Chapter

Rediscovering Kemp's Ridley Sea Turtle (*Lepidochelys kempii*): Molecular Analysis and Threats

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Abstract

Sea turtles are reptiles that have inhabited the earth for 100 million years. These are divided into 2 families (Cheloniidae and Dermochelyidae) and 7 species of sea turtles in the world: the leatherback turtle (*Dermochelys coriacea*); hawksbill turtle (*Eretmochelys imbricata*); Kemp's ridley (*Lepidochelys kempii*); olive ridley (*L. olivacea*); Loggerhead turtle (*Caretta caretta*); flatback sea turtle (*Natator depressus*) and green turtle (*Chelonia mydas*). In particular, Kemp's ridley is included in the red list of IUCN categorized as "critically endangered". The most important site around the Word is in Rancho Nuevo, Tamaulipas, Mexico. Where 80–95% of the world's nesting is concentrated. Other nesting areas are Tepeguajes and Barra del Tordo, in Tamaulipas, and with less intensity in Veracruz (Lechuguillas and El Raudal beaches) and South Padre Island, Texas, USA. They deposit an average of about 90 eggs and hatching takes 40 to 60 days. Therefore, they are vulnerable to different anthropogenic activities and sources of pollution, such as heavy metals, which can cause toxic effects that are harmful to the turtles, damage their physiology and health. To understand the real situation about health and genetic parameters it is necessary to analyze biochemical and molecular factors in this species.

Keywords: Kemp's ridley, molecular analysis, nesting beaches, pollution

1. Introduction

For decades, the Kemp's ridley (*Lepidochelys kempii*) was one of the most elusive sea turtles, and its nesting sites were unknown. Study, ingenuity and a home movie shed light on what for many was a riddle. The first references occurred when fishers off the coast of Florida, USA reported that they caught "demonic turtles". The turtle's morphology was described as flat and grey with a large head and that they were very active when they were caught in fishing nets which resulted in broken nets. The encounters were reported to researchers, and Samuel Garman, a prominent herpetologist and ichthyologist at the Harvard Museum of Comparative Zoology, was the first person to describe the turtle characteristics and, it is thanks

to him that the Kemps ridley sea turtle received its name. In 1880, Garman named the species after Richard Kemp who had a fascination for natural history and sent specimens of the turtles to Garman for study. It was Kemp who first thought the turtles were hybrids from a mating between green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles, hence their nickname of bastard turtles [1].

Another authority in the kemps ridley story was Archie Carr, who wrote several publications on the species. Despite this, he was unable to locate the turtles nesting sites. However, one of his disciples and collaborators, Peter Pritchard, continued this work and established the species morphometric measurements and other key information, such as foraging sites, but the search for the nesting sites of the mysterious Kemp's ridley was unsuccessful.

1.1 Discovery

It took two further researchers and several years to start to unravel the mystery. One crucial player in the discovery was Ing. Andrés Herrera, a rancher and lover of the outdoors who lived in Tamaulipas, Mexico. Ing. Herrera listened to locals talk of beaches where sea turtles nested along the coasts of Tamaulipas. Luckily Andres was also a light aircraft pilot, armed with information that turtles nested on specific beaches in Tamaulipas during the spring, and he decided to start flying his aeroplane at that time to try and observe them. According to Ing. Herrera, on 18th June 1947, one of these flights was successful when he encountered a mass nesting event, or "*arribada*", which occurred on the beaches of the town of Rancho Nuevo near Barra Calabazas, Tamaulipas. He landed the aeroplane and made the famous film, "the film of Ing. Herrera". This footage not only documented Kemp's ridley nesting but also that this was a mass nesting event or "arribada" which was happening in broad daylight which was different to other sea turtles such as the green turtles which nest at night [2].

Another key to solving the kemps ridley riddle was the biologist Henry Hildebrand from the University of Corpus Christi who had heard and read about the stories of the *arribada* and the place where said nesting had taken place, as well as the writings of Archie Carr. At the beginning of the 1960s, Hildebrand learned footage made by Ing. Herrera and travelled to Tamaulipas to ask for permission to present it at the 1961 meeting of the American Society of Ichthyologists and Herpetologists at the University of Texas. After several decades of uncertainty, Hildebrand in 1963 presented the mystery of where this elusive turtle nested to the scientific community. Later, Carr and Hildebrand's reports estimated that the film's famous *arribada* accounted for around 40,000 Kemp's ridley nesters in a single day [3, 4].

1.2 Population

The Kemp's ridley turtle population baselines is an estimated 40,000 nesting females in a single day of an arribada. However, in 1966, the largest arribada was reported to include just 1,317 turtles [5] and the number of nests laid continued to decrease until the 1970s, when records show that in 1978, the number of nests fell below 1,000 [6].

Later, in the mid-1980s, only 702 nests were recorded at Rancho Nuevo, Tamaulipas. However, from 1985 to 1990, nesting stabilised, with nest numbers ranging from 702 to 839. Finally, the USA and Mexico introduced legislation to reduce bycatch, protect major nesting sites, and establish a binational agreement. These crucial changes contributed to the increase and later recovery in the numbers of nesting turtles, which increased from approximately 1,000 nests in the mid-1990s to over 21,000 nests on the beaches of Tamaulipas in 2009 [7–9].

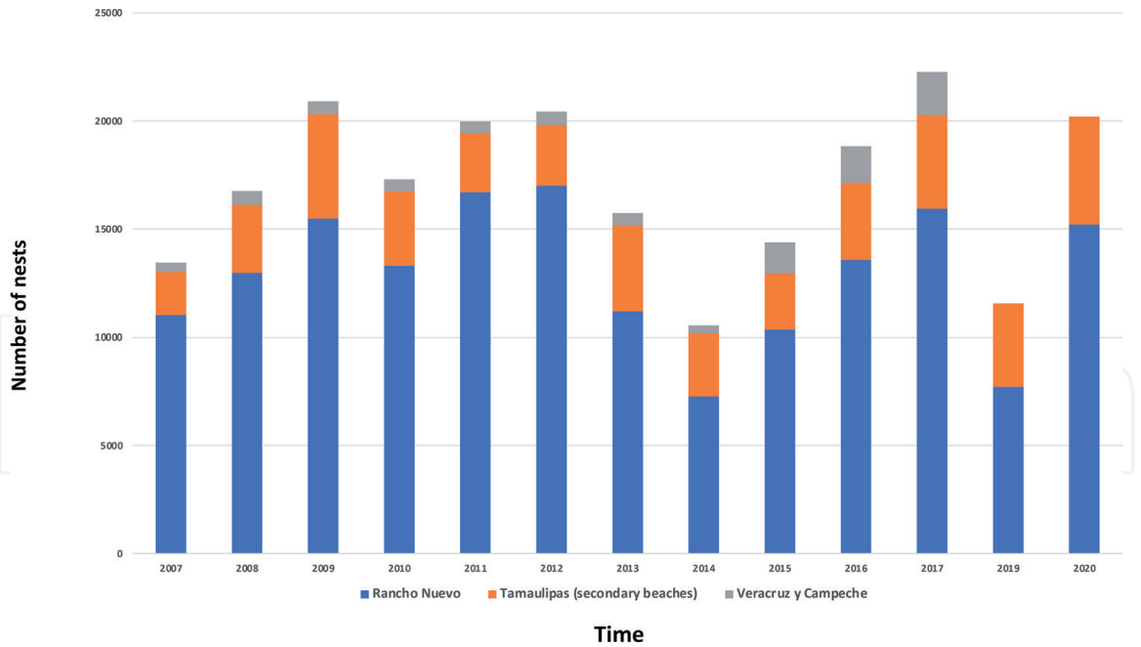

Figure 1.
Number of protected Kemp's ridley (Lepidochelys kempii) nests in Mexico from 2007 to 2020 [10].

By the 2011–2012 nesting season, the kemps ridley turtle population had stabilised at 21,000 nests. However, in 2013 numbers of nesting turtles fell rapidly to around 16,000, and the downward trend continued with just 12,000 nests recorded in 2014. Over the last five years (2015–2020), an average of 1,500 nests have been protected per season at the Rancho Nuevo Sanctuary compared to the 3,868 on the other Tamaulipas beaches. During the 2020 nesting season, there were encouraging results with 15,210 nests in Rancho Nuevo and 4,995 from other Tamaulipas beaches (**Figure 1**). It is important to note that data for 2018 was unavailable and the data for 2019 and 2020 do not include the number of nests for beaches found outside of Tamaulipas state [10].

2. Biology (lifecycle and reproduction)

2.1 Morphology

The Kemp's ridley sea turtle (*Lepidochelys kempii*) is one of the seven species of sea turtles that exist worldwide. The species belongs to the Cryptodira turtles of the Cheloniidae family and is a direct relative of the olive ridley sea turtle *Lepidochelys olivacea* (**Table 1**). Studies on Kemp's ridley sea turtle genetics show that there is only one population within this species, which evolved and has existed for approximately 2.5–3.5 million years [11]. The Kemp's ridley is the smallest sea turtle and is an endemic species to the Gulf of Mexico.

The Kemp's ridley (**Figure 2**) has usually a carapace as wide as it is long and contains five pairs of costal scutes that overlap the bony carapace (**Figure 2A, B**) and a triangular head with a slightly hooked bill (**Figure 2C-E**). The hatchlings are dark in colour on both sides. Adults generally present a greyish-green carapace with a pale yellowish plastron Each of the front flippers has a claw, while the rear flippers can present one or two claws. Juveniles have a dark grey carapace with a yellowish-white plastron, and adults develop an olive-grey or dark green carapace and a cream or yellowish plastron [12].

2.2 Taxonomy and nomenclature

Domain	**Eukaryota**
Kingdom	Animalia
Phylum	Chordata
Class	Reptilia
Order	Testudines
Family	Cheloniidae
Genus	*Lepidochelys*
Species	*kempii*

Table 1.
Kemp's ridley sea turtle (Lepidochelys kempii) taxonomy [13].

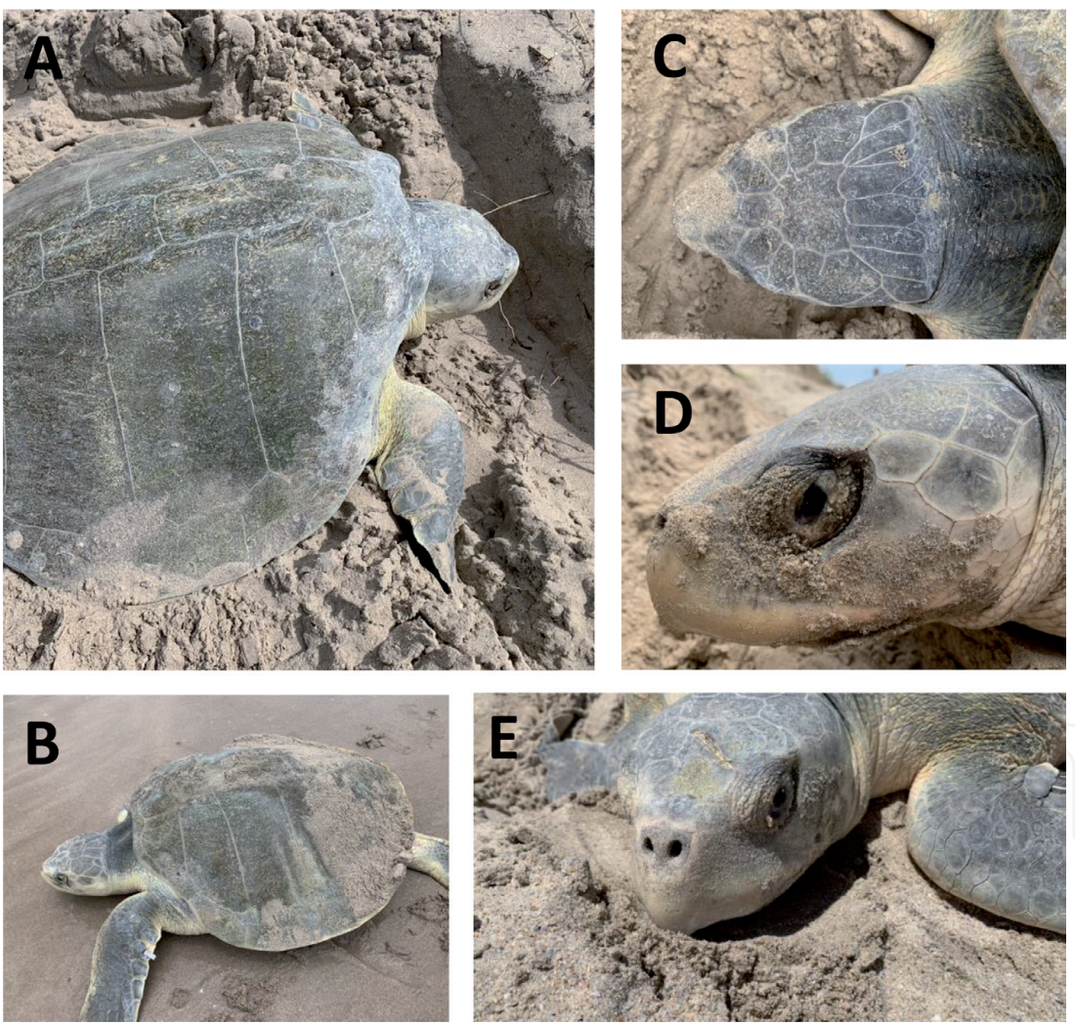

Figure 2.
Pictures showing general morphology of Kemp's ridley. (A and B) general view; (C) Aeria l view; (D) Lateral view; and (E) Frontal view. Pictures courtesy: Fátima Y. Camacho-Sánchez.

2.3 Life cycle

The Kemp's ridley sea turtle presents characteristics that differentiate it from other species, in addition to its small size and restricted distribution, this species, nests during the day, and as with the olive ridley sea turtle nests on mass in a

phenomenon known as an "arribada". Kemps ridley females deposit an average of 90–100 eggs per nest. These turtles nest 2 to 3 times per season, with an interval of 14–28 days between laying each clutch of eggs. Another distinctive feature are the pores located in their plastron, known as "Rathke's gland", through which they secrete a substance that is considered a pheromone [14].

2.4 Biogeography

Globally the Kemp's ridleys sea turtle is represented by one unique population which presents one of the most restricted distributions of all sea turtle species. Kemp's ridleys are found primarily in the Gulf of Mexico and secondarily in the Atlantic Ocean, on the south-eastern coasts of the United States (USA). Approximately 70% of the population nests on a beach with a linear extension of 30.4 km, located in Rancho Nuevo, in Tamaulipas, Mexico. Ninety-seven per cent of the population lives in an area of 146 km that includes Rancho Nuevo beach and surrounding areas. A small proportion of the population is found on the coast of Veracruz, Mexico and the coast of Texas, USA (**Figure 1**). In southern Texas, South Padre Island is considered a secondary rookery for Kemp's ridley nesting after Rancho Nuevo [15].

On rare occasions, they are found off the coasts of Canada, Bermuda, Azores, Madeira and in the Mediterranean Sea [16]. However, the Gulf of Mexico is more than just the nesting ground for Kemp's ridley turtles. In addition to navigating neritic areas, both hatchling and juvenile Kemp's ridley turtles use oceanic areas of the Gulf of Mexico during their development, by entering the open ocean to forage. During migration, turtles can be found in the Northwest Atlantic and the Mediterranean Sea (**Figure 2**), for example, Bermuda, Canada, France, Ireland, Portugal and the United Kingdom [13, 16].

The Kemp's ridley turtle shares many characteristics with other sea turtle species in terms of its life cycle. Female turtles nest on beaches, depositing their eggs beneath the sand. The eggs hatch after approximately 45–58 days and the hatchlings migrate to the sea. The following years are spent on foraging grounds where they develop into adults at which point, they return to their natal area to reproduce and nest. During the sea turtle lifecycles, three key phases occur, and these are divided by ecosystem: terrestrial phase, neritic phase, and oceanic phase.

2.5 Terrestrial zone (Hatching)

The Kemps ridley turtle uses terrestrial ecosystem during three stages of its life cycle: nesting females, eggs, and hatchlings. The principal Kemp's ridley nesting beach at Rancho Nuevo is characterised by small dunes of variable size, with vegetation that improves stability. The sand is small and fine-grained, and the beach is high energy. The nesting season runs from March to August [15]. Mass nesting events or *arribadas* occur mainly in early April through the July period. These phenomena can be triggered by meteorological conditions represented by strong winds, primarily from the north, or by a change in atmospheric pressure [17].

Kemp's ridley nesting typically occurs during the day; however, reports of sporadic cases of nocturnal nesting exist in Texas. Clutch incubation lasts between 45–60 days, and climatic conditions act directly on embryo development with incubation duration and hatchling sex ratios affected by temperature. Studies have shown that sex ratios are male-biased at the beginning of the nesting season when temperatures are lower. In contrast, nests produce mainly female hatchlings when laid during the second arribada when temperatures are highest [18].

2.6 Neritic zone (juvenile stage and adult stage)

The hatchlings, after being released on the sand, immediately begin their trajectory to the ocean. The hatchlings swim using the energy provided by the nutrients found in the yolk of their egg, which lasts for a maximum of 4 days, during this period, the hatchlings migrate to ideal areas for their development. Hatchlings enter the surf and orient seaward into the waves, diving as they break, thus being swept seaward by the motion of the waves without significant energy expenditure. Like other species, the hatchlings can perceive the waves' movements to guide them towards the sea, the same mechanism they use on the way back to the coast, recognising the magnetic orientation. Juveniles reach adulthood in neritic waters, which are their most frequently used habitat. The Gulf of Mexico's northern coast is home to the majority of kemps ridley sea turtles, principally in waters no greater than 37 m deep. Although kemps ridley turtles may consume algae, their diet is primarily carnivorous and primarily based on crabs, but occasionally includes clams, shrimp, jellyfish and some fish species. Turtles may also scavenge in benthic areas on marine debris [15].

2.7 Oceanic zone

The hatchlings that manage to reach oceanic or pelagic areas stop actively swimming and enter a passive state, allowing themselves to be guided by the ocean currents to foraging grounds where they remain as they develop into juveniles. Although kemps ridley sea turtles are considered a carnivorous species, individuals have been seen feeding on sargassum [15]. During their oceanic phase, juvenile turtles can be divided into two groups: the current system of the northern and western Gulf of Mexico or the Gulf Stream of the Northwest Atlantic. However, a small proportion of the population finds its way to the Mediterranean Sea. During this period that lasts between 1 to 4 years, juveniles complete their oceanic feeding phase to return to surface waters within the Gulf of Mexico and the northwest Atlantic, in US waters, where they continue to forage.

3. Status

3.1 Threats

Sea turtle populations face multiple threats which need research and monitoring to understand how these threats are detrimental to their populations and conservation. Overlooking the multiple threats that species face throughout their life cycle puts the success of recovery plans at risk [19].

3.2 Illegal hunting: direct consumption and illegal trade

Globally, fishing and eggs collection for human consumption are the principal causes of the drastic reduction in sea turtle populations, and the Kemp's ridley is no exception. In the late 1960s, excessive capture of adult kemps ridley turtles and their eggs, contributed to the drastic reduction by almost 99% of the Kemp's ridley nesting population. Turtle eggs are considered an aphrodisiac in some countries [19]. The consumption of turtle eggs is not as frequent in the United States of America and Mexico as it was before the federal government bans on harvest and consumption. However, despite governmental agencies and non-governmental organisations efforts with protection and public awareness programs, consumption continues in some coastal communities.

3.3 Climate change

Climate change can have a severe impact on turtle populations. All reptiles, including sea turtles, are dependent on environmental temperature, which they use to regulate their physiology [20]. Sex determination in sea turtles is directly dependent on environmental temperature. Therefore, projected anthropogenic climate change will alter the sand temperature, affecting the primary sex ratios and increasing the risk of instability in the composition of sea turtle populations.

3.4 Marine pollution: rubbish and oil

Sea turtles can mistake plastic objects floating in the water column for food, such as jellyfish. If ingested these inorganic materials can choke or cause obstructions in the turtle digestive systems. They can also become entangled in discarded fishing lines and nets, drowning or unable to feed or swim. Trash on beaches can trap hatchlings and prevent them from reaching the sea.

During 2010 an accident occurred on the Deepwater Horizon platform that triggered an oil spill from the platform into the Gulf of Mexico This event negatively impacted the marine ecosystem, including the kemps ridley turtle as their essential habitats and migratory routes were affected by the spill [21]. However, the extent to which this oil spill was responsible for the decrease in the Kemp's ridley population observed in the years following the disaster is unknown, with other factors possibly contributing to the decline. Continued monitoring is essential to understanding the long-term effects of this and other oil spill events.

3.5 Bycatch

Every year thousands of turtles are accidentally caught by the shrimp fishery. Sea turtles must surface to breathe, so prolonged periods trapped in submerged fishing gear leads to drowning. Fisheries that use longlines and gillnets are also major causes of sea turtle mortality [22]. Although laws requiring the use of sea turtle excluder devices in shrimp fishery exist, turtles continue to become trapped and drown in these nets.

3.6 Coastal development: beach modification and human presence

Uncontrolled coastal development has destroyed beaches that are essential for nesting. The lights coming from roads and buildings confuse hatchlings and disorienting them away from the sea. Vehicles used in beach restoration projects, including dredging and sand nourishment projects, damage near-shore foraging areas and beaches, and can also destroy nests and hatchlings. These activities referred to as "beach maintenance" have been reported on Padre Island, where machinery has prevented hatchlings from reaching the water, leaving them trapped and exposed to vehicle traffic [15].

3.7 Marine biotoxins

Harmful algal blooms in the oceans are natural phenomena that occur as a result of increasing temperature, alteration of ocean currents, intensification or weakening of local nutrient upwelling, and heavy precipitation and storm events causing changes in land runoff [23]. Brevetoxins are a group of biotoxins produced mainly by algae *Karenia brevis*, which is the cause of the main harmful algal blooms (HABs) along the coast of south-west Florida, with periodic blooms throughout the Gulf of Mexico [24].

Sea turtles are affected by marine biotoxins present in the water column, in aerosols generated by waves, or through the consumption of contaminated prey [25]. In recent years, the impacts of these phenomena have caused or contributed to sea turtle mass mortality events in *C. mydas*, *C. caretta*, *L. olivacea* and *L. kempii* [26].

4. Studies

4.1 Satellite telemetry

Studies that follow in water movements of sea turtles and other marine vertebrates often use tagging to shed light on migration routes and local movements. The most common ways to tag sea turtles are to apply external metallic tags with unique codes usually to the turtle flippers which allow the individual turtle to be identified. This method requires the turtle to be recaptured and does not supply information on the routes taken from release and capture position. The advent of satellite telemetry in the 90s and improving technology has allowed researchers to follow the routes taken by sea turtles and better understand habitat use and connectivity [27].

Satellite tagging of Kemp's ridley sea turtles, as with other sea turtle species requires the attachment of platform terminal transmitters PTT to the turtle's carapace using epoxy glue. The study of the migration of kemps ridley turtles began in 1995 and has focused mainly on post-nesting females. These marked females confirmed that Kemp's ridley turtles keep mainly to the Gulf of Mexico, and forage near the North Texas and Louisiana coasts in the USA. Satellite tracking of juvenile turtles shows that they live off the coast of Veracruz and that they nest mainly in Tamaulipas and more specifically in Rancho Nuevo Sanctuary. Additionally, information on foraging site fidelity is easily obtained from satellite telemetry studies. Furthermore, studies have also indicated that male Kemp's ridley turtles typically remain off the coast of Tamaulipas, close to the principal nesting area [28].

Satellite telemetry has also shed light on nesting and nest-site fidelity. Tracked females have been shown to nest one to three times at the same site. Additionally, the distance females migrate from nesting sites to the open ocean decreased as the number of times they returned to nest increased. Somewhat surprisingly satellite telemetry has shown that turtles do not forage during the inter-nesting period, and they minimise energy loss by spending most of their time resting, limiting movement.

Of the studies on female kemps ridley movements between nesting events, information is only available for three individuals. These studies found that after nesting one tagged female travelled north [29] from the main nesting site at Rancho Nuevo Sanctuary; two turtles have travelled south from Rancho Nuevo with one covering a distance of ~100 km Further studies are required to understand Kemps ridley movements between nesting events.

4.2 Biochemical studies

Sea turtles play an important role in ocean ecosystems, by maintaining healthy seagrass beds, beaches, estuaries, and reefs. In turn, they are considered sentinel species for ecosystem health, since their longevity and physiology provide essential and early information on marine and coastal habitats and the local environments in which they live, which provides a quick risk diagnosis. These reptiles are particularly susceptible to pathogens such as parasites, bacteria, fungi and viruses. Examples of these parasites are for bacteria: *Vibrio* spp. *Pseudomonas* spp., *Enterococcus* spp., *Aeromonas*, *Cytophaga*, and others; for fungi: *Fusarium*

species *Fusarium solani*, *Fusarium oxysporum*, F. *solani*, and *Pseudallescheria boydii Fusarium keratoplasticum*; and viruses manly *Herpesviridae* [29]. In turn, they risk spreading diseases when in contact with other species or populations. Sea turtles are also susceptible to the toxicity and bioaccumulation of environmental pollutants that can affect their health, causing a deterioration in their immune system, and increasing the risk of them developing diseases due to the exposure to pollutants [30, 31].

Therefore, as part of sea turtle protection activities, incorporating a population health assessment program and identifying diseases and abnormalities in wildlife organisms is a priority. However, the lack of knowledge about the physiology of these reptiles makes it difficult to establish criteria to measure the health of their populations and to be able to distinguish between "normal" physiology and the presence of pathologies [30, 32].

Multiple techniques exist to diagnose organism health, including the analysis pollutant concentrations, physical evaluation and disease detection. However, haematological methods are the most widely used as indicators of sea turtle health, since they provide information on immune, cellular and humoral factors, which are necessary for the response to adverse factors, through the blood which can indicate pathological changes in the organisms. Therefore, blood parameters are a non-invasive diagnostic tool that can be used to evaluate and monitor the health status of wildlife [33].

The evaluation of blood parameters, generally accompanied by a detailed clinical history, clinical signs, physical examination and other diagnostic tests, is an important tool for the evaluation of the health of a population through the establishment of blood reference intervals (BRI) and has made it possible to determine and evaluate the functioning of organs and confirm possible diseases in progress. However, in the case of sea turtles, there is difficulty in defining the blood reference values considered normal parameters due to the variability between sea turtles' geographic areas, ecological habitat, populations, sexual maturity, reproductive status, diet and migration. For this reason, therefore, blood parameters must be established for each species and region [31, 33].

Previous studies on the establishment of blood biochemistry reference values in other sea turtle species have found that the values are influenced by ecological implications, the season of the year and the reproductive stage. For example, adult turtles that feed in neritic habitats had low levels of creatinine (Cr), alkaline phosphatase (ALP), Phosphorus (P), Sodium (Na), Magnesium (Mg), unlike adult turtles that feed in ocean habitats [34].

By sex, significant differences have been observed in blood parameters in total protein (PT), albumin (ALB), Calcium (Ca), cholesterol (CHOL), triglycerides (TRIG), cell pack volume (PVC) and total bilirubin (TBIL) being higher in females due to its association with vitellogenesis and folliculogenesis, on the other hand, in males the levels of BUN (blood urea nitrogen) and glucose (GLU) are higher since they are associated with the lack of food during mating. In foraging areas, no significant relationship has been found between the cell packet volume (PCV) with respect to the size and sex of sea turtles, rather PVC is related to feeding and stress on the body. Other parameters such as glucose (GLU) may present higher levels in juveniles than adults, particularly in adult females since they require more energy for the nesting process [35].

Finally, a significant difference has been observed in visibly sick sea turtles compared to healthy individuals, for example in turtles with fibropapillomatosis, the values of total protein (PT), albumin (ALB), cholesterol (CHOL) and triglycerides (TRIG) are lower due to the chronicity and severity of this disease, while in turtles with necrotic or traumatic lesions lower values of total protein (PT), albumin

(ALB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been reported [36].

Some studies on sea turtle blood biochemistry from the northern Gulf of Mexico have included samples from *L. kempii* turtles, which, although the principal objective of these studies was not to establish blood reference intervals (**Table 2**), the results obtained contribute knowledge on the possible differences that the species may present by age or sex, and that factors such as diet do not influence the preprandial and postprandial hematological and plasma parameters in *L. kempii*, unlike other species of sea turtles such as *C. mydas*, in which the biochemical levels of total protein (PT), albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and postprandial cholesterol (CHOL) increased significantly. The authors mention that this is due to the type of diet of each species, since juvenile *L. kempii* turtles are carnivorous and consume mainly crabs, while juvenile *C. mydas* turtles change their diet from carnivorous to herbivorous when mature [37].

Parameter	Massachusetts Juveniles Foraging area [12]	North Carolina Juveniles Foraging area [38]	North Carolina Adults Foraging area [39]
Sample size (n)	26	10	4
VPC	NA	NA	NA
Total protein (gdL-1)	2.6 ± 0.4	−2.4 – 17.6	2.6 ± 0.9
Albumin (gdL-1)	1.0 ± 0.2	−7.11 – 7.7	0.9 ± 0.3
Globulin (gdL-1)	1.7 ± 0.3	−7.7 – 23.8	1.7 ± 0.6
A/G ratio	0.6 ± 0.1	NA	NA
Total Bilirubin (mgdL-1)	NA	NA	NA
Creatinine (mgdL-1)	0.25 ± 0.11	−33.3 – 50.0	NA
BUN (mgdL-1)	33 ± 22	NA	68.3 ± 20.7
Glucose (mgdL-1)	141 ± 50	−13.1 – 16.9	112.3 ± 48.8
Cholesterol (mgdL-1)	334 ± 141	−2.6 – 10.0	NA
Triglycerides (mgdL-1)	NA	−11.8 – 25.1	NA
Enzymes			
ALKP (UL-1)	285 ± 417	−9.9 – 12.9	NA
ALT (UL-1)	26 ± 50	−100 – 50	NA
AST (UL-1)	610 ± 50	−3.9 – 14.4	108.8 ± 54.9
GGT (UL-1)	3 ± 2	NA	NA
CK (UL-1)	21,979 ± 24,298	−2.4 – 15.8	2,412.3 ± 2,235.4
AMYL (UL-1)	NA		NA
Elements			
Calcium (mgdL-1)	6.6 ± 1.1	−11.5 – 4.5	13.5 ± 9.7
Phosphorus (mgdL-1)	7.4 ± 1.2	−1.1 – 6.9	7.5 ± 4.0

PVC = Packed cell volume, BUN = Blood urea nitrogen, ALKP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutamyl transpeptidase, CK = Creatine phosphokinase, NA = Not analysed.

Table 2.
Reference values of blood biochemistry for different populations of clinically healthy Lepidochelys kempii sea turtles [12, 38, 39].

The impact of gillnets on the sea turtle health, where a significant increase in enzymes such as lactate dehydrogenase (LDH) and creatinine kinase (CK), Phosphorus (P), calcium (K) and glucose (GLU) was observed the longer a turtle was trapped in the net. The authors concluded that the level of stress to which the organism is subjected to alters the blood parameters values. On the other hand, kemps ridley turtles are exposed to pollutants such as metals and organochlorine pesticides from an early age, and even low concentrations of these pollutants are associated with changes in biochemical parameters, which affects the health and behaviour of these organisms [38].

Although the *L. kempii* turtle is considered one of the sea turtle species at the greatest risk of extinction, blood biochemistry reference values have not been established. Therefore, blood biochemistry studies should be prioritised for this species as understanding the health status and disease patterns for these turtles will help secure their future.

4.3 Heavy metal studies

Globally, ocean pollution is a high-impact problem, which is generated from multiple sources and anthropogenic activities, such as fisheries, urban development, mining, production of agricultural and industrial products and oil refineries. These activities significantly increase pollutant levels in marine ecosystems and negatively impact the health of the organisms that inhabit them. Among the primary pollutants found in marine ecosystems are organochlorine compounds, solid waste, plastics (micro and macro), pharmaceuticals, hydrocarbons and heavy metals. Heavy metals are of great importance in ecotoxicology, due to their high toxicity and duration in the environment, since bacteria cannot degrade them over short periods [40–42].

Although some metals are essential for organisms' biochemical processes (Cu, Mn, Mg, Se, Cr, As, Na, K and Mo), high levels of these elements can affect organism health. On the other hand, toxic metals (Hg, Pb and Cd) are elements that are unnecessary in organisms and can alter metabolic pathways and develop diseases and even death [35, 42, 44]. Heavy metals are acquired mainly through diet, and bioaccumulate in specific organs or tissues, such as Cd in the kidney and Pb in bones, by affinity. Through biomagnification processes heavy metals can affect the entire trophic web, with the greatest impact observed in the "top" organisms, such as sea turtles, altering blood biochemistry and other health parameters, since they have the ability to act as endocrine disruptors and produce carcinogenic effects, as well as affecting the fertility of eggs [33, 34, 41].

On the other hand, high concentrations of heavy metals such as Cu, Fe and Pb have been related to the presence of fibropapillomas, a disease which seriously threatens sea turtle health. For this reason, heavy metals such as Cd, Hg and Pb are the pollutants with the greatest impact on these reptiles, even affecting the embryos during vitellogenesis due to the vertical transmission from the female to her eggs; for example, Pb mimics as Ca as its reserves are depleted during nesting seasons and can be transferred to eggs. For these reasons, heavy metals have been identified as a risk to the health of sea turtle populations worldwide.

Although the issue of heavy metals in sea turtles has been widely studied, most studies are based on the analysis of tissues collected from dead individuals. However, this does not increase our understanding on the bioavailability, bioaccumulation and toxicology of heavy metals in sea turtles or their immune response to these pollutants, which is why blood is currently used as analysis tissue, which reflects exposure to short-term pollutants, although it has been observed that there is a relationship between metal levels in the blood with respect to that in other

SCL	Weight	Area	n	Hg	As	Cd	Cu	Pb	Se	Zn	Author
46.9 ± 5.0	16.6 ± 5.0	F	24	0.04 ± 0.04	6.84 ± 1.98*	0.02 ± 0.01*	NA	0.01 ± 0.004*	4.11 ± 1.83*	NA	46
NA	NA	F	106	0.018(0.0005–0.06)	NA	NA	0.52(0.21–1.3)	0.001(0.00–0.03)	NA	7.5(3.28–18.9)	47
46.3 ± 7.1	17.6 ± 7.4	F	18	0.01 ± 0.0092	NA	0.007 ± 0.05	0.470.06	0.02 ± 0.03	NA	3.9 ± 1.47	48
65 ± 3.3	NA	N	18	0.06 ± 0.04	NA	0.01 ± 0.01	0.40 ± 0.09	0.05 ± 0.02	NA	22.70 ± 12.6	48
36.1 ± 7.4	7.4 ± 6.2	F	91	0.01 ± 0.01	NA	0.01 ± 0.005	0.41 ± 0.11	0.03 ± 0.03	NA	6.71 ± 4.46	48

F = Foraging. N = Nesting. NA = Not analysed.
**Analysis performed on red blood cells. Mean (min-max) when the standard deviation is not reported.*

Table 3.
Heavy metal concentrations reported in different areas (mean ± standard deviation, μg g^{-1} wet weight) in L. kempii blood [46–48].

tissues such as the liver and kidneys. Another benefit of using blood tissue is that an organism immune response can be observed between the levels of essential (Zn, Cu and Se) and toxic (Hg, Pb and Cd) metals, which suggests that there is a detoxification process by the organism due to the formation of metallothioneins (MT), which are proteins that function as a detoxifying agent.

At present, it is unknown to what degree chemical pollutants are harmful sea turtle health, since the concentration of each metal vary between species and populations, due to the fact that several factors influence the pollutant load, such as habits, diet and the levels of pollutants found in its food, sex, age and physical condition, exposure time, as well as the local habitat characteristics and climatic conditions [40–44]. For this reason, the monitoring of heavy metals in sea turtles must be carried out for each species on a regional and population level.

Sea turtles are sentinel species due to their sensitivity to environmental pollutants which can be analysed in their tissues and reflect the bioaccumulation and behaviour of pollutants such as heavy metals in the environment in which they live. This allows for the identification of potential threats to the environment and health [45].

Regarding the Kemp's ridley turtle (*Lepidochelys kempii*), different authors point out that their population within the Gulf of Mexico is exposed to the heavy metal concentrations present in water and sediments, highlighting that the main sources of contamination in the area are due to large oil spills, such as IXTOC-I in 1979 and Deepwater Horizon in 2010, which increased the concentrations of Pb and other toxic elements, causing potential risks to biota. However, few studies [46] have been carried out on the levels of heavy metals in this sea turtle (**Table 3**), these studies observed that the concentrations of Pb increased with turtle size in a foraging area in Texas, USA, while in another foraging area in Louisiana, USA, Cu and Hg accumulated in higher concentration related to size. On the other hand, this relationship was not observed in the population of the southeast Atlantic. These regional differences were attributed to geographic differences, sources of contamination and levels of metals in the blue crab (*Callinectes sapidus*), the main component of the Kemp's ridley's diet.

These differences observed in Kemps ridley turtles and the interspecific variability of the pollutant load due to the factors described above, show the importance of periodically monitoring the levels of heavy metals in the Gulf of Mexico and in this sea turtle, which would expand knowledge about the levels of these pollutants in the Gulf of Mexico and the impact on the health of this species, contributing to better care and management in *L. kempii* sea turtle conservation programs.

4.4 Molecular studies

The study and analysis of nucleic acids in sea turtles has different approaches which depend on what type of nucleic acid is used to understand what, how and when to conduct molecular analysis. The application of genetics to improve species conservation efforts is an area with great potential, and there is a growing interest in this research.

All organisms or protein entities (such as viruses that contain DNA or RNA inside them) have different nucleic acid sequences (DNA or RNA). Variations are due to factors, such as differences between two individuals, which can be caused due to the time and space where they live, their biology, reproductive success, demographics, places and time of migrations and even natural selection. All the genetic information of each species is stored in its DNA; therefore, if its genome is analysed, it is possible to obtain this information for almost any evolutionary process. In conservation, the acquisition of this knowledge is important for identifying

closely related individuals and mitigate inbreeding or exogamy and minimise the loss of genetic variation.

Although taxonomic studies based on morphological characteristics are used to classify individuals or species, molecular analyses are useful in studying sea turtles providing data on their evolution, populations, phylogeny, and how to implement conservation management plans based on molecular results. The study of DNA sequences can provide important information on sea turtle history and data related to their reproductive behaviour and ecology [46]. On the other hand, conservation objectives based on genetics present an opportunity to acquire a greater understanding of a population's status and management for genetic diversity preservation and prevent the persistent risks that affect populations. To better understand sea turtle species, studies should be directed towards those that provide precise data, such is the case of molecular markers.

Thus, molecular markers applied amongst other things to genetic studies, have relevance in countless studies in multiple species, their application in conservation provides information that helps in the understanding of evolutionary history, demography and ecology of endangered species. Information related to species distribution, biology and population dynamics is required to develop efficient and successful conservation strategies. Thanks to the development of molecular analyses and their application in sea turtle conservation, researchers have generated information that has contributed to species recovery [49]. Likewise, the application of molecular techniques can provide a breadth of information on different areas for conservation. However, many techniques present limitations which are subject to their correct use and development, including the correct selection of molecular markers. Therefore, we can consider that genetic or molecular markers are DNA sequences with known locations within a genome, a gene or in a non-coding region. They are generally directly or indirectly associated with the gene's function where they are located or the function of a contiguous gene. Some factors that must be taken into account in the use of molecular markers are the following: variation in the ability to detect differences between individuals, their application costs, ease of use, consistency, multiple capacities (evaluate several loci at the same time) and repetition.

These markers can be small sequences, such as polymorphisms (change or changes of one or more bases within a given sequence, in at least 1% of the population) of a single nucleotide (SNP, single nucleotide polymorphism) or long such as microsatellites [50]. Molecular markers are indispensable tools for determining genetic variation and biodiversity with a high degree of precision and reproducibility.

There are two possibilities within the study of molecular markers: nuclear DNA (nDNA) or in mitochondrial DNA (mDNA) markers. DNA from the mitochondrial genome provides information based on maternal lineage and population dispersion, which is used for analysis in molecular-phylogenetic studies, thanks to its ability to analyse evolution, since it evolves faster than nDNA, resulting in an accumulation of differences between nearby species. The mDNA can estimate gene flow and population history. The information found in the mDNA is conserved, with the absence of introns, short intragenic regions, and few duplications. Markers based on nDNA also called the nuclear genome, provide information on the genetic flow inherited by the male or males, as well as their polygamous reproduction habits with females from different nesting areas, in the same way resolving the paternity question for each nest.

Starting in the 90s, studies on sea turtles using different molecular markers notably increased. These studies have been crucial to increasing our understanding sea turtle ecology. Among the research carried out on sea turtles, one can find

species cataloguing and biodiversity inventories [51], identification of illegal sea turtle products, population structure and historical biogeography, phylogenies, female philopatry, male philopatry, multiple paternity, hybridisation, sex ratio [52], epigenetic factors, recombination processes, gene selection and drift that can generate different genealogical histories.

For molecular studies of Kemp's ridley, we should consider different options and marker types. Before going into detail, we need to keep in mind that these studies require a methodical and systematised process, not only for the type of molecular analysis, but during the entire process, from the organisms capture, sampling, DNA extraction, amplification and sequencing and its bioinformatic analysis. Although there are large amounts of information on sea turtle species, molecular studies in the endemic kemps ridley turtle are limited. Therefore, here we will present a general panorama of molecular studies in sea turtles.

Worldwide, biodiversity loss is accelerating due to multiple factors including land exploitation, excessive deforestation, drastic climate change, invasive species and emerging pathogens. Natural resource management focuses on accelerating the inventory of biological diversity, understanding its function and integrating its use in the sustainable development of human society. In this sense, due to the disciplinary crisis in conservation biology and thanks to the evolution of molecular information, a goal was established in which it is necessary to incorporate several technologies to accelerate, ensure and increase the precision of decision-making for conservation. One crucial approach in advancing conservation medicine was centralised database creation, such as the barcode database and DNA records.

In 2003, Paul Hebert and his working group [53] at the University of Guelph, Canada, proposed implementing a coding sequence for cytochrome c oxidase subunit I (COI or CoxI) of the mitochondrial genome as a universal marker for animal species identification. The proposal was based on simulating the universal identifier as a "barcode" used to identify commercial products, their year of manufacture, batch, cost, or simple identification within a warehouse. Thanks to Hebert and his working group's novel work, in May 2004, the Consortium for the Barcode of Life (CBOL) was created, which currently has 130 organisations from 40 countries [54].

Cytochrome c oxidase subunit I (COI, or cox1) is a fragment of the mitochondrial multienzyme complex, which occurs as a transmembrane system related to the mitochondrial matrix. The region used for barcode generation is approximately 648 bp in length. In 2003, the COI gene was designated as standard "barcode" due to its absence of introns, low exposure to recombination, haploid genetic condition, variety of copies that allow easy DNA recovery, and high mutation rate that allows distinction between closely related species, being used as a universal marker for population genetic analysis, identification, phylogeographic studies and cataloguing of all species in all taxa of the animal kingdom. It presents a diversity of nucleotides in certain regions of the gene, a characteristic that allows the discrimination of probable closely related species [55].

The use of this marker with a molecular taxonomic approach was carried out for species-level differentiation studies among 5 sea turtle species. The consumption of sea turtle products and by-products is an activity that often occurs in communities that live close to these species. The application of the "barcode" to identify illegal trafficking of sea turtle meat, eggs, carapace and other by-products helps counteract and combat these illegal activities. It is also useful in identifying stranded animals with a high level of decomposition or when traces of these species are present. Studies have used this mitochondrial fragment to identify samples of hawksbill sea turtle *Eretmochelys imbricata*. In this sense, the use of the mtDNA COI marker is useful for creating phylogenies and population dynamics and contributes directly to conservation actions for sea turtles [50, 56]. Advantages of this marker include the

high level of barcode variation between specimens, which helps in the identification of cryptic species and thus its use in species identification, biodiversity and conservation studies. The barcode can also be used to facilitate species identification when this is difficult due to the organism ontogeny or due to only receiving an individual's remains. However, disadvantages exist when using this marker, such as the lack of clarity between the genetic separation values between intra- and interspecific divergence in the selected marker. The discovery of new species cannot be confined solely to a universal barcode, since this marker has errors in the genetic separation values for certain taxonomic groups.

Another commonly used marker is the control region in mDNA, which contains a displacement loop, known as the "D-loop", believed to be the most rapidly evolving region of the mitochondrial genome in most vertebrates. This structure contains most of the regulatory elements for the mitochondrial genome expression and is used to study intraspecific population structures. It has proven useful in the study of sea turtles, with molecular markers derived from the control region used to identify sea turtle natal origins and in the case of the loggerhead turtle (*Caretta caretta*) used to demonstrate their transpacific migration. In 1994, another study designed primers from the control region and amplified a 496 bp fragment for this control region in leatherback turtles [57].

The control region is a ubiquitous characteristic of vertebrate mitochondrial genomes, its name is due to its structure that consists of a triple chain of ~0.8-1 kb and is located between the genes that encode tRNAPro on the light strand (L -strand) and tRNAPhe on the heavy strand (H-strand). This morphology is created by the displacement of the parental H-strand by a DNA of 0.6–0.8 kb complementary to the L-strand. The control region measures between 880 and 1400 bp, and in some species, it can be greatly extended due to its repeated sequences. However, its reduced size and the increased understanding of its replication and transcription mechanisms, its sequence availability in many species, this region represents a good marker to analyse the non-coding regulatory genome to the evolution in the time of the establishment of mammals about 150 million years. In the late 1990s, Dutton et al. described the control region's use, in conjunction with recapture information, to test the philopatry hypothesis. Naro-Maciel et al. conducted a study using the control region as a marker for the DNA barcode, highlighting that although this region is useful in numerous species conservation studies, it does not meet all the criteria for use as a barcode sequence [50, 57].

One advantage of this genetic marker in sea turtle molecular studies is its high degree of variability. It is the most variable region of the mitochondrial and nuclear genome, evolving ten times faster than nDNA and can be used for species identification, even reaching the sub-species level. It degrades slower than nDNA and can be recovered from samples after long storage periods.

5. Conclusion

This chapter showed different stages of sea turtles, one in particular Kemp's ridley. Since that sea turtle has as a unique site to nest, it is important to understand what the real situation about the cycle of life, anthropogenic threats, genetic diversity, and pollution. Not only, for the turtles but for human beings. To see that, previous studies in sea turtles were describe in tissue samples such as carapace, kidney, liver, heart, etc. Another kind of used sample was blood. Blood as test tissue allows information on recent exposure to anthropogenic pollutants, it is inexpensive and easy to acquire. Also, it represents a relationship between the concentrations of heavy metals with other tissues, since the blood transports them through the

circulatory system to these. Biochemical analyzes could let us see if some population of this sea turtle is healthier than others o maybe understands if spills, such as the one in 2010 in the Gulf of Mexico, impacts in this species.

On the other hand, there are few molecular studies on these turtles, all of them in secondary nesting areas and with specimens from those places. This chapter would contribute to pointing out molecular studies in mtDNA sequences such as the COI gene, the control region, or in nuclear DNA microsatellite sequences. Which can reveal this species is important to the marine ecosystem conservancy, surely due to its location limited to a single geographical area, which is the Gulf of Mexico. Finally, for many years this species was unknown until brave and clever people decided to act and to protect them. They gave a legacy and showed how marvelous the nesting and hatching are. Now, it is a great opportunity to study with modern tools them and to understand if those sea turtles are healthy and strong to transmit their genes to the next generations of Kemp's ridley. In the end, we showed the state of the art of the smallest, unique, and elusive sea turtle.

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Conflict of interest

The authors declare no conflict of interest.

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