Paresis as a limiting factor in the reproductive efficiency of a nesting colony of *Lepidochelys olivacea* in La Escobilla beach, Oaxaca, Mexico

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Short Report

Keywords: Escobilla, health, Lepidochelys, muscle, rear-flippers, paresis.

Posted Date: March 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1442543/v1

Abstract

Rear flippers are crucial in the nesting process of olive ridley turtles (Lepidochelys olivacea), so any impact on them could constitute a limiting factor in reproductive efficiency. Muscle weakness of the rear legs has been observed in some nesting females on La Escobilla beach in Oaxaca state, Mexico; however, this disorder has not been sufficiently researched. The aim of this study was to identify and describe this problem in a nesting colony of L. olivacea in La Escobilla. We obtained the biochemical profiles of eight females with clinical signs of muscle weakness of the rear legs, that could not build the incubation chamber for their nest. In order to compare their blood characteristics, we selected eight seemingly healthy turtles that successfully built their nests, laid eggs through oviposition and covered the nest. We found no significant differences in most of the blood parameters, except for Creatinine-Kinase (CK). Female turtles with muscle weakness presented significantly higher concentrations of CK ($t = 2.1448$, d. f.= 2, $p<0.0001$) when compared to the healthy turtles. CK is an appropriate enzyme for identifying the integrity of the muscle cell and is a muscle damage indicator. Our hypothesis is that the paresis observed in the rear legs of the female turtles in La Escobilla could be a chronic debilitation caused by a gradual exposure to biotoxins such as saxitoxins.

Introduction

The olive ridley turtle (*Lepidochelys olivacea*) is one of the most abundant sea turtle species and classified as vulnerable by the International Union for Conservation of Nature (Abreu-Grobois and Plotkin 2008). In Mexico, all sea turtles are also classified as endangered species through the regulation NOM-ECOL-059-2010, and additionally, they are considered a priority species for conservation.

The olive ridley turtle has a circumtropical distribution and inhabits the Atlantic, Pacific and Indic Oceans, living mainly in the northern hemisphere (Bjorndal 1997). On the American continent, their most notable nesting beaches are located in Costa Rica (Cornelius et al. 1991, Fonseca et al. 2009), Panama (Cornelius et al. 2007, Honarvar et al. 2016), Nicaragua (Stewart 2001, Hope 2002), and Mexico (Peñaflores et al. 2000, Campbell 2007).

In Mexico, one of the world's major nesting sites for this species is La Escobilla beach in Oaxaca state; where impressively large mass synchronous nesting aggregations – called arribadas – occur (Márquez and Van Dissell 1982). Despite their importance, relatively little is known about the health status or the presence of clinical signs of a disease in the nesting colony (Mashkour et al. 2020). In addition to human activity and predation, diseases are another important factor that have contributed to the decrease in the population of sea turtles (Wallace et al. 2010).

In olive ridley nesting activity, rear flippers play a key role in building the nest cavity (egg chamber); hence, the depth of a nest chamber is dependent on the size of a female's rear flippers (Rusli 2019). Therefore, rear flippers are crucial in the nesting process, and any impact on them could constitute a limiting factor in reproductive efficiency.

On La Escobilla beach, various nesting turtles with muscle weakness of the rear legs have frequently been observed during the arribadas, a situation that is widely known empirically; however, the disorder has not been sufficiently researched. Until now, no studies on this subject have been performed on any nesting colony of *Lepidochelys olivacea*; thus, the aim of this study was to identify and describe this problem in a nesting colony of *Lepidochelys olivacea* in La Escobilla, Oaxaca, Mexico. This information will contribute to the protection of the species and allow for long-term health assessments and monitoring.

Materials And Methods

This study was conducted on La Escobilla beach, located in the municipality of Santa Maria Tonameca on the southwest Mexican Pacific coast (15°43’37.56” N, 96°44’49.23” W). The beach is approximately 25 km long, and the olive ridley turtles mainly nest along an 8-km strip at its western end.

During the development of a research project focused on assessing blood parameters in nesting olive ridley turtles (*L. olivacea*) on La Escobilla beach, we encountered several females that displayed difficulties in making the egg chamber during the arribada events (September, October and November) of the 2021 nesting season.

We obtained the biochemical profiles of eight females with clinical signs of muscle weakness of the rear legs that could not build the incubation chamber; as a result, after many attempts they ended up laying their eggs on the surface level of the sand (Figure 1). Additionally, in order to compare their blood characteristics, we selected eight seemingly healthy turtles that successfully built their nests, laid eggs through oviposition and covered the nest. Previously, we conducted an external inspection of all the turtles evaluated to determine the existence of traumatic injuries.

Biometric data of all the turtles were taken according to the methodology described by Bolten (2000), including curved carapace length (CCL) and curved carapace width (CCW), which were measured using a flexible fiberglass tape (measurement error ± 0.5 cm, Limpus et al. 1983). The weight of each turtle was measured by suspending the turtle attached to a digital scale (MH-C 100 model, Mini Crane Scale).

Afterward, we collected blood samples from the dorsal cervical sinus, using a 1.5’-gauge needle and a 5-ml syringe and transferred the samples into Vacutainers ® containing lithium heparin as an anticoagulant (Owens and Ruiz 1980). Prior to sampling, the puncture area was cleaned. Samples were stored in refrigeration at 4°C until laboratory processing at the Universidad del Mar (not more than eight hours after sampling).

A whole blood sample was centrifuged in an Eppendorf model 5430 centrifuge at 3000 rpm for 10 min. We used plasma in preference to serum because in reptiles clot formation is unpredictable, changing biochemical values and occasionally producing hemolysis in the blood samples (Bolten and Bjorndal 1992). Plasma was placed posteriorly into 1.6-ml cryogenic vials. Sixteen plasma parameters were recorded using the automated blood chemistry analyzer Celercare V5 (Kabla Veterinary DX) to establish

the sea turtles' health profiles (Anderson et al. 2011, Espinoza-Romo et al. 2018). Additionally, we repeated the sampling process in the healthy turtles.

Analyzed parameters were divided into three groups: 1) nutrients and metabolites: Albumin (ALB; g dL^{-1}), total protein (TP), globulin (GLO), Albumin/Globulin (A/G) ratio, glucose (GLU), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CRE), blood urea nitrogen/creatinine (BUN/CRE) ratio, total bilirubin (TBIL); 2) enzymes: amylase (AMY), Alanine aminotransferase (ALT), creatinine kinase (CK), alkaline phosphatase (ALP); and 3) electrolytes: calcium (Ca) and phosphorus (P).

Results are presented as mean, range and standard deviation (SD). Data normality and homoscedasticity were assessed using Kolmogorov-Smirnov and Levene tests, respectively. Differences in blood parameters between groups (with and with clinical signs) were assessed employing a *t*-student test. We performed all analyses using Past 4.08 statistical software (Hammer et al. 2001).

Turtle samples were collected under the SGPA/DGVS/03919/21 permit granted by SEMARNAT, Mexico.

Results

Descriptive statistics of SCL, WCL, CCL, CCW, weight and blood parameters of the olive ridley turtles are presented in table 1. We found no significant differences in most of the blood parameters, except for CK.

Female turtles with muscle weakness presented significantly higher concentrations of CK ($t = 2.1448$, d. f.= 2, $p<0.0001$) when compared with healthy turtles. During the external examination of the turtles, there was no evidence of recent traumatic injuries. However, one of the turtles with evident muscle weakness (paresis) of the rear legs laid eggs on the surface of the sand, after several failed attempts to perform the incubation chamber (Fig. 1).

Discussion

Although olive ridley turtles are the most abundant sea turtle species globally, knowledge regarding their health on mass nesting beaches remains limited. We found similar values in most blood parameters between turtles with clinical signs of paresis and seemingly healthy turtles; however, CK was highlighted as the muscle damage indicator.

CK is an appropriate enzyme for identifying the integrity of the muscle cell; and is considered a specific muscle enzyme; that is, it increases in the bloodstream when a muscle disease is present (Perrault et al. 2012, Anderson et al. 2013). With this in mind, paresis is characterized as an inability of muscles to perform their usual functions. Its physiopathology is related to the motor function of the voluntary tracks consisting of the upper and lower motor neurons, peripheral nerves, neuromuscular plate and muscle fibers, so damage to any of these structures causes a paresis that depends on the degree of the injury.

Except for Espinoza-Romo et al. (2018), there are no previous reports on CK values in *L. olivacea*. Those authors showed a CK mean of 245.3 ± 386 for *L. olivacea* in northern Sinaloa, Mexico. On the contrary, we found high CK values in turtles with obvious muscle problems of the rear legs; however, the apparently healthy females showed values close to those reported by Espinoza-Romo et al. (2018). Because there are no papers published about muscle weakness (paresis) in *L. olivacea*, we compared our results to *Caretta caretta*, since this species has some similarities in their eating habits, sharing an analogous position in the food chain.

Previous studies on several species have reported different degrees of paresis. Jacobson et al. (2006) describes clinical signs of a neurological disorder in subadult loggerhead sea turtles (*Caretta caretta*) in south Florida, USA. In this study, there was no evidence of heavy metal toxicosis and organophosphate toxicosis as possible causes; instead, the clinical changes observed resulted from combined spirorchiid parasitism and possible chronic exposure to a novel toxin present in the diet of *Caretta caretta*.

Herrera-Galindo et al. (2015) reported several dead sea turtles (*Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys olivacea*) on the coast of Oaxaca, Mexico. This study described the presence of salps and cells of *Pyrodinium bahamense* in the turtles' stomach contents. Later, Ley-Quiñónez et al. (2020) presented evidence of Paralytic Shellfish Poisoning (PSP) causing mass mortality of sea turtles in Puerto Vallarta, Jalisco, Mexico.

PSP has been identified as the most toxic and dangerous syndrome along the Pacific coast (Sierra-Beltrán et al. 1998). Two dinoflagellate species (*Gymnodinium catenatum* and *Pyrodimium bahamense*) produce saxitoxin that is associated with these PSP events (Ochoa et al. 1997, Cusik and Sayler 2013). These phenomena affect the entire trophic web, principally primary consumers, but organisms such as fish, marine mammals and sea turtles that feed on planktivorous species may also be affected (Garate-Lizarraga et al. 2004, Sellner et al. 2003).

In animals and humans, clinical signs of saxitoxin intoxication include muscular paralysis and pronounced dyspnea, which – if not promptly treated – can result in death from respiratory paralysis (Hallegraeff 1993). Although lethal doses of saxitoxins have not been defined for sea turtles or other reptiles (González-Barrientos et al. 2019), constant ingestion of this dinoflagellate species could probably provide enough toxin to produce muscle weakness of the rear legs in sea turtles.

Later, González Barrientos et al. (2019) presented evidence of abnormal clinical signs in stranded green sea turtles (*Chelonia mydas*) that were exposed to saxitoxins and tetrodotoxins on the southern Caribbean coast of Costa Rica. Among the stranded turtles, two live green turtles exhibited extreme paresis. Saxitoxicosis in green turtles appears to have resulted from opportunistic foraging on the Caribbean sharp-nose puffer (*Canthigaster rostrata*).

Although specific causes of muscle weakness (paresis) in olive ridley turtles remain unknown, we hypothesize that the paresis observed in the rear legs of the female turtles in La Escobilla could be a chronic debilitation due to a gradual exposure to biotoxins such as saxitoxins. It is important to mention

that this debilitation could be a limiting factor in the reproductive efficiency of a nesting colony of *Lepidochelys olivacea* in La Escobilla, Oaxaca; therefore, we recommended initiating a continuous monitoring program to follow the occurrence of paresis in subsequent years in order to better document its prevalence and to follow the progression of this muscle weakness among sea turtles.

Declarations

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Universidad Autónoma del Estado de México (Code: 18-AGO-2021-1).

Consent for publication

The authors declare that we agree with the process of publication of this work.

Availability of data and materials

There is no database available.

Competing interests

The authors declare that they have no conflict of interest.

Funding

The authors did not receive support from any organization for the submitted work.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Alejandra Buenrostro Silva, Jesús García Grajales, Petra Sánchez Nava and María de Lourdes Ruiz Gómez. The first draft of the manuscript was written by Alejandra Buenrostro Silva and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank the Universidad Autónoma del Estado de México (UAEMex) and its Division of Postgraduate Studies (PCARN) for the logistics and facilities provided. To Universidad del Mar for the facilities provided to project CUP: 2IR2104. Research permits were obtained from Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT: SGPA/DGVS/03919/21). In addition, special thanks go to A.T. Rosewicz for her revisions and suggestions that improved the English of this manuscript. JG-G, PS-N, and MLRG thank the Sistema Nacional de Investigadores (SNI) for its grant.

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Tables

Table 1. Biometric data and blood chemistry of the olive ridley turtles with clinical signs of muscle weakness of the rear legs (paresis) and seemingly healthy turtles in La Escobilla beach, Oaxaca, Mexico.

	Turtles with paresis		Seemingly healthy	
	Mean (SD)	Range	Mean (SD)	Range
Biometric data				
Weight	36.2 ± 6.71	27.55 – 45.45	36.29 ± 3.56	31 – 42
CCL	65.97 ± 4.18	61.5 – 72.1	65.77 ± 2.59	63 – 69.5
CCW	69.3 ± 4.91	62.6 – 78.7	69.6 ± 2.78	65.9 – 73
Blood chemistry				
ALB	1.01 ± 0.16	0.8 – 1.3	1.15 ± 0.23	1 – 1.7
TP	3.41 ± 0.49	2.8 – 4.2	3.6 ± 0.4	3.2 – 4.5
GLO	2.4 ± 0.45	1.9 – 3.3	2.4 ± 0.2	2.2 – 2.8
A/G	0.44 ± 0.09	0.3 – 0.6	0.46 ± 0.07	0.4 – 0.6
GLU	76.6 ± 25.56	42 – 107	106.6 ± 9.4	94 – 120
BUN	17.4 ± 13.94	7.68 – 50.2	9.68 ± 1.8	7.39 – 12.6
CHOL	234.1 ± 48.06	176 – 339	244 ± 69.48	178 – 396
CRE	0.74 ± 0.23	0.46 – 0.99	0.65 ± 0.21	0.31 – 0.99
BUN/CRE	29.75 ± 33.98	11 – 109	16.5 ± 5.83	9 – 26
TBIL	0.21 ± 0.07	0.12 – 0.34	0.23 ± 0.07	0.16 – 0.39
AMY	338.5 ± 129.16	143 – 552	305.4 ± 84.9	239 – 487
ALT	2.5 ± 0.92	1 – 3	24.1 ± 9.64	11 – 45
CK *	**1921.5 ± 771.79**	**1133 – 3143**	**250 ± 108.89**	**87 – 393**
ALP	21.6 ± 5.52	13 – 31	2.875 ± 0.35	2 – 3
P	8.6 ± 1.4	6.98 – 10.55	8.2 ± 1.19	6.84 – 9.78
Ca	6.8 ± 1.74	4.9 – 10.1	5.6 ± 1.9	2 – 7.9

* Denote significant differences.

CCL curved carapace length, CCW curved carapace width.

ALB Albumin, TP total protein, GLO globulin, A/G Albumin/Globulin ratio, GLU glucose, BUN blood urea nitrogen, CHOL cholesterol, CRE creatinine, BUN/CRE blood urea nitrogen/creatinine ratio, TBIL total

bilirubin; AMY amylase, ALT Alanine aminotransferase, CK creatinine kinase, ALP alkaline phosphatase, Ca calcium, P phosphorus.

Figures

Figure 1

Individual of *Lepidochelys olivacea* with evident muscle weakness (paresis) of the rear legs laid eggs on the surface of the sand in La Escobilla beach, Oaxaca, Mexico.