Olive Ridley as Environmental Bioindicator - A Review

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Abstract - Olive Ridley Sea Turtles are listed as vulnerable under IUCN. Characterization of different molecular parameters rather than scaling the correlation of different pollutants to different proteins and genes of this animal is necessary to deduce the path of their sustainability related to reptiles' biodiversity. These turtles show a large geographical range of migration and are exposed to different stressors i.e. biochemical as well as biophysical marine environment and hence are considered as interesting bio-indicators too. Monitoring these turtle populations for the toxicological effects of different persistent organic pollutants and different heavy metals is the fundamental approach for their protection and sustainability.
This review includes the analysis of several toxicological studies that characterize the pollutants accumulation nature in different tissues of the Olive Ridley as well as the nature of excretion, and effects of pollutants on different molecular, morphological, and developmental parameters.
Olive Ridley was surveyed in several geographical marine habitats like in the pelagic Pacific longline revealed a high ingestion rate of black and blue plastic fibers with microbeads, and thermoplastics by different methods, and most of them accumulated in their large intestine. The amount of plastic ingested was found to be correlated to POP (Persistent Organic Pollutants) in their fats and the result analysis rather than review revealed higher ∑DDT (dichlorodiphenyltrichloroethane ethane and metabolites) than ∑PCB (Polychlorinated biphenyl). The POPs were maternally transferred to the hatchlings, resulting in developmental abnormalities. Cd, Zn was found to be highest in blood and yolk with the presence of Hg, Cu, and Ni, in the excreta of egg-laying females. Evaluation of the metals in nesting females showed the highest renal Cd level in any sea turtle. POP concentrations were unrelated to the amounts of ingested plastic in olive ridleys, suggesting that their exposure to POPs is mainly through prey. The findings suggest variations in the animal's biological parameters are caused by the environment rather than marine pollution. Several research outcomes indicate carapace asymmetry is significantly associated with pollutant levels in adult Olive Ridley and could be used as a biomarker for some metal accumulation. The rate of gene expression of different anti-oxidants like Superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) was increased mainly in the liver tissue with heavy metal exposure is indicative of both their increased adaptability as well as their acceptance as

a bio-indicator. P-nitrophenyl acetate esterase activity (EA) and cortisol concentrations in the serum of marine Olive Ridley are also found to have immense applications be used as a biomarker as their amount in the serum of the animal was found to be positively correlated to metal and metalloid pollution in their respective marine environment. The malformed embryos tested and confirmed to have higher levels of Hg and the embryos with *Schistosomusreflexu*s syndrome (SR), had higher DNA methylation than normal. A potential accumulation of Polychlorinated biphenyl (PCB) that is CYP1A substrate was found, providing the Olive Ridley with biotransformation capacity.
Thus, Olive Ridley tends to accumulate organic and inorganic pollutants in their long life and can be harnessed as useful bioindicators. Moreover, the usefulness of blood for biomonitoring with respect to pollutants was also established.

Keywords*: *Olive Ridley, Bioindicators, Plastics, Heavy metals, Antioxidant, DNA methylation, Pollutants.

I. INTRODUCTION

The Olive Ridley turtle (*Lepidochelys olivacea*) is a species distributed throughout the tropics. Its largest populations are found in the Pacific and Indian Oceans [1]. Like all marine turtles, the Ridley turtle is considered an endangered species (IUCN 2012) and is listed in Appendix I of the Convention of International Trade in Endangered Species (CITES). The main threats that have brought turtles to this precarious situation are, among others, their exploitation of meat, eggs, and shells in certain regions around the world, by-catch, global warming, loss of nesting sites, and environmental pollution.[2,3] According to several authors, anthropogenic-induced stressors, such as coastal pollution, can exacerbate other adverse impacts and seriously compromise the ability of sea turtles to adapt to climate change.[4] In this context, it is necessary to study biological markers for the evaluation of pollution effects on sea turtles. Possible toxic metals (such as Cd and Pb) are contaminants of important environmental relevance due to their high risk of toxicity and persistence. [5]Lead (Pb) and cadmium (Cd) are among the elements most studied in aquatic environments, and both have been associated with different pathologies in many vertebrate species, such as bone necrosis, endocrinological alterations, and immune and reproductive problems.[6]

II. MATERIALS AND METHODS

Polychlorinated Biphenyls and Biotransformation enzymes of 3 species of Sea Turtle from the Baja California Peninsula of Mexico

Samples of liver tissue were collected from stranded wild-born loggerhead, green, and olive ridley turtles during 2003-2006. They were found primarily in the waters surrounding Baja California and Baja California Sur.[7] Turtles were necropsied and a sample of liver tissue was placed in aluminum foil and stored on ice for transport and then stored a -80C until analysis.
Approximately 3 g of liver tissue from four loggerheads, three green, and four olive ridley turtles was spiked with PCB 65 surrogate standard prior to homogenization with anhydrous

sodium sulfate, followed by extraction with hexane using an ultrasonic disruptor (550 Sonic Dismembrator; Fisher Scientific) according to EPA method 3550B as described else. The extract was evaporated to 2 mL and 250 mL were removed for gravimetric lipid determination. The remaining extract was treated by vigorous vortexing with 2 mL of hexane-cleaned concentrated sulfuric acid. The acid layer was then back-extracted two times with hexane and the hexane fractions were combined and evaporated to 0.25 mL for gas chromatograph–electron capture detector (GC-ECD) analysis. Quantification of individual PCB congeners (IUPAC PCB8,18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128,138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206, and 209) was performed using the relative response factors generated from the calibration curve of standards (Mullin et al. 1984[8]). The subcellular fractions were isolated by sequential centrifugation at 20,000g for 30 min and then at 100,000g for 90 min, both performed at 4C to prevent protein degradation. Due to limited biomass, profiles of CYP isoforms in sea turtles were evaluated by Western blotting using CYPantiserum raised against fish CYP isoforms because antibodies for sea turtle CYP isoforms are not available. Microsomal proteins (50 lg per lane), along with molecular-weight markers (See Blue Plus2; Invitrogen, Carlsbad, CA, USA) were resolved using polyacrylamide gels [sodium dodecyl (SDS)-PAGE, 10% gradient].

P-Nitrophenyl Acetate Esterase activity and Cortisol as Biomarkers of metal pollution in the blood of Olive Ridley Turtles

Forty-four nesting female turtles were randomly selected. Blood samples were carefully taken from the dorsal cervical sinus, using a 5-mL syringe with 21″needles. The turtles showed no alteration in their behavior after sampling. Neck region was carefully cleaned using ethanol then blood was collected. For EA and cortisol analysis, blood samples were immediately transferred into empty tubes and centrifuged at 2000 rpm for 10 min at room temperature to obtain serum, which was transferred into − 20 °C at Eppendorf® microtubes. For metal analysis, the complete blood samples were stored in 1.5 Eppendorf® microtubes at − 20 °C until analyses. Blood samples were pretreated as previously described [9]. Inorganic element concentration was determined using an Inductively Coupled Plasma Optical Emission Spectrophotometer. EA was analyzed in an automated clinical chemistry analyzer by measuring the hydrolysis of p-nitrophenol acetate to p-nitrophenol as described by Haagen and Brock (1992), with some modification[10]. Cortisol was analyzed in an automated chemiluminescent immunoassay.

Trace metals (Cd, Ni, Cu, Zn) in blood and eggs of sea turtle Lepidochelys olivacea from a nesting colony in Oaxaca, Mexico

Eggs and blood of L. olivacea were sampled from EB, Oaxaca, Mexico during the nesting season 2005–2006 during the third ‘‘arribada’’ event, between 10 and 14 August 2005.
After biometric measurements, blood and egg samples were collected from each female turtle; blood samples were taken from the dorsal cervical sinus using a sterile plastic syringe and needle in order to collect 5–10 ml that were immediately placed in an acid-washed polyethylene tube. All blood samples were kept under fresh conditions (4°C) and were transported to the laboratory. Once at the lab, these eggs and blood were stored at -20C.
Egg samples were rinsed with deionized water (Milli-Q;18.3 MX/cm) to remove any particulate matter that might have adhered. Next, eggs were weighed and sized and subsequently separated into shells, albumen, and yolk. Blood and pooled samples of eggs

were freeze-dried (72 h at -49°C and 133 9 10-3 bar) and then powdered. Powdered samples (0.25 g) were digested with quartz-distilled concentrated nitric acid (5 mL) in microwave equipment (CEM; MDS 2000) under established conditions (MESL 1997). The digested material was finally diluted to 25 ml using purified (Chelex-100 resin; Bio-Rad; 100–200mesh) deionized water and stored in a polyethylene container for further analysis. Analyses were made by flame atomic absorption spectrophotometry (FAAS) for Zn; in the case of Cd, Cu, and Ni, graphite furnace atomic absorption spectrophotometry (GFAAS) was used. [11–13]

Relationship between plasma biochemistry values and metal concentration in nesting Olive Ridley

Blood samples were taken from the dorsal cervical sinus, using a 5-mL syringe with a 21″ needle. For the biochemical parameters, blood was placed in repose into a tube without anticoagulant (Vacutainer®) for approximately 2 h to separate serum from the red cellular package. Then, the serum was transferred into microtubes (Eppendorf®). For metal analysis, the whole blood samples were stored in 1.5 microtubes (Eppendorf®). All samples were kept at − 20 °C until analyses. The biochemical constituents of the serum were measured using an automated clinical chemistry analysis The biochemical panel included alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, albumin, cholesterol, glucose, proteins, triglycerides, urea, and P-nitrophenyl acetate esterase activity (EA). Cortisol was analyzed in an automated chemiluminescent immunoassay. Prior to metal analysis, acid digestion was performed using 0.5 g of the sample into 4 mL ofHNO3 (69%) and 1 mL of H2O2 (33%) mixed in special Teflon reaction tubes in microwave digestion system36672 Environ SciPollut Res (2018) 25:36671–36679 (UltraClave-Microwave Milestone) for 20 min at 220 °C and finally diluted with 25 mL of double deionized water (MilliQ). Metal concentrations were determined using an inductively coupled plasma optical emission spectrophotometer (ICPOES, ICAP 6500 Duo Thermo) following descriptions from [10]. All concentrations are expressed in μg in wet weight.

III Review of Results

Polychlorinated Biphenyls and Biotransformation enzymes of 3 species of Sea Turtle from the Baja California Peninsula of Mexico

PCB Congeners 28,81,195,209 were not detected while 105,138,153constituted 33-63% of tPCB concentrations.[10]Mean PCB concentrations were 18.1, 10.5, and 15.2 for loggerhead, green, and olive ridley turtles, respectively. However, no significant differences were found in the tPCB concentrations among species ($p = 0.451$). Pentachlorinated biphenyls also contributed considerably to PCBs.[14]

Hepatic microsomal protein showed a high degree of variability in the patterns of bands among individuals; some turtles exhibited a singlet band, whereas others displayed doublets. No correlations were observed between CYP expression with tPCB or PCB metabolic groups.

p-Nitrophenyl Acetate Esterase Activity and Cortisol are used as Biomarkers

The detected concentrations of EA, cortisol, and chemical elements in blood are recorded. All of the samples analyzed exhibited values above the detectable limit for all elements, except cortisol, which was below DL in many experimental cases as reported. Zn was the metal with the highest concentrations in blood, whereas Pb was the element with the lowest concentrations of ions.

Trace metals (Cd, Ni, Cu, Zn) in blood and eggs of sea turtle Lepidochelys olivacea from a nesting colony in Oaxaca, Mexico

The metal concentrations in L. olivacea from EB were reported as follows: for Zn, yolk; for Cu, eggshell; for Cd, blood; for Ni, eggshell showed maximum level, and concentrations in other parts are given in the following figure.

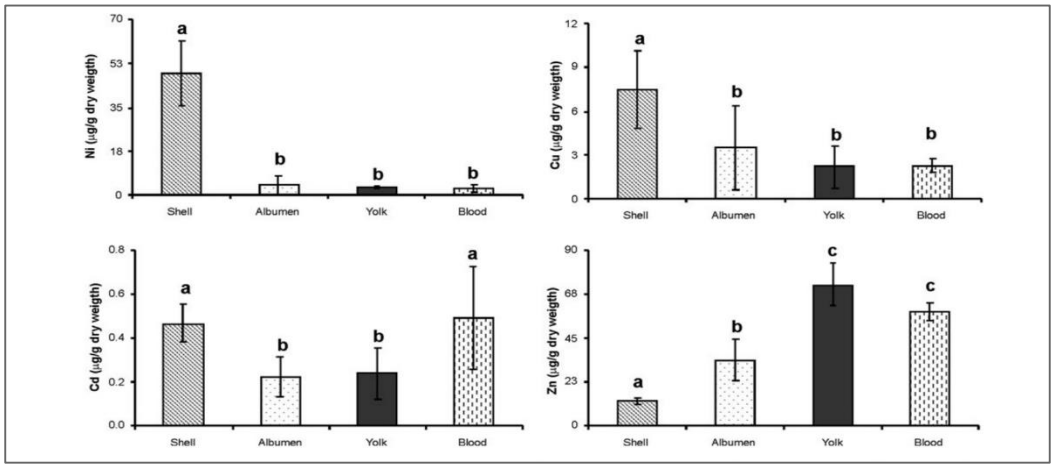

Figure-Metal concentrations in analyzed materials in *L.olivacea*

The multiple comparisons of means of metals evidence various significant (p\0.05) differences: The Cu and Ni mean concentrations were higher in the eggshell than the other tissues; Zn levels were different among shell, albumen, and yolk. The Cd concentration was higher in blood and eggshell, whereas the Zn concentration was higher in yolk.
For the correlation between the weights of eggs and the concentration of metals analyzed in each fraction of eggs, only a defined pattern was evidenced and significant (p\0.05); Ni in albumen had a negative tendency with the natural logarithm of the weight of eggs; clearly, it indicates that when the egg size is increased, the Ni content in albumen is reduced. On the other hand, in the case of a load of metal in the blood of the nesting females versus the metal levels found in the fractions of eggs, direct linear relationships were not significantly correlated (p\0.05); only Ni in eggshell versus the natural logarithm of Ni in blood showed a significant (p\0.05) correlation, which indicates that when Ni is increased in the female turtles, the concentration of Ni is increased in the eggshell.[15–17]

Relationship between plasma biochemistry values and metal concentration in nesting Olive Ridley

All inorganic elements in whole blood as included in several experimental studies (n = 100) were above the detection limit (<0.01 µg g−1). Zinc presented the highest concentration with

a mean ± standard deviation of 7.7 ± 2.4 μg g−1; it was followed by Se with 6.72 ± 3.0, As 1.27 ± 0.9, Sr 1.02 ± 0.45, Cu 0.52 ± 0.2, Mn 0.41 ± 0.12, Cr 0.17 ± 0.07, Cd 0.12 ± 0.05, Ni 0.07 ± 0.07, Ti 0.03 ± 0.08, and finally Pb, with 0.02 ±0.01 μg g−1.Strontium had four positive relationships with ALT, AST, urea, and albumin; titanium also had four positive relationships with creatinine, urea, cholesterol, and cortisol; lead had two positive relationships (AST and glucose) and one negative (EA); cadmium had two negatives with creatinine and glucose; arsenic had two positives (AST and urea) and two negatives (glucose and cholesterol); selenium had two positives (creatinine and glucose) and two negatives (urea and EA); zinc had three negatives (AST, albumin, and creatinine) and one positive (EA); copper one positive (cholesterol) and one negative (ALT); and, finally, chrome had only one negative relationship with cholesterol.

IV. CONCLUSIONS

In conclusion, PCB levels in loggerhead, green, and olive ridley turtles from the Baja California region of Mexico are generally lower than those reported from more polluted regions of the globe. The presence of dioxin-like congeners and relatively high TEQs along with a lack of CYP1A expression suggest a potential mechanism of accumulation of group 1 congeners. Future studies might clarify the contributions of CYP and GST isoforms. Different results of the reviewed works indicate that some inorganic elements could have a significant effect on p-nitrophenyl acetate esterase activity and on cortisol in Olive Ridley turtles and could be considered useful biomarkers related to the differential contamination in this species. The lack of studies regarding concentrations of understudied elements in this species, such as Ti and Sr in marine turtles, and the weak but insignificant correlations detected between EA and Cd, Pb, and Ti, and those between cortical and Sr, As, and Se, highlight the necessity for further research in this field to identify the biological responses to each one of these elements. Results confirm the importance of this component in the accumulation of trace metals in marine turtles. On the basis of the nesting season, the excretion rates of trace metals through egg-laying were estimated; Cd was 0.2%, whereas Cu, Zn, and Ni were 5.9, 4.1, and 20.1%, respectively. It indicates that egg-laying is not a major route for transferring nonessential metals (perhaps with the exception of Ni), but essential metals are transferred at a higher rate, possibly as a source mechanism for the hatchlings. Evidence from reviewed articles suggests several associations between metal concentrations and biochemical parameters.

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