Pacific loggerhead sea turtle (*Caretta caretta*) from Baja California Sur, Mexico

César Paúl Ley-Quiñónez<sup>1,2,3</sup> • Natalia Alejandra Rossi-Lafferriere<sup>4</sup> • Teresa Leticia Espinoza-Carreon<sup>1</sup> • Catherine Edwina Hart<sup>2,3</sup> • Sherwood Hoyt Peckham<sup>5,6</sup> • Alfredo Alonso Aguirre<sup>7</sup> • Alan Alfredo Zavala-Norzagaray<sup>1,2,3</sup>

Received: 12 June 2016 / Accepted: 2 February 2017 / Published online: 25 February 2017 © Springer-Verlag Berlin Heidelberg 2017

**Abstract** This study investigated selected trace elements toxicity in sea turtles *Caretta caretta* population from Baja California Sur (BCS), Mexico, by analyzing associations among Zn, Se, Cu, As, Cd, Ni, Mn, Pb, and Hg with various biochemical parameters (packed cell volume, leukocytes, and selected blood parameters), and whether their concentrations could have an impact on the health status of sea turtles. Blood samples from 22 loggerhead (*C. caretta*) sea turtles from BCS, Mexico, were collected for trace elements on biochemistry parameter analyses. Significant associations among trace element levels and the biochemistry parameters were found: Cd vs ALP ( $R^2 = 0.874$ , *p* 0.001), As vs ALP ( $R^2 = 0.656$ ,

Responsible editor: Philippe Garrigues

César Paúl Ley-Quiñónez cleyq@ipn.mx

- <sup>1</sup> Instituto Politécnico Nacional, CIIDIR-SINALOA, Guasave, Sinaloa, Mexico
- <sup>2</sup> Grupo Tortuguero de las Californias A.C, La Paz, Baja California Sur, Mexico
- <sup>3</sup> Investigación, Capacitación y Soluciones Ambientas y Sociales A.C, Tepic, Nayarit, Mexico
- <sup>4</sup> Wildlife Conservation Society, Bronx, NY, USA
- <sup>5</sup> SmartFish, La Paz, Baja California Sur, Mexico
- <sup>6</sup> Center for Ocean Solutions, Stanford University, Pacific Grove, CA, USA
- <sup>7</sup> Department of Environmental Science and Policy, George Mason University, Fairfax, VA, USA

p 0.001), Mn vs ALP ( $R^2 = 0.834$ , p 0.001), and Ni vs LDH ( $R^2 = 0.587$ , p 0.001). This study is the first report of the biochemical parameters of the North Pacific loggerhead sea turtle (*C. caretta*) from Baja California Sur, Mexico, and it is the first to observe several associations with toxic and essential trace elements. Our study reinforces the usefulness of blood for the monitoring of the levels of contaminating elements and the results suggest that, based on the associations with health clinical parameters, high levels of Cd and As could be representing a risk to the North Pacific loggerhead population health.

Keywords Sea turtle · Caretta caretta · Biochemistry parameters · Trace elements · North Pacific loggerhead

### Introduction

The sea turtle is in the final links of the webs of life with few predators, which has allowed him to develop and adapt to climate change of the planet during his evolutionary development (Spotila 2004). But now, all the species of this reptile are in a status of endangerment (SEMARNAT 2010; CITES 2015) that are the result of human pressures (Gardner and Nichols 2001; Aguirre and Lutz 2004; Broderick et al. 2006). The loggerhead sea turtle, *Caretta caretta*, with only nine populations at the global level, has been strongly impacted, particularly the North Pacific loggerhead (Aguirre et al. 2016). As a result of this, studies on the aspects of population, anthropogenic and natural threats, and biointoxication are important (Seminoff et al. 2014).

Anthropogenic activities have generated and pouring different pollutants to the environment, like solid residues, organic persistent pollutants, and heavy metals (Clark 2001; Green-Ruiz and Páez-Osuna 2004; Green-Ruiz et al. 2005; Kucuksezgin et al. 2006), strongly impacting on the health of ecosystem and living beings (Aguirre and Lutz 2004; Wilcox and Aguirre 2004). In particular, toxic trace elements like Cd, Hg, Pb, As, and even essential elements, that are in high concentrations can be harmful to organisms, are relevant in ecotoxicology, because these inorganic pollutants tends to accumulate, concentrate, and biomagnify through the food chain (Storelli et al. 2005; Camacho et al. 2013), mainly affecting species in the end links like sharks and sea turtles (Aguirre and Lutz 2004; Tabor and Aguirre 2004; Frías-Espericueta et al. 2014; Zavala-Norzagaray et al. 2014).

Several studies have pointed to exposure of sea turtles to contaminant like trace elements could affect various functional processes (Aguirre et al. 2006; Camacho et al. 2013) and like potential synergic etiologies of fibropapillomatosis and other diseases in sea turtles (Aguirre and Starkey 1994), even in low concentrations (Day et al. 2007). However, most studies about trace metal concentrations in sea turtle are developed in tissues collected postmortem like the liver, heart, muscle, and kidneys (Aguirre et al. 1994; Maffuci et al. 2005; Frias-Espericueta et al. 2006; Gardner et al. 2006; Storelli et al. 2008). In recent years, some studies analyzed the use of blood to determinate the heavy metals and metalloid concentrations in sea turtles (Day et al. 2007; Day et al. 2010; Ley-Quinonez et al. 2011; Camacho et al. 2013; Ley-Quinonez et al. 2013; Zavala-Norzagaray et al. 2014), concluding that the blood is a promissory tissue as analysis matrix that has made it possible to measure trace element levels with excellent results (Day et al. 2005; Day et al. 2010; Ley-Quinonez et al. 2011; Zavala-Norzagaray et al. 2014), and these levels showed a significant correlation among blood with other tissues (van de Merwe et al. 2010). On the other hand, blood biochemistry has been used as an invaluable tool for monitoring the health and status of wildlife, for analyzing potential sources of contamination and disease (Aguirre and Balazs 2000), and possibly for observing processes of absorption, accumulation, and circulation of trace elements and their potential toxicity in sea turtle (Ley-Quinonez et al. 2013), however, only a few studies have analyzed the effect of selected trace elements on the health of sea turtles, e.g., low Hg concentration in blood of Caretta caretta showed negatively correlation with lymphocyte cell counts and aspartate aminotransferase (Day et al. 2007), or other trace elements like Cd and As showed correlations with biochemistry parameters which could have a negative effect on the health of sea turtles (Camacho et al. 2013). The aim of this study was to analyze selected trace element toxicity in sea turtle C. caretta population from Baja California Sur (BCS), Mexico, by analyzing the existence of associations among these elements with various biochemical parameters, and whether their concentrations could have an impact on the health status of sea turtles.

#### Materials and methods

#### Sampling

In July and August of 2008, blood samples from 22 loggerhead (*C. caretta*) sea turtles were collected from Vizcaino Peninsula ( $27^{\circ} 50' 00'' \text{ N/115}^{\circ} 05' 40'' \text{ W}$ ) to Bahía Magdalena ( $24^{\circ} 30' 00'' \text{ N/112}^{\circ} 00' 00'' \text{ W}$ ), BCS, México. Loggerhead turtles were captured by hand from small fishing boats, measured and weighed. Curve carapace length (CCL) and curve carapace width (CCW) were measured with a flexible tape measure.

Samples were taken by venipuncture from the dorsal postoccipital sinuses following manual restraint (Owens 1999). Blood samples were collected using 21 gauge needles and 5-ml or 10-ml syringes. Five percent to eight percent of reptile body weight, and 10% of this volume may be safely collected (Sikes IV and Klaphake 2008). Eight milliliters of blood of each sea turtle captured was collected, 3 ml for trace element and 5 ml for biochemistry parameters. Blood samples were transferred into vacutainer tubes containing lithium heparin and placed on blue ice until processing at the wet lab in the field station between 2 and 8 h from the time of capture.

0.5 g of each blood specimen (wet weight) was subjected to an acid digestion using a mixture of 5 ml HNO<sub>3</sub>, HCl and H<sub>2</sub>O<sub>2</sub> in a proportion of 2:2:1. Sample digestions were performed in a microwave system (ANTON PAAR) for 35 min. The digestions were analyzed with an optical emission inductively coupled plasma atomic spectrophotometer (ICP-AES) model OPTIMA 4300TM DV (Perkin Elmer) using the procedure described by Ley-Quiñonez et al. (2011). The elements analyzed were Zn, Ni, Cu, Mn, Se, Cd, As, Pb, and Hg. Limits of detection (LOD) for each element were 0.01  $\mu$ g g<sup>1</sup> for Cd, As, Ni, Hg, and Pb, and 0.04  $\mu$ g g<sup>1</sup> for Zn, Cu, Mn, and Se. Multielement standard SIGMA 6000 (Perkin Elmer) was used for the spectrophotometer calibration curves. During the digestion process, the lobster hepatopancreas reference material for trace elements, TORT-2 (National Research Council of Canada, Ottawa) was run with the samples. Percentages of recovery of all elements were among 87 and 95%.

Packed cell volume (PCV) was measured using capillary tubes spun for 5 min with a microcentrifuge model ZO-1 (LW Scientific Inc.). Five blood smear slides were prepared immediately on board and allowed to air dry. Plasma total protein was recorded using a small hand-held refractometer model VET-360 (REICHERT). Plasma was separated by centrifuge COMPACT II model 420225 (Becton Dickinson) at 3000 rpm for 15 min and pipetted into 1.6-ml cryogenic vials. The white blood cell layer was collected in one cryogenic vial per sample. Plasma, WBC, red blood cells, and whole blood samples were stored in a freezer until transfer for further biochemical analysis. Blood plasma parameters were measured at IDEXX Laboratories, West Sacramento, CA. Plasma samples (40 μL)

were analyzed using a chemistry analyzer model VetTest 8008 (IDEXX) and commercial kits, according to the IDEXX specifications. Quality Control F1620 (IDEXX) was analyzed once a month, to verify that equipment was functioning properly (IDEXX 2014). Selected blood parameters are the most frequently used in sea turtles (Aguirre 1996; Camacho 2013). Total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine phosphokinase (CPK), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphorus, cholesterol, glucose, sodium, globulin, and potassium were quantified. In addition, we calculated values of albumin/globulin (A/G) and sodium/potassium (Na/K) ratios. Blood smears were fixed in methanol and stained using the HEMA® stain set protocol (Romonowsky type, Fisher Scientific, USA) at the American Museum of Natural History. Blood smear slides were reviewed for leukocytes and classified based on the morphologic features (Casal and Orós 2007) using an Accuscope compound microscope with 40× lens and Neubauer hemocytometer, according to the Natt and Herrick method (Camacho et al. 2013). The estimated count method is performed by counting the total number of leukocytes in ten consecutive fields multiplied by 1000 to obtain the estimate. Two hundred leukocytes were counted and classified as heterophil, lymphocyte, monocyte, eosinophil, or basophil to determine relative leukocyte differential counts (Deem et al. 2009). The article of Sikes IV and Klaphake's Hematology reptile was used as cell identification guide (Sikes IV and Klaphake 2008). Counting an identification cell was made by trained personnel.

#### Statistical analyses

Mean, standard error (SE), and range data were reported for curve carapace length (CCL), weight, trace elements, and values for each blood parameter. Kolmogorov-Sminov test was used as a normality test. The correlations among statistical variables were determined using a simple regression model  $R^2 > 50\%$  and p value as statistical indicators. The CCL and weight were used as a relative indicator of age determination in sea turtles (Gardner et al. 2006; Ley-Quinonez et al. 2013).

## Results

Mean, standard error (SE), curved carapace length (CCL), weight, and metal concentrations for 22 loggerhead turtles are summarized in Table 1. Low numbers of epibionts (20) on the specimen plastron suggest healthy animals and may be a useful clinical indicator of good health (Gelli et al. 2008; Deem et al. 2009; Flint et al. 2010). Table 1Mean, standard error (SE), and curved carapace length (CCL),weight, and blood trace element concentrations for loggerhead turtles(Caretta caretta) from Baja California Sur, Mexico

Variable	Mean $\pm$ SE	Range
Carapace length (cm)	69.0 ± 1.1	54.0-83.5
Weight (kg)	$48.5 \pm 2.7$	17.0-80.0
Zinc	$44.8 \pm 2.6$	28.0-75.0
Copper	$2.8\pm0.3$	<lod (13)<="" 1.0="" td=""></lod>
Manganese	$0.6 \pm 0.7$	<lod (16)<="" 2.6="" td=""></lod>
Nickel	$1.5 \pm 1.9$	<lod (15)<="" 13.2="" td=""></lod>
Cadmium	$1.8 \pm 0.4$	1.3–3.7
Arsenic	$4.0 \pm 1.2$	0.3-23.6
Selenium	$6.14 \pm 1.4$	1.2–14.4

n = 22; trace element concentration in micrograms per gram wet weight; range (min-max) followed by the number of samples above the LOD in parenthesis if  $\leq 16$ . Limits of detection (LOD) for each element were 0.01 µg g<sup>-1</sup> for Cd, As, Ni, Hg, and Pb and 0.04 µg g<sup>-1</sup> for Zn, Cu, Mn, and Se. Trace element concentrations previously published by Ley-Quiñónez et al. (2011)

ND no determined, LOD limit of detection

Trace element concentrations were previously published by Ley-Quiñónez et al. (2011). Samples for the trace elements were collected from the same sampled turtles for biochemical and hematological analysis. Trace element distribution was Zn > Se > Cu > As > Cd > Ni > Mn. Pb and Hg concentrations were below of the detection levels of the calibration curve used (Table 1). No correlations were found among trace elements analyzed in blood and the size or weight of the turtles ( $R^2 \quad 0.50$ ;  $p \quad 0.05$ ).

Mean packed cell volume was 34.76% (±0.77, range 25.00–44.00%). Heterophils were the most numerous leukocytes found, followed by lymphocytes. No basophils or hemoparasites were detected in any of the 22 turtles sampled. For lactate dehydrogenase (LDH) and uric acid, only 16 turtles and one turtle could be analyzed, respectively. Blood parameter values are summarized in Table 2.

Heterophils, lymphocytes, and alanine phosphatase were significantly correlated with body length and weight. Similar correlations obtained for body length and weight could be explained by the linear relationship between these two variables ( $R^2 = 0.94$ ). Lymphocyte percentage (%Lc) and ALP demonstrated a positive correlation with CCL (%Lc:  $R^2 = 0.624, p = 0.004$ ; ALP:  $R^2 = 0.501, p = 0.029$ ) and weight (%Lc:  $R^2 = 0.639; p = 0.003$ ; ALP:  $R^2 = 0.0.485, p = 0.035$ ). Statistically, there were no relations among other biochemistry parameters vs size or weight ( $R^2 = 0.50; p = 0.05$ ).

Significant associations among Cd vs Cu ( $R^2 = 0.949$ , p = 0.001) or trace element levels and the biochemistry parameters were found: Cd vs ALP ( $R^2 = 0.874$ , p = 0.001), As vs ALP ( $R^2 = 0.656$ , p = 0.001), Cu vs ALP ( $R^2 = 0.877$ ,

**Table 2**Mean, standard error (SE), and biochemical and hematologicvalues for loggerhead turtles (*Caretta caretta*) from Baja California Sur,Mexico

Variable	Mean ± SE	Range
PCV (%)	$34.7 \pm 0.7$	25.0-44.0
Heterophil (%)	$56.1 \pm 1.1$	40.0-70.0
Lymphocyte (%)	$33.2 \pm 1.4$	20.0-45.0
Eosinophil (%)	$5.8 \pm 1.3$	0.0-12.0
Monocyte (%)	$4.95\pm0.8$	2.0-9.0
Basophil (%)	ND	ND
$ALP (U l^{-1})$	$137.0\pm9.2$	15.0-395.0
ALT (U $l^{-1}$ )	$5.8 \pm 2.4$	0.0-24.0
AST (U $l^{-1}$ )	$155.6\pm10.3$	23.0-520.0
$CPK (U l^{-1})$	$340.4 \pm 12.5$	51.0-1077.0
LDH (U $l^{-1}$ )	$7.8 \pm 4.0$	39.0-0 (16)
$GGT (U l^{-1})$	$2.2 \pm 0.6$	1.0-4.0
Albumin (g $dl^{-1}$ )	$1.5 \pm 0.2$	1.1-2.1
Total protein (g dl <sup>-1</sup> )	$4.5 \pm 0.2$	3.4–5.3
Globulin (g $dl^{-1}$ )	$3.0\pm0.1$	2.3-3.7
BUN (mg $dl^{-1}$ )	$135.6 \pm 3.3$	58.0-206.0
Creatinine (mg dl <sup>-1</sup> )	$0.1 \pm 0.2$	0.0-0.4
Cholesterol (mg dl <sup>-1</sup> )	$225.5 \pm 4.4$	104.0-342.0
Glucose (mg $dl^{-1}$ )	$102.9\pm2.0$	72.0-144.0
Calcium (mg dl <sup>-1</sup> )	$6.1 \pm 0.7$	2.3-10.8
Phosphorus (mg dl <sup>-1</sup> )	$6.9\pm0.7$	3.2-12.3
Potassium (meq $l^{-1}$ )	$4.0 \pm 0.3$	2.8-5.6
Sodium (meq $1^{-1}$ )	$152.5\pm0.2$	145.0–156.0
A/G ratio	$0.5\pm0.1$	0.4–0.7
Na/K ratio	1.9	27.0-55.0

Range (min-max) followed by number of samples analyzed in parenthesis if  $\leq 16$ 

n = 22, ND no determined, ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, CPK creatine phosphokinase, LDH lactate dehydrogenase, GGT gamma-glutamyl transferase, BUN blood urea nitrogen, A/G albumin globulin ratio, Na/K sodium potassium ratio, PCV packed cell volume

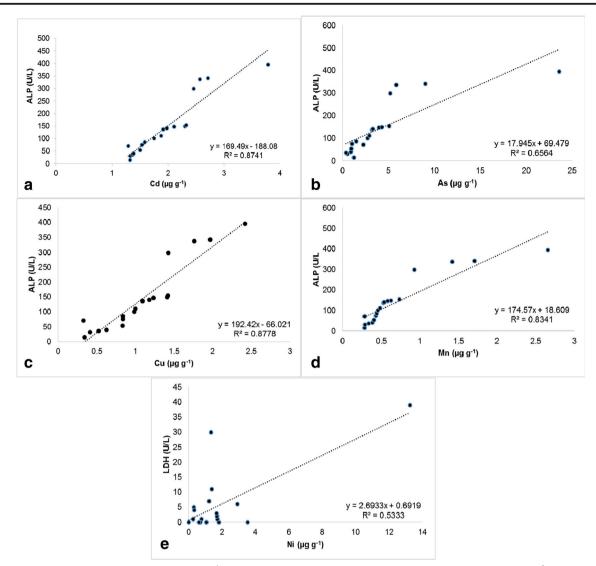
p = 0.001), Mn vs ALP ( $R^2 = 0.834$ , p = 0.001), and Ni vs LDH ( $R^2 = 0.587$ , p = 0.001) (Fig. 1).

#### Discussion

Sea turtles are considered sentinel species and provide an overview of the possible ecosystem health (Aguirre and Lutz 2004); however, it is necessary to understand the impact of contaminants such as some heavy metals and metalloids may pose to sea turtle populations and establish health parameters and biochemical reference intervals (RIs) for each species and regional populations (Storelli and Marcotrigiano 2003). There is limited available data on blood values and reproductive status for sea turtles, and establishing baseline information remains a priority for conservation management (Aguirre and Balazs 2000; Deem et al. 2009). Although the RIs did not differ between turtle sex and maturity, so these factors do not influence the health status of turtles (Bolter and Bjorndal 1992; Flint et al. 2010). Several studies concluded that the RIs in sea turtles must be considered regionally (Deem et al. 2009: Flint et al. 2010): therefore, comparisons between the results of this study and previous studies are complicated and possibly inaccurate, because the species behavior (Samour et al. 1998), foraging areas (Whiting et al. 2006), methods of blood collection, handling, processing, and biochemical analysis must be a source of possible variation (Bolter and Bjorndal 1992). Other important point is the limitation in accessing individuals for sampling and constrained resources, e.g., the population of the North Pacific Loggerhead C. caretta is one of the nine populations most strongly impacted (Aguirre et al. 2016), and our study area is a foraging area (Tomaszewicz et al. 2015). Seminoff et al. (2014) estimated an annual abundance of 43,226 loggerhead turtle; however, the number of loggerheads sighted each year ranged from 230 to 309 with a density of 0.650 turtles  $\text{km}^{-2}$ . which widely reduces the capture possibility of a specimen; however, this study is useful as the first to report of biochemical parameters and to observe several association with toxic and essential trace elements. No significant differences were observed among the means of the distinct biochemistry parameters and morphometric of the individuals sampled. The results obtained are consistent with those of (Flint et al. 2010), who concluded that there is no relationship among sex or size in sea turtles based on biochemistry parameters.

Trace element accumulation in sea turtles present differences between both intraspecific and interspecific populations from different areas (Aguirre and Balazs 2000), and previous studies highlighted the need to understand toxicological effect of selected metal accumulation in sea turtle and their immune response to it (Storelli and Marcotrigiano 2003; Ley-Quinonez et al. 2011); however, such studies remain limited. The rank order of the blood concentration of the trace elements evaluated is consistent with the rank order of the blood element concentration report by Camacho et al. (2013), only Mn concentration varied, presenting the lowest level in *C. caretta* from Cape Verde, West Africa. The authors conclude that metal concentrations have potential adverse effects on the *C. caretta* hematological and biochemical parameters.

Ni and Mn levels also presented a positive relationship with ALP (Fig. 1). Ni and Mn are considered essential for organisms like for some plants, bacteria, and invertebrates (Clark 2001; Liu et al. 2008; Ley-Quinonez et al. 2011); however, high concentrations in organisms can develop different diseases, e.g., Ni, As, and Se cause negative effect on white blood cells (Camacho et al. 2013). Some algae and marine plants accumulate high levels of Ni, as defense against predators and



**Fig. 1** Relation among blood metal concentration ( $\mu g g^1$ ) with clinical blood parameters for loggerhead sea turtles. **a** Cd vs ALP ( $R^2 = 0.874, p 0.001$ ). **b** As vs ALP ( $R^2 = 0.656, p 0.001$ ). **c** Mn vs ALP ( $R^2 = 0.834, p 0.001$ ). **d** Cu vs ALP ( $R^2 = 0.877, p 0.001$ ). **e** Ni vs LDH ( $R^2 = 0.587, p 0.001$ )

pathogens (Baker et al. 2000), while higher levels of Mn was found in the kidney and muscle for other loggerhead populations (Sakai et al. 2000b; Gardner et al. 2006); however, the role of the Mn and its accumulation in sea turtles are not clear (Storelli et al. 2003; Ley-Quinonez et al. 2013).

Blood Pb levels in sea turtles show differences between the turtle size (Day et al. 2005; Wang 2005; Camacho et al. 2013), possibly like a reflect of different eating habits during lifespan, in oceanic zones while are small size, and neritic in large size (Eckert et al. 2000; Eder et al. 2012). Our study doesn't show differences among the turtles size, and the load of this contaminate appears to be low in North Pacific loggerhead sea turtle from BCS (Gardner et al. 2006; Ley-Quinonez et al. 2011) and other species sea turtles from Mexican Pacific (Frias-Espericueta et al. 2006; Gardner et al. 2006; Paez-Osuna et al. 2010a; Ley-Quinonez et al. 2013; Zavala-

Norzagaray et al. 2014 are possibly a reflect of the feeding areas (Camacho et al. 2013), apparently pristine or nearly pristine grounds (Paez-Osuna et al. 2010a).

Previously, other studies observed a response of biochemical parameter caused by Hg and organochlorine (OC) contaminant levels in loggerhead blood (Keller et al. 2004; Keller et al. 2005; Day et al. 2007; Day et al. 2010) and concluded that both, the Hg as OC contaminants, even in low concentrations may be affecting the health of loggerhead sea turtles. Day et al. (2007) observed that Hg exposure, even in low concentration, can raise some blood parameter levels like AST or CPK and conclude a negative impact on *C. caretta* sea turtle immune function. Keller et al. (2004) found that persistent OC contaminants were correlated positively with AST activity and negatively with ALP activity, suggesting a possible hepatocellular damage. Cd and As are metals with great ecotoxicological importance. Mean concentrations of Cd and As in *C. caretta* blood were higher than those reported in previous studies on other sea turtle species (Kenyon et al. 2001; Guirlet et al. 2008; Paez-Osuna et al. 2010b; van de Merwe et al. 2010; Camacho et al. 2013). In fresh water turtles, Cd might affect gonadal developmental during embryonic and postnatal stages and enhance the apoptosis rate (Kitana and Callard 2008); Paez-Osuna et al. (2010b) suggest that Cd concentration in *L. olivacea* sea turtle from Mexican coast, even at low levels, might be a hazard to the eggs by maternal transfer.

Cd has a particular importance in the Northwest of Mexico, there are natural benches on the Baja California peninsula, as well as waste from phosphorus exploitation in the region, Likewise, conditions in the Gulf of California, a semienclosed sea of high primary productivity, and upwelling, contribute the bioavailability of Cd in the food chain (Storelli et al. 2008; Ley-Quinonez et al. 2011), particularly in the species carnivorous feeding on calcareous organisms such as crabs, main food of the loggerheads turtle; however, there is a lack of knowledge in this ecotoxicological research (Ley-Quinonez et al. 2013). Our results show an association among blood Cd and As levels with ALP enzyme. Firstly, the elevation of ALP may be caused by Cd and As levels, as it is observed with other pollutants could suggest a possible liver damage (Keller et al. 2004; Keller et al. 2005; Day et al. 2007; Innis et al. 2008; Camacho et al. 2013). Other possibility may occur due to an incorrect excretion process (Barile 2008), resulting in organism suffering a series of toxic effects and the development of pathologies such as fever, anorexia, hepatomegaly, melanosis, cardiac arrhythmia, and pulmonary and neurological diseases (Cantilena 2008). Maffucci et al. (2005) observed highly significant correlations between Cd and Cu or Zn in the liver and kidney of C. caretta and mentioned the detoxification process by metallothionein that are effective against the toxicity of Cd in sea turtles. These results are consistent with our study, and a relationship between Cd and Cu was also observed, which contributes to the data mentioned above during MT production, an increase in both values was observed as a result of possible liver or kidney damage (Storelli and Marcotrigiano 2003; Storelli et al. 2008; Camacho et al. 2013). The above supports the relationship of Cd vs Cu such relationships are common in liver and kidney during metallothionein (MT) production (Sakai et al. 2000a; Sakai et al. 2000b; Storelli and Marcotrigiano 2000; Anan et al. 2002; Storelli et al. 2005; Storelli et al. 2008; Paez-Osuna et al. 2010b; Zavala-Norzagaray et al. 2014). Proteins function as toxic metal chelates (Ley-Quinonez et al. 2013) and partake in the excretion process of these contaminants (Gardner et al. 2006; Garcia-Fernandez et al. 2009).

Although the change in blood biochemistry caused by different diseases is similar in reptiles and mammals (Heard et al. 2004; Camacho 2013) and heavy metals and metalloids have potential adverse effects on the hematological and biochemical parameters of sea turtles (Suzuki et al. 2012), explaining the nature of each correlation remains speculative (Innis et al. 2008), due to probable involvement of immune responses and the pathologies that can develop. Hematologic characteristics and plasma chemistry values enable the detection of potential sources of contamination, disease, and the determination of baseline health values (Bolter and Bjorndal 1992; Aguirre and Balazs 2000). Moreover, blood characteristics in animals indicate physiologically and also specific values in relation to disease (Kakizoe et al. 2007). Even though the effects of potential toxic metals and other organic pollutants on vertebrates are known (Keller et al. 2004; Innis et al. 2008), their toxic effects and the immune response in wildlife are still unknown, particularly in sea turtles (Aguirre et al. 1994; Camacho et al. 2013).

In conclusion, this study further supports the use of blood as an ideal tissue for health assessments and heavy metal monitoring.

According to the physical examination, the North Pacific loggerhead sea turtle (*Caretta caretta*) population from Baja California Sur, Mexico, presents apparent good health; however, the results from this study suggest that, based on the associations with health clinical parameters, high levels of heavy metals such as Cd and As could be representing a risk to the North Pacific loggerhead population health. Therefore, further studies allowing for observation of the possible short-and long-term effects of these contaminants on the health of the sea turtles are needed.

Blood analysis can be used not only to diagnose sea turtle diseases but also to assess the health status of populations. Therefore, establishing baseline blood chemical profiles for healthy, wild populations of endangered sea turtle species has become a high priority. Information on sea turtle health can help to establish clinical attention in sea turtles. It is necessary to highlight the importance of support for program development and management plans that allow the performance of studies by trained personnel in the long term, contributing to the establishment of different factors for each sea turtle population, like abundance, densities, biochemical reference intervals, and medical attention to unhealthy turtles.

Acknowledgements This work was supported by the CIIDIR-SIN IPN, GRUPO TORTUGUERO, and EcoHealth Alliance (formerly known as Wildlife Trust). The first author acknowledges the participation of the Lopez Mateos Bay community, Baja California Sur, México. This research was performed under Mexican regulation and laws under a permit provided by SEMARNAT (Secretaría de Medio Ambiente and Recursos Naturales) Permit number SGPA/DGVS/03481/09. Partial funding was provided by the Project SIP-IPN 20090831 from Instituto Politecnico Nacional. The authors would like to thank CONACYT for support through a Ph.D. scholarship.

#### References

- Aguirre AA (1996) Plasma biochemistry values of green turtles (*Chelonia mydas*) with and without fibropapillomas in the Hawaiian Islands. Southwest Fisheries Science Center. Administ Report H-96-10C
- Aguirre AA, Balazs GH (2000) Blood biochemistry values of green turtle, *Chelonia mydas*, whith and whithout fibropapillomatosis. Comp Hematol Int 10:132–137
- Aguirre AA, Balazs GH, Zimmerman B, Spraker TR (1994) Organic contaminants and trace metals in the tissues of green turtles(*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. Mar Pollut Bull 28:109–114
- Aguirre AA, Bolen L, Stasiak I, Zavala-Norzagaray AA, Nichols WJ (2016) Ecology, management, and conservation policies of loggerhead turtles in the North Pacific. In: Aguirre AA, Sukumar R (eds) Tropical conservation: perspectives on local and global priorities. Oxford University Press, New York, NY, p 520
- Aguirre AA, Gardner SC, Marsh JC, Delgado SG, Limpus CJ, Nichols WJ (2006) Hazards associated with the consumption of sea turtle meat and eggs: a review for health care workers and the general public. EcoHealth 3:141–153
- Aguirre AA, Lutz PL (2004) Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator ? EcoHealth 1:275–283
- Aguirre AA, Starkey EE (1994) Wildlife disease in U.S. national parks: historical and coevolutionary perspectives. Conserv Biol 8:654–661
- Anan Y, Kunito T, Sakai H (2002) Tanabe S. Subcellular distribution of trace elements in the liver of sea turtles Mar Pollut Bull 45:224–229
- Baker A, McGrath S, Reeves R, Smith J (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: Terry N, Bañuelos G, Vangronsveld J (eds) Phytoremediation of contaminated soil and water. FL, Boca Raton, pp 85–107
- Barile F (2008) Principles of toxicology testing. CRC Press, Boca Ratón, FL
- Bolter AB, Bjorndal KA (1992) Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas: sizespecific and sex-specific relationships. J Wildl Dis 28:407–413
- Broderick AC et al (2006) Are green turtles globally endangered? Glob Ecol Biogeogr 15:21–26
- Camacho M (2013) La sangre como marcador de utilidad clínica y toxicológica: estudio de los efectos de los contaminantes persistentes en tortugas marinas. Doctoral Europea, Universidad de las Palmas de Gran Canaria
- Camacho M et al (2013) Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): results from a nesting colony from Cape Verde, West Africa. Mar Environ Res 92:15–22. doi:10.1016/j.marenvres.2013.08.002
- Cantilena LR (2008) Clinical toxicology. In: Klaassen CD (ed) Casarett and Doull's toxicology: the basic science of poisons, 7th edn. McGraw-Hill, Kansas City, Kansas, pp 1109–1122
- Casal AB, Orós J (2007) Morphologic and cytochemical characteristics of blood cells of juvenile loggerhead sea turtles (*Caretta caretta*). Res Vet Sci 82:158–165. doi:10.1016/j.rvsc.2006.07.017
- CITES (2015) Appendix lists species that are the most endangered among CITES-listed animals and plants. UICN. http://www.cites.org/eng/ app/index.php. Accessed May 12 2015
- Clark RB (2001) Marine Pollution, 5th edn. Oxford University Press, New York
- Day RD, Christopher SJ, Becker PR, Whitaker DW (2005) Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. Environ Sci Technol 39:437–446
- Day RD et al (2010) Comparison of mercury burdens in chronically debilitated and healthy loggerhead sea turtle (*Caretta caretta*). J Wildl Dis 46:111–117
- 🖉 Springer

- Day RD, Segars AL, Arendt MD, Lee AM, Peden-Adams MM (2007) Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (*Caretta caretta*). Environ Health Perspect 115:1421–1428
- Deem SL et al (2009) Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (*Caretta caretta*) along the coast of Georgia. USA J Wildl Dis 45:41–56. doi:10.7589/0090-3558-45.1. 41
- Eckert KL, Bjorndal KA, Abreu-Grobois FA (2000) Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group
- Eder E, Ceballos A, Martins S, Pérez-García H, Marín I, Marco A, Cardona L (2012) Foraging dichotomy in loggerhead sea turtles *Caretta caretta* off northwestern Africa. Mar Ecol Prog Ser 470: 113–122
- Flint M, Morton JM, Limpus CJ, Patterson-Kane JC, Murray PJ, Mills PC (2010) Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (*Chelonia mydas*). Vet J 185:299–304
- Frías-Espericueta MG, Cardenas-Nava NG, Márquez-Farías JF, Osuna-López JI, Muy-Rangel MD, Rubio-Carrasco W, Voltolina D (2014) Cadmium, copper, lead and zinc concentrations in female and embryonic pacific sharpnose shark (*Rhizoprionodon longurio*) tissues. Bull Environ Contam Toxicol 93:532–535. doi:10.1007/s00128-014-1360-0
- Frias-Espericueta MG, Osuna-Lopez JI, Ruiz-Telles A, Quintero-Alvarez JM, Lopez-Lopez G, Izaguirre-Fierro G, Voltolina D (2006) Heavy metals in the tissues of the sea turtle *Lepidochelys olivacea* from a nesting site of the northwest coast of Mexico. Bull Environ Contam Toxicol 77:179–185. doi:10.1007/s00128-006-1048-1
- Garcia-Fernandez AJ et al (2009) Heavy metals in tissues from loggerhead turtles (*Caretta caretta*) from the southwestern Mediterranean (Spain). Ecotoxicol Environ Saf 72:557–563. doi:10.1016/j.ecoenv. 2008.05.003
- Gardner S, Fitzgerald S, Vargas B, Rodríguez L (2006) Heavy metal accumulation in four species of sea turtles from the Baja California peninsula, Mexico. Biometals 19:91–99. doi:10.1007/s10534-005-8660-0
- Gardner SC, Nichols WJ (2001) Assessment of sea turtle mortality rates in the Bahía Magdalena region, Baja California Sur, México. Chelonian Conserv Bi 4(1):197–199
- Gelli D et al (2008) Establishing physiological blood parameters in the loggerhead sea turtle (*Caretta caretta*). Eur J Wildl Res. doi:10. 1007/s10344-008-0214-7
- Green-Ruiz C, Ruelas-Inzunza J, Paez-Osuna F (2005) Mercury in surface sediments and benthic organisms from Guaymas Bay, east coast of the Gulf of California. Environ Geochem Health 27:321–329. doi:10.1007/s10653-004-5741-x
- Green-Ruiz CR, Páez-Osuna F (2004) Potential bioavailability of heavy metals in surface sediments from the Altata-Ensenada del pabellon lagoon, SE gulf of California. J Coastal Res 20:1126–1134
- Guirlet E, Das K, Girondot M (2008) Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat Toxicol 88:267–276
- Heard D, Harr K, Wellehan J (2004) Diagnostic sampling and laboratory tests. In: Girling SJ, Raiti P (eds) Manual of reptiles, 2nd edn. British Small Animal Veterinary Association, Quedgeley England, pp 71– 86
- IDEXX (2014) IDEXX VetTest chemistry analyzer operator's manual. Maine, USA
- Innis C, Tlusty M, Perkins C, Holladay S, Merigo C, Weber Iii ES (2008) Trace metal and organochlorine pesticide concentrations in coldstunned juenile Kemp's ridley turtles (*Lepidochelys kempii*) from Cape Cod, Massachusetts. Chelonian Conserv Bi 7:230–239
- Kakizoe Y, Sakaoka K, Kakizoe F, Yoshii M, Nakamura H, Kanou Y, Uchida I (2007) Successive changes of hematologic characteristics

and plasma chemistry values of juvenile loggerhead turtles (*Caretta caretta*). J Zoo Wildl Med 38:77–84

- Keller JM, Kucklick JR, Stamper MA, Harms CA, McClellan-Green PD (2004) Associations between organochlorine contaminant concentrations and clinical health parameters in Loggerhead Sea turtles from North Carolina, USA. Environ Health Perspect 112:1074– 1079
- Keller JM, McClellan-Green PD, Kucklick JR, Keil DE, Peden-Adams MM (2005) Effects of organochlorine contaminants on loggerhead sea turtle immunity: comparison of a correlative field study and in vitro exposure experiments. Environ Health Perspect 114:70–76
- Kenyon LO, Landry AM, Gill GA (2001) Trace metal concentrations in blood of the kemp's ridley sea turtle (*Lepidochelys kempii*). Chelonian Conserv Biol 4:128–135
- Kitana N, Callard IP (2008) Effect of cadmium on gonadal development in freshwater turtle (*Trachemys scripta*, *Chrysemys picta*) embryos. J Environ Sci Health A 43:262–271. doi:10.1080/ 10934520701792753
- Kucuksezgin F, Kontas A, Altay O, Uluturhan E, Darilmaz E (2006) Assessment of marine pollution in Izmir Bay: nutrient, heavy metal and total hydrocarbon concentrations. Environ Int 32:41–51
- Ley-Quinonez C, Zavala-Norzagaray AA, Espinosa-Carreon TL, Peckham H, Marquez-Herrera C, Campos-Villegas L, Aguirre AA (2011) Baseline heavy metals and metalloid values in blood of loggerhead turtles (*Caretta caretta*) from Baja California Sur, Mexico. Mar Pollut Bull 62:1979–1983. doi:10.1016/j.marpolbul.2011.06. 022
- Ley-Quinonez CP et al (2013) Selected heavy metals and selenium in the blood of black sea turtle (*Chelonia mydas* agasiizzi) from Sonora, Mexico. Bull Environ Contam Toxicol 91:645–651. doi:10.1007/ s00128-013-1114-4
- Liu J, Goyer RA, Waalkes MP (2008) Toxic effects of metals. In: Klaassen CD (ed) Casarett and Doull's toxicology: the basic science of poisons, 7th edn. McGraw-Hill, Kansas City, Kansas, pp 931– 979
- Maffucci F, Caurant F, Bustamante P, Bentivegna F (2005) Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (*Caretta caretta*) from the Western Mediterranean Sea (southern Italy). Chemosphere 58: 535–542
- Owens DW (1999) Reproductive cycles and endocrinology. In: Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M (eds) Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4,
- Paez-Osuna F, Calderon-Campuzano MF, Soto-Jimenez MF, Ruelas-Inzunza JR (2010a) Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific: concentration, isotopic composition and maternal transfer. Mar Pollut Bull 60:433– 439. doi:10.1016/j.marpolbul.2009.10.004
- Paez-Osuna F, Calderon-Campuzano MF, Soto-Jimenez MF, Ruelas-Inzunza JR (2010b) Trace metals (Cd, Cu, Ni, and Zn) in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony of Oaxaca. Mexico Arch Environ Contam Toxicol 59:632– 641. doi:10.1007/s00244-010-9516-3
- Sakai H, Saeki K, Ichihashi H, Kamezaki N, Tanabe S, Tatsukawa R (2000a) Growth-related changes in heavy metal accumulation in green turtle (*Chelonia mydas*) from Yaeyama Islands, Okinawa, Japan. Arch Environ Contam Toxicol 39:378–385
- Sakai H, Saeki K, Ichihashi H, Suganuma H, Tanabe S, Tatsukawa R (2000b) Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar Pollut Bull 40: 701–709

- Samour JH, Howlett JC, Silvanose C, Hasbún CR, Al-Ghais SM (1998) Normal haematology of free-living green sea turtles (*Chelonia mydas*) from the United Arab Emirates. Comp Haematol Int 8: 102–107
- SEMARNAT (2010) Protección ambiental Especies nativas de México de flora y fauna silvestres - Categorías de riesgo y especificaciones para su inclusión, esclusión o cambio - Lista de especies en riesgo. NOM-059-SEMARNAT-2001, México
- Seminoff JA et al (2014) Loggerhead sea turtle abundance at a foraging hotspot in the eastern Pacific Ocean: implications for at-sea conservation. Endang Species Res 24:207–220
- Sikes JM IV, Klaphake E (2008) Reptile hematology veterinary clinics. Exot Anim 11:481–500
- Spotila JR (2004) Sea turtles: a complete guide to their biology, behavior, and conservation. The Johns Hopkins University Press, Baltimore
- Storelli MM, Barone G, Storelli A, Marcotrigiano GO (2008) Total and subcellular distribution of trace elements (Cd, Cu and Zn) in the liver and kidney of green turtles (*Chelonia mydas*) from the Mediterranean Sea. Chemosphere 70:908–913
- Storelli MM, Ceci E, Storelli A, Marcotrigiano GO (2003) Polychlorinated biphenyl, heavy metal and methylmercury residues in hammerhead sharks: contaminant status and assessment. Mar Pollut Bull 46:1035–1039. doi:10.1016/S0025-326X(03)00119-X
- Storelli MM, Marcotrigiano GO (2000) Total organic and inorganic arsenic from marine turtles (*Caretta caretta*) beached along the Italian coast (South Adriatic Sea). Bull Environ Contam Toxicol 65:732– 739
- Storelli MM, Marcotrigiano GO (2003) Heavy metal residues in tissues of marine turtles. Mar Pollut Bull 46:397–400. doi:10.1016/S0025-326X(02)00230-8
- Storelli MM, Storelli A, D'Addabbo R, Marano C, Bruno R, Marcotrigiano GO (2005) Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. Environ Pollut 135:163–170. doi:10.1016/j.envpol. 2004.09.005
- Suzuki K et al (2012) Relationships between carapace sizes and plasma major and trace element status in captive Hawksbill Sea Turtles (*Eretmochelys imbricata*). J Vet Med Sci 74:1677–1680. doi:10. 1292/jvms.12-0099
- Tabor GM, Aguirre AA (2004) Ecosystem health and sentinel species: adding an ecological element to the proverbial "canary in the mineshaft". EcoHealth 1:226–228
- Tomaszewicz CNT et al (2015) Age and residency duration of loggerhead turtles at a North Pacific bycatch hotspot using skeletochronology. Biol Conserv 186:134–142. doi:10.1016/j.biocon.2015.03.015
- van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY (2010) Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*). Mar Pollut Bull 60: 579–588
- Wang HC (2005) Trace metal uptake and accumulation pathways in Kemp's Ridley Sea turtles (*Lepidochelys kempii*). Texas A&M, College Station, TX
- Whiting SD, Guinea ML, Limpus CJ, Fomiatti K (2006) Blood chemistry reference values for two ecologically distinct populations of foraging green turtles, eastern Indian Ocean. Comp Clin Pathol 16:109– 118
- Wilcox BA, Aguirre AA (2004) One Ocean. One Health EcoHealth 1: 211–212
- Zavala-Norzagaray AA, Ley-Quiñónez CP, Espinosa-Carreón TL, Canizalez-Román A, Hart CE, Aguirre AA (2014) Trace elements in blood of sea turtles *Lepidochelys olivacea* in the Gulf of California. Mexico Bull Environ Contam Toxicol 93:536–541. doi:10.1007/s00128-014-1320-8

Environmental Science & Pollution Research is a copyright of Springer, 2017. All Rights Reserved.