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# Diet and captive care of sea turtles

literature review

# A tengeri teknősök tartása és táplálása

irodalmi áttekintés

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#### 1. Abstract

Rescue and rehabilitation centres have a significantly role in sea turtle conservation. Captive care of sea turtles is challenging because of their special requirement. Apart from health issues or injuries before captivity, when turtles are first presented to a rehabilitation facility, they are often malnourished and/or emaciated. Therefore, the aim of this literature review is to examine previous research throughout the years concerning nutritional and rearing management of sea turtles during captivity.

First in chapter 1, a brief description of the different sea turtle species (Green turtle [Chelonia mydas], Loggerhead [Caretta caretta], Kemp's ridley [Lepidochelys kempii], Olive ridley [Lepidochelys olivacea], Hawksbill [Eretmochelys imbricata], Flatback [Natator depressa] and Leatherback [Dermochelys coriácea]. Diets vary within species and different age groups. Then findings in nutritional parameters and management of captive sea turtles are discussed. This includes the Ca:P ratio, followed by vitamin D, amino acids, and protein requirements. Some researchers suggested that carapace anomalies in sea turtles could be due to an insufficient vitamin D supplementation during captivity. Also, more study is needed to determine long term consequences of limited UV exposure. Finally, specialise diets and techniques are suggested based on the proposed research.

Chapter 3 deals with environmental enrichment, proposing various methods found in the literature, in order to strengthen individuals mentally and physically; and therefore, release them successfully into their natural habitat. Afterwards, basic requirement concerning the size of the water tanks and water quality is also discussed.

The last chapter describes the common diseases in sea turtles during and before rehabilitation. These diseases are as follow: malnutrition, bloating-floating, stress, trauma and injuries, debilitated turtle syndrome and cold stunning, gastrointestinal disorders, bacterial and viral infections. Meanwhile, blood parameters in healthy and stranded sea turtles are described.

Based on the aim and objectives of my literature review to what findings I encountered, it can be concluded that some fields and/or areas of study still need more update and recent bibliographic references. For example, a lack of information is available on the precise nutritional requirement of each species. Also, little information is available on captive rearing of certain sea turtle species, especially Olive ridley.

# 2. Összefoglaló

A mentésre és rehabilitációra szakosodott központoknak jelentős szerepe van a tengeri teknősök megóvásában. Speciális igényeik miatt a tengeri teknősök fogságban tartása kihívást jelentő feladat. Függetlenül a fogságba kerülésük előtti egészségi állatótól vagy sérülésektől a rehabilitációs központokba kerülő teknősök sok esetben alultápláltak, illetve lesoványodottak. Ezért az irodalmi áttekintés célja, hogy az elmúlt évek publikációi alapján áttekintést adjon a fogságban tartott tengeri teknősök táplálási- és tartási igényeiről.

Az 1. fejezet röviden bemutatja a tengeri teknős fajokat (Közönséges levesteknős [Chelonia mydas], Álcserepesteknős [Caretta caretta], Atlanti fattyúteknős [Lepidochelys kempii], Olajzöld fattyúteknős [Lepidochelys olivacea], Közönséges cserepesteknős [Eretmochelys imbricata], Flatback teknős [Natator depressa] és Kérgesteknős [Dermochelys coriácea], amelyek táplálkozását befolyásolja a faj és az életkor is. Ezt követően a tengeri teknősök fogságban alkalmazott táplálása kerül bemutatásra, amely magában foglalja a Ca:P arányt, valamint a D-vitamin, aminosav és fehérje igényt. A kutatások egy része szerint a fogságban tartott egyedeknél kialakuló hátpáncél elváltozások hátterében a nem megfelelő D-vitamin ellátás áll. további vizsgálatokat igényel annak megállapítása, hogy mik a korlátozott UV-fénynek következményei. való kitettség hosszútávú Az irodalmi adatok alapján megfogalmazásra kerültek a táplálása- és etetési technikára vonatkozó ajánlások is.

A környezetgazdagítási lehetőségeket mutatja be a 3. fejezet. A javasolt módszerek alkalmazásával javítható az egyedek mentális és fizikai állapota, amely szükséges a természetbe való visszaengedéshez. Az összefoglaló tartalmazza a víztartályok méretére és a vízminőségre vonatkozó alapvető követelményeket is.

Az utolsó fejezet bemutatja a rehabilitáció előtti és alatti időszakban előforduló gyakori egészségügyi problémákat, minta az alultápláltság, felfűvódás, stressz, trauma és sérülések, lehűlés, emésztőszervi betegségek, valamint bakteriális és vírusos fertőzések. Ezen felül a dolgozat ismerteti az egészséges és lehűlt testhőmérsékletű ("cold-stunned") teknősök vérparamétereit is.

A kitűzött célok és az eredmények alapján levonható az a következtetés, hogy bizonoys témakörök esetében szükséges lenne további vizsgálatok evégzése. Példaként említhető, hogy kevés információ áll rendelkezésre az egyes fajok pontos táplálási szükségleteiről. Szintén korlátozottak az ismeretek némely teknősfaj, mint a Olajzöld fattyúteknős tartási igényeiről.

# **Table of content**

1. Abstract	1
2. Összefoglaló (Abstract in Hungarian language)	2
3. Introduction	5
4. Literature Review	7
4.1 Introduction of the sea turtle species	
4.1.1 Natural diet	
4.2. Diet in captivity	
4.2.1 Nutritional requirements	
4.2.1.2 Vitamin D <sub>3</sub>	
4.2.1.3 Essential and nonessential amino acids	
4.2.1.4 Protein requirements	13
4.2.2 Feed ingredients	
4.2.3 Feeding techniques	
4.3 Environmental enrichment	
4.3.1.1 Dietary supplements	
4.3.1.2 Freezing Feed	
4.3.1.3 Drilled pipes and feeding mat	23
4.3.2 Enrichment Devices	
4.3.3 Analysing the effectiveness of enrichment programs	
4.4 Husbandry	
4.4.2 Debilitated turtles	
4.4.3 Water quality	
4.4.4 Water temperature	28
4.4.5 Water pH	
4.4.6 Ultra violet light	
4.4.7 Hatchlings	
4.5.1 Common disorders during captivity	
4.5.1.1 Malnutrition	
4.5.1.2 Bloating-Floating	
4.5.1.3 Stress	
4.5.2 Pre-existing conditions	
4.5.2.1 Trauma and injuries	
4.5.2.3 Gastrointestinal Disorders	
4.5.2.4 Bacteria and antibiotic-resistance	
4.5.2.5 Herpes virus	32
4.5.3 Blood biochemistry and haematology	33
5. Materials and methods	36
6. Conclusions	36
7. Summary	37
0 Deferences	20

9. Acknowledgments	43
10. Copyright declaration	44

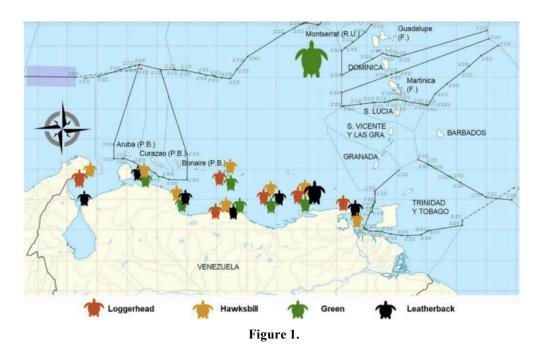
#### 3. Introduction

Species and entire ecosystems have been going extinct for millions of years as a natural evolutionary process. Today, however, marine ecosystems are going extinct due to abrupt changes brought by humans. Overexploitation, marine pollution, habitat destruction, invasive species predation and climate change are the most important threats causing marine species to decline at a rate never seen before in history. Such a threats have far-reaching social, economic and biological effects, all of which could jeopardise our own existence (Atalah, 2020). Much can be learned about the condition of the planet's environment by simply looking at sea turtles. Nowadays, all seven species of sea turtles are designated as endangered or threatened by the International Union of Conservation of Nature (IUCN) (Kenney, 2019).

Rehabilitation programs, relocation, and release have become a very important part in the veterinary medicine. As a result, the number of sea turtles released back into the wild after injuries, is rising over time despite the lack of disclosure of the outcomes from these efforts (Innis et al. 2019). Handling of sea turtles, transportation protocols, quarantine, data evaluation, facility requirements, analgesia and treatment of injuries and illness, euthanasia and water system diagrams are important aspects during the rehabilitation program. However, feeding methods, environmental enrichment, nutritional requirements and husbandry are equally important in order to rehabilitate patients. Bluvias and Eckert (2010) describe that a high percentage of sea turtles coming into rehabilitation are nutritionally deficient, if not emaciated. Therefore, knowledge about sea turtle's feeding habits and nutritional requirements is crucial for relatively efficient treatments to restore body mass and eventually release them back into their natural habitat. Sick or injured turtles may worsen rather than improve if there are subjected to unnecessary stress during rehabilitation and do not receive sufficient care or proper nutrition. Hence, the main mission at rescue centres is to provide both medical and adequate diet.

The objective of this comprehensive literature review is to summarize the most common diseases found in sea turtles before and during rehabilitation, describe nutritional requirements and recommend optimal environmental enrichment methods and diets, specific to each species. The primary goal is to display baseline knowledge from a nutrition point of view, to promote population recovery because even at diminished population levels, they play an important role in marine ecosystems by maintaining healthy sea grass beds and coral reefs, providing habitat for other

individuals, balancing marine food webs and facilitating nutrient cycling from water to land (Wilson et al, 2010). Along the Caribbean coast of my home country Venezuela, five critically endangered species of sea turtles can be found (Figure 1) nesting and feeding, with the exception of Olive ridley. Conservation and rehabilitation of these species is an important issue in this area, since according to the Red Data Book from the Conservation Monitoring Centre of the International Union of Conservation of Nature (IUCN), hunting of adult females and the persistent poaching of their eggs are the two main reasons why numbers of nesting turtles has been declining in a large area of the Caribbean Sea (Guada and Sole, 2000). Moreover, the increase of cruises and yachts, has brought issues related to the discharge of waste, anchoring, and the destruction of coral reefs and sea grass in which sea turtles, especially Green turtles can forage and hide. For this reason, if sea turtles become extinct, both the marine and beach ecosystem will weaken.

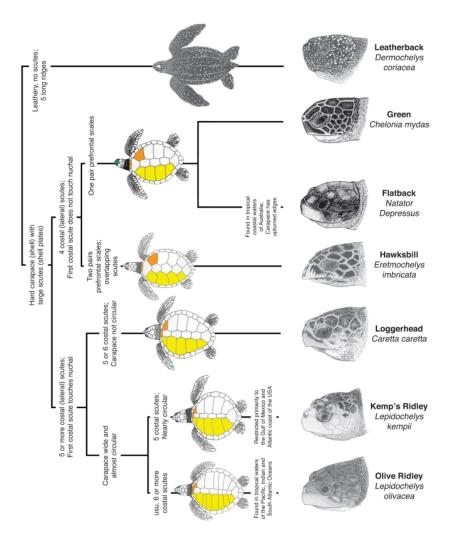


Distribution of sea turtles in Venezuela (Courtesy: Barrios and Garcia, 2020)

#### 4. Literature review

# 4.1 Introduction of the sea turtle species

Seven different species of sea turtles are recognised scientifically in our oceans (Figure 2); six belonging to the *Cheloniidae* family, includes the Green turtle (*Chelonia mydas*), Loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), Olive ridley (*Lepidochelys olivacea*), Hawksbill (*Eretmochelys imbricata*) and Flatback (*Natator depressa*). Meanwhile, the seventh specie so called Leatherback (*Dermochelys coriácea*) belongs to the *Dermochelyidae* family (Kennedy, 2019). According to the IUCN red list, all seventh species are designated as endangered or threatened (Bluvias and Eckert, 2010). Corresponding to the Turtle Island Restoration Network (2018) Kemp's ridley are consider to be critically endangered species, with a single primary breeding site being a short section of beach near Rancho Nuevo, Mexico.



**Figure 2**. Sea turtle identification key (Courtesy: seaturtle.org)

#### 4.1.1 Natural diet

Understanding sea turtle nutrition is critical for their effective conservation. A balanced meal, comparable to that of a wild diet, is provided as part of the rehabilitation process to assist wounded, sick, malnourished, and debilitated turtles to recover weight and improve physical condition before being released back into their natural environment. Differences among life stages, geographic location, temporal distributions with predominant prey species dictated by local availability and morphology are keys factors for determining their feeding strategies (Seney, 2016). Such variations are critical for giving a species-specific feed and formulating nutritionally balanced feed.

Diets vary within species and different age groups. An approximate list of different adult and hatchling sizes, weights and natural diet depending on the species is shown in Table 1. Green turtles, particularly hatchlings and juveniles, eat invertebrates in addition to their herbivorous diet of terrestrial plants, algae, and sea grass. Moreover, much of their consumption is unintentional in some circumstances but deliberate in others, classifying them as omnivores marine species (Nagaoka et al, 2011). It is a frequent misconception to feed captive Green turtles with a strict lettuce diet, which bears some poor resemblance to a diet of sea grass in terms of nutrients (Bluvias and Eckert, 2010).

Opportunistic carnivores comprise other species like Kemp's ridley and Loggerhead marine turtles. In contrast, juvenile and adult Hawksbill and Leatherback turtles are consider sponge and gelatinous prey specialists (Seney, 2016). Therefore, Leatherbacks should not be kept in captivity due to its particular requirements on a special diet based entirely on jellyfish. Nevertheless, specialised facilities with highly educated and experienced staff would be required in this instance. On the other hand, Leatherback hatchlings have been successfully raised, but only for a few weeks to a few months, and their health and development have suffered as a result (Bluvias and Eckert, 2010).

A recent research with Loggerheads, revealed that the most prevalent prey items found in the stomach contents of stranded individuals were crabs (*Brachyura*), fish (*Osteichthyes*), shrimp (*Penaeidae*), gastropods (*Busycon spp.*), horseshoe crabs, bivalve mollusks (*Spisula solidissima*) and other invertebrates including tunicates, sponges, sea cucumbers, and soft coral in decreasing order; indicating that they are indeed opportunistic carnivores (Molter et al., 2021). Nutritional analysis of common prey items of loggerhead turtles is shown in Table 2.

**Table 1.** Size, weight and diet of sea turtles

Species	SCL Hatchling	SCL Adult	Weight*	Natural diets
Green Turtle (Chelonia (mydas)	45-57mm	59- 117cm	96-186 kg	Hatchlings&Juveniles: Omnivorous Adults: Herbivorous
Loggerhead (Caretta caretta)	38-55mm	70- 125cm	170-182 kg	Carnivorous
Hawksbill (Eretmochelys imbricata)	39-50mm	63- 94cm	78-91kg	Hatchlings: omnivorous Juveniles&Adults: spongivorous
Kemp's Ridley (Lepidochelys kempii)	38-46mm	58- 75cm	32-49 kg	Carnivorous
Olive Ridley (Lepidochelys olivacea)	40-50mm	56- 78cm	35-45 kg	Omnivorous
Leatherback (Dermochelys coriacea)	56-63mm	114- 176 cm CCL	200-916 kg	Gelatinivorous
Flatback (Natator depressa)	56-66mm	81- 97cm	80-90 kg	Omnivorous

<sup>\*</sup> Adult female

CCL, Curved carapace length; SCL, straight carapace length.

From: Wyneken J., Mader D. R., Weber III E. S., Merigo C (2005) 76 Medical Care of Sea Turtles. In: Rudolp P., Stringer S (eds) Reptile Medicine and Surgery. Saunders Elsevier Inc. 11830 Westline Industrial Drive St. Louis, Missouri 63146. pp 972-1007

Seney, E.E (2016) Diet of Kemp's Ridley Sea Turtles Incidentally Caught on Recreational Fishing Gear in the Northwestern Gulf of Mexico, Chelonian Conservation and Biology 15 (1) 132-137

**Table 2.** Prey items in stomach content of loggerhead turtles

Parameters	Horseshoe carb	Whelk	Cannonball jellyfish	Blue carb
Gross energy		578 (kcal/g)	_	2,328(kcal/g)
Moisture (%)			_	_
Dry matter	29.1 (%)	75.3 (%)	5.3 (%)	31.1 (%)
Crude protein	73.7 (%)	11.4 (%)	30.8 (%)	38.1 (%)
Crude fat	1.9 (%)	1.3 (%)	2.5 (%)	2.6 (%)
Ash	17.46 (%)	84.48 (%)	59.82 (%)	51.55 (%)
Calcium	1.35 (%)	32.16 (%)	0.67 (%)	16.05 (%)
Phosphorous	0.38 (%)	0.08 (%)	0.26 (%)	1.81 (%)
Vitamin A	<0.0045(IU/g)	_	_	_
Vitamin E	3.53(mg/kg)		_	

Nutritional contents are presented as dry matter basis.

From: Molter C. M., Norton T. M., Hoopes L. A., Nelson Jr. S. E., Kaylor M., Hupp A., Thomas R., Kemler E., Kass P. H., Arendt M. D., Koutsos E. A., Page- Karjian A (2021) Health and nutrition of loggerhead sea turtles (*Caretta caretta*) in the southeastern United States. J Anim Physiol Anim Nutr 00:1–15

# 4.2 Diet in captivity

# 4.2.1 Nutritional requirements

Knowledge regarding appropriate nutritional demands for sea turtles is indispensable since proper feeding based on each species nutrition requirements will result in vigorous and healthy individuals during captivity. Additionally, parameters like daily feed intake or adequate amount of nutrients are equally important. For instance, Green turtles consume the equivalent of only 0.24% to 0.33% of their body weight each day (Bjorndal, 1980).

# 4.2.1.1 Calcium and phosphorous

Sea turtles possess a broad skeletal material, in which the levels of calcium and phosphorous are of prime importance in their diet. Moreover, the Ca:P ratio (serum or plasma) in captive reptiles, is a considerable therapeutic relevance parameter. Therefore,

an appropriate feeding of captive sea turtles can beneficially improve plasma Ca:P ratios.

Prior research by Abreu et al (2000) suggest that a diet consisting of 20% squid and 80% teleosts (shrimp, smelt, herring, mackerel) can positively affect Ca:P ratios in Loggerhead and mimic wild turtles Ca:P ratio (1.3:1). Another, approach is to reduce the amount of squid since they are low in calcium as shown in Table 3, increase food item high in calcium such as crabs and mussels, and add calcium supplements in tablet form. However, the form of calcium supplementation can affect absorption, and the bioavailability of calcium carbonate is unknown in sea turtles (Stringer et al., 2010).

**Table 3.** Calcium and phosphorous levels of sea turtle diet

Seafood item	Calcium (mg/100g)	Phosphorous (mg/100g)
Squid (unspecified)	27	193
Shrimp (Aztecus sp.)	89	258
Smelt (Osmeridae family)	567	534
Herring (Harengus sp.)	20	280
Mackerel (Scrombus sp.)	8	307

From: Abreu-Grobois F.A., Briseño-Dueña R., Márquez R., Sarti L (2000) Proceedings of the Eighteenth International Sea Turtle Symposium. In: U.S. Dep. Commer. NOAA Tech. Memo. NMFS-SEFSC 436

In reptiles, further research suggests that an optimal Ca:P ratio should range from 1.2:1 to 1.5:1. However, in the case of nesting females or growing juveniles turtles, a Ca:P ratio approaching 2 would be desirable (Choromnaski et al., 1987).

Finally, a common misconception during the rehabilitation programs of Loggerheads turtles is through the implementation of a "natural" diet consisting of shrimp, crabs and fishes, all of which have a considerably greater phosphorous content than calcium; therefore encountering low plasma Ca:P ratios (below 1.0:1) in these turtles. It is hypothesised that feeding in the wild only with shrimp, crab and fish, is not a concern since their diet is supplemented with a larger variety of foods items, including sea grass (Choromanski et al, 1987).

#### **4.2.1.2** vitamin **D**<sub>3</sub>

Vitamin D<sub>3</sub> is involved in calcium metabolism and distribution (Choromnaski et al., 1987) and therefore, it is assumed that turtle carapaces are subject to a number of growth anomalies, some of which might be caused by inadequate vitamin D supplementation (Purgley et al., 2009). Stringer et al., 2010 suggested that Green turtles in rehabilitation maintain adequate levels, perhaps due to the slow breakdown of the large quantity of calcium deposited in green turtle carapace and bones allows captive turtles to stay asymptomatic for long periods of time, especially when they are not subjected to the metabolic needs of reproduction. However, another study suggested that Green turtles kept in outdoor facilities reached vitamin D<sub>3</sub> serum concentrations of 60–70 nmol/L; while turtles kept in indoor facilities reached levels of 5– 15 nmol/L for about 4-5 months following indoor confinement and continued to decline for 6-8 years (Purgley et al., 2009).

Wild sea turtles receive plenty of sunlight to meet their vitamin D<sub>3</sub> needs in their natural habitat. Nevertheless, only a few study have reported normal serum levels of 25-hydroxivitamin D in turtles; wild turtles range between 16.1–72.1 nmol/L and 17.2-64.6 nmol/L for turtles in rehabilitation (Stringer et al., 2010). Finally, there is a wide range of commercial supplements providing appropriate levels of vitamin D<sub>3</sub>, calcium and phosphorous for captive sea turtles. Different brands and pertinent amount of each tablet are shown in Table 4.

**Table 4.** Supplements from different brands

Per tablet	<u>SeaTabs</u> ®	Mazuri <sup>®</sup> Vita-Zu <sup>®</sup> Shark/Ray tablets	Pfizer's Pet-
Cholecalciferol (Vitamin D <sub>3</sub> )	20 I.U.	690 I.U	400 I.U
Calcium	-	-	17.5%-21.0%
Phosphorous	-	-	14.0%

From: Bluvias J. E. and Eckert K. L. (2010) Marine Turtle Trauma Response Procedures: A Husbandry Manual. Wider Caribbean Sea Turtle Conservation Network (WIDECAST). Technical Report 10:100

### 4.2.1.3 Essential and non-essential amino acids

Amino acids that can be synthesized by the organism are considered nutritionally nonessential, but those that must be obtained from feed, are considered nutritionally essential. For instance, the following studies were conducted on amino acids by Wood (2009); suggesting out of eighteenth amino acids of nutritional significance, nine are consider to be nutritionally essential (lysine, histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and tryptophan), eight nonessentials (glutamic acid, aspartic acid, glycine etc) in hatchling Green turtles. On the other hand, hatchlings are able to synthesise arginine, but not in sufficient amounts to sustain maximal development. Nevertheles, arginine may not be considered semi-essential but rather nonessential, if adult turtles can synthetize enough when transitioning later into an herbivorous diet.

# 4.2.1.4 Protein requirement

Protein demand is of great importance in species such as Olive ridley hatchlings, being carnivorous and therefore; exhibiting a high protein demand. Over time, an extensive research has developed on the determination of optimum protein requirements in hatchlings by Abreu et al (2000). Consequently, the best growth response in terms of final body weight and maximum feed intake efficiency was observed by offering diet D40 as shown in Table 5; containing 43% protein and feeding three to four times per day. The obtained protein/energy rate (P:E) value was 118.48 g protein/Kcal (45%, 7% lipids and 3.79 Kcal/g.)

The method introduced by Kanghae et al., (2014) concluded that there is a variety of artificial diets that can be provide to Green turtles. As shown in Table 6, four diets classified fresh diet (FD); carnivorous fish diet (CFD); omnivorous fish diet (OFD) and carnivorous shrimp diet (CSD), were offered to Green turtles. However, feeding with a carnivorous fish diet can provide optimal growth and better-feed utilisation, as well as in terms of behaviour, reduce aggression along with good appetite. Suggesting that CFD is a suitable choice among the commercially available diets for head-started juvenile Green turtles in conservation.

**Table 5.** Nutritional value and ingredients of diet D45 and D40

	Diet (protein l	evels (g/kg -1)
Formula*	<u>D45</u>	<u>D40</u>
Fish meal	24.29	20.00
Head shrimp meal	7.30	9.13
Meat meal	21.88	18.02
Wheat meal	6.35	6.35
Fish oil	2.11	2.42
Corn oil	0.90	0.90
Dextrin	10.21	16.23
	Nutrients con	itents (g/kg -1)
Protein	45.00	40.00
Lipids	7.00	7.00
Fibre	2.78	2.94
Ash	7.52	6.82
NFE	23.44	22.96
ME (Kcal/100g)	379.78	376.67
Protein	43.19	39.57

<sup>\*</sup> Fixed ingredients present in all diets were (g/kg -1): 8.96 Soya meal, 3.38 Spinach meal 10.57 Corn meal, 0.01 BHT, 1 Lecithin, 0.10 Vitamins prepared mixture, 0.20 Minerals prepared mixture, 0.12 Vitamin C, 0.02 Coline, 0.50 CaCO3, 0.10 CaHPO4, 2 Carboximetil cellulose.

From: Abreu-Grobois F.A., Briseño-Dueña R., Márquez R., Sarti L (2000) Proceedings of the Eighteenth International Sea Turtle Symposium. In: U.S. Dep. Commer. NOAA Tech. Memo. NMFS-SEFSC 436

**Table 6.** Proximate chemical compositions of the experimental diets used for rearing juvenile Green turtles.

Chemical composition	<u>FD</u>	<u>CFD</u>	<u>OFD</u>	CSD
Crude protein	54.13±0.46	48.75±0.05	18.52±0.09	42.45±0.06
Crude lipid	6.20±0.38	6.98±0.22	3.83±0.07	6.75±0.11
Crude fibre	5.30±0.11	1.77±0.12	12.31±0.44	1.78±0.15
Crude ash	12.44±0.01	12.14±0.02	12.75±0.01	13.25±0.01
Nitrogen free extract	21.93±0.61	30.36±0.26	52.59±0.45	35.77±0.20

Chemical composition is expressed as % of dry weight

Data from triplicate analyses are expressed as mean  $\pm$  SEM.

FD, fresh diet; CFD, carnivorous fish diet; OFD, omnivorous fish diet; CSD, carnivorous shrimp diet. From: Kanghae H., Ninwat S., Thongprajukaew K., Intongcome A., Kittiwattanawong K (2014) Performance of Head-Started Green Turtles, *Chelonia mydas* (Linnaeus 1758) fed a commercial diet. Asian Fisheries Science 27:164

In regards of adult individuals, the only sea grass herbivore in the Caribbean sea is the Green turtle which, prior to over exploration of their population, was certainly the major sea grass (*Thalassia testudinum*) consumer in tropical and subtropical waters, like the Caribbean Sea (Bjorndal, 1980). Therefore, knowledge on the nutritional content of sea grass, shown in the Table 7, is fundamental since it allows predicting nutrient requirements.

Green turtles feeds selectively, maintaining grazing plots of young blades of sea grass which are higher in protein and lower in lignin levels compared to ungrazed older leaves of sea-grass; therefore, by re-grazing, individuals are selecting a food that is higher in protein and lower in lignin, considering it forms complexes with cellulose and hemicellulose, and hence limits the activity of digestive enzymes (Bjorndal, 1980). On the other hand, a change from a diet of sea grass to algae (*Sargassum* sp. or *Batophora* sp.) would require radical changes from their cellulose secreting gut microbes to microbes secreting enzymes capable of breaking down algae carbohydrates; resulting in low digestibility (Bjorndal, 1980).

**Table 7.** Nutrient component of blades of Turtle-grass (*Thalassia testudinum*) from grazed patches. Organic matter values are presented as percent of dry matter, energy as KJ g-1 of organic matter, and the remaining values as percent of organic matter.

Component	Mean quantities of blades consumed from September to August
Organic matter	74.1 (0.82)
Energy	18.9 (0.17)
NDF	58.9 (1.10)
ADF	49.9 (1.53)
Lignin	4.6 (0.29)
Protein	-

NDF: Neutral detergent fibre; ADF: Acid detergent fibre; SD: Standard deviation, -: Not determined Cellulose constitutes the major component of NDF (averaging 45.3%), while hemicellulose and lignin average only 9.0% and 4.6%, respectively.

From: Bjorndal, K. A (1980) Nutrition and grazing behaviour of the green turtle *Chelonia mydas*. Marine biology 56 (2) 147-154

#### **4.2.2** Feed ingredients

A recent research conducted by Bloodgood et al (2020) at the Georgia Sea Turtle Center revealed that the intestinal bacterial phylum of Green turtles is largely composed of *Fiirmicutes* and *Bacteroidetes*; therefore, when offering a diet with high percentage of vegetables, *Firmicutes* population increases in the gut and *Bacteroidetes* decreases. In conclusion, some rehabilitation centers feed Green turtles early in rehabilitation with a seafood-based diet, high in animal protein, in order to combat poor appetite and emaciation; later switching to a vegetable-based diet in order to restore the normal gastrointestinal flora before release into the wild (Bloodgood et al., 2020). On the other hand, Abdelrhman et al (2016) suggested that the gastrointestinal bacterial community of Loggerheads is made up of *Firmicutes* (66%), *Proteobacteria* (23%), and *Bacteroidetes* (6.2%). The unusual prevalence *Firmicutes* in a carnivorous species still is unknown. However, phylogeny, in addition to diet, influences bacterial communities, and closely related species have homologous microbes; this might explain why Green turtles and Loggerhead turtles have almost similar microbiomes.

Loggerheads yearlings should be feed with a balanced gelatine diet as shown in Table 8 and Table 9, in order to growth stronger and heavier and additionally improve

plasma Ca:P ratio from 1.15:1 to 1.30:1 (Choromanski et al, 1987). Although, if the gelatine diet is to be used for juveniles or adults that are healthy and not in need of additional caloric intake, a maintenance value should be use (Bluvias and Eckert, 2010).

**Table 8.** Composition of gelatine diet for Loggerhead yearlings

Ingredients of Staple Diet	Weight (g)	% of diet
Trout chow	425	8
Fish, various species	565	10.6
Squid (visera removed)	282	5.3
Peeled shrimp	282	5.3
Carrots (fresh, washed)	142	2.7
Spinach (fresh or frozen)	142	2.7
Gelatine (unflavored)	450	8.5
Water	2800 mL	53

From: Wyneken J., Mader D. R., Weber III E. S., Merigo C (2005) 76 Medical Care of Sea Turtles. In: Rudolph P., Stringer S (eds) Reptile Medicine and Surgery. Saunders Elsevier Inc. 11830 Westline Industrial Drive St.Louis, Missouri 63146. pp 972-1007

Captive Kemp's ridleys beyond their first year of life should be given a variety of natural foods, especially crustaceans, crabs (*Callinectes sapidus*, *Callinectes ornatus*), and a nutritious, dry, pelletized, floating diet such as PURINA's turtle chow. On the contrary, oily or fatty fish should indeed be avoided as a food source for captive Kemp's ridleys because they promote fatty liver degeneration and steatitis, which usually results in death (Fontaine et al., 1988).

In most situations, especially for short-term care, a product of comparable nutritional content – particularly for protein, fat, carbohydrates, and vitamins – could be substituted. Dog food (small chunk), for example, might substitute for trout chow, which is widely used in the aquaculture sector. On the other hand, commercial diets such as AquaMax and Zeigler Finfish Silver are widely used as well (Crude protein: 40% min., Crude fat: 10% min., Crude fiber: 4% min., Moisture: 12% min., and Ash: 8% min) (Bluvias and Eckert, 2010).

**Table 9.** Supplements of gelatine diet for Loggerhead yearlings

Supplements	Weight (g)	% of diet
Sea Tabs (Pacific Research Labs, Inc El Cajon, Calif) #4	500 mg tabs	0.04
Amino acid Complex 1000 #4	500 mg tabs	0.04
Spirolina (Lightforce, Santa Cruz, Calif)	50 mL powder (28g)	0.5
Rep-Cal (Rep-Cal Research Labs, Los Gatos, Calif)	200 mL powder (180g)	3.4

From: Wyneken J., Mader D. R., Weber III E. S., Merigo C (2005) 76 Medical Care of Sea Turtles. In: Rudolph P., Stringer S (eds) Reptile Medicine and Surgery. Saunders Elsevier Inc. 11830 Westline Industrial Drive St.Louis, Missouri 63146. pp 972-1007

Feeding intervals have a direct effect on feed utilization according to a recently published study; two per day with long time interval (at 08:00 and 16:00) is optimal for Hawksbill sea turtle (Jualaong et al., 2021). Twice a day feeding is recommended for juvenile Green turtles in their head-started propagation. This technique promotes growth, reduces feed consumption, and improves health and carapace quality (Kanghae, 2017)

# 4.2.3 Feeding techniques

Bluvias and Eckert (2010) recommend two feeding techniques; Primarily, the most basic feeding method is to hand-toss prepared food into the tank. Food products should be distributed rather than concentrated in one place, in order to encourage foraging. Additionally, assisted feeding which consist of occasional persuasion before they will eat live food. Furthermore, another recent technique so called "Drowning feeders", described as a non-contact feeding method, avoids throwing food items and limits human interaction to better prepare them for the wild and eliminate the issue of the birds to catch sea turtle food at the surface (Gaspar, 2017).

According to Songnui et al (2017), another important factor to be considered is the type of pellets and water depth. Therefore, turtles reared in a 15 cm water depth and fed with floating pellets exhibits superior growth (specific growth rate 2.76% body weight/day) and feed utilisation (feeding rate 1.42% body weight/day, feed conversion ratio 0.61 g feed/g gain, protein efficiency ratio 3.76 g gain protein); suggesting that the

rearing programs of post-hatching Green turtles should provide a 15 cm water depth tank and the preferred type of feed is floating (vs. sinking) pellets. Table 10 Illustrates different chemical compositions of two pellet types.

**Table 10**. Commercial floating or sinking feed pellets for marine carnivorous fish expressed as g/kg-1 on DM basis.

Chemical composition	Sinking pellet	Floating pellet
Moisture	75.9	68.8
Crude protein (DM)	474.1	466.8
Crude lipid (DM)	85.5	80.8
Crude fiber (DM)	23.4	29.4
Ash (DM)	117.7	111.5
NFE (DM)	299.3	311.5

From: Songnui A., Thongprajukaew K., Kanghae H., Satjarak J., Kittiwattanawong K (2017) Water depth and feed pellet type effects on growth and feed utilisation in the rearing of green turtle (*Chelonia mydas* Linnaeus, 1758). Aquatic Living Resources 30 (18) 2-8

Lastly, another beneficial technique described by Kanghae et al (2017) is presoaking feed pellets in water, prior to use in feeding, which contributes to the successful rearing of juvenile Green turtles. The benefits of pre-soaking may be related to the dilution effect that avoids overfeeding. In contrast, feeding un-soaked pellets has been reported to lower survival rate, growth and feed utilisation. It is important to keep in mind that increasing feed moisture should not be pursue because it would make the feed pellets fall apart, so the highest and optimal practical soaking ratio is suggested to be 0.7.

#### 4.3 Environmental enrichment

Environmental enrichment means is the execution of various methods within an artificial environment in order to optimize the life quality of captive animal. Captive settings are often safer than wild habitats, but they can also be less demanding and engaging. However, a more rich and dynamic environment is proposed in order to

provide more compelling cognitive activities. According to Monreal-Pawlowsky et al. (2017) based on the structure of the enrichment provided, there are five distinct types of enrichment: sensory, cognitive, structural, social and nutritional.

The enrichment program can begin after it is determined that the individual has acclimated to the new enclosure by monitoring hunger or indicators of discomfort (increased RR, fast movements, swimming against the tank, attempting to escape, or extended immersions) (Monreal-Pawlowsky et al, 2017). The goal is to enhance or contribute to the demonstration of species-specific behaviour such as exploration, foraging, and tactile stimulation. Although, no previous research on sea turtles has revealed that enrichment devices influences foraging behaviour (Kanghae et al, 2021). In the same way, when enrichment is present there is a decrease in resting and repetitive pattern swimming around the tank, together with an increase in random swimming and focused behaviour such as bitting, pushing and rubbing against items (Therrien et al, 2007). On the other hand, when feeding is restricted, fighting and biting can happen among sea turtles (Kanghae et al, 2021). Therefore, the introduction of enrichment devices might lessen the incidences of hostile or disruptive behaviours during captive programs (Therrien et al, 2007). Nevertheless, some authors have also suggested that the introduction of novel stimulation should ensure that the animal interacts with the enrichment devices both voluntary and exploratory, and that increase activity is not elicited as a result of disturbance due to stress (Therrien et al, 2007). It is advised that blood samples be taken once a month to ensure that parameters, particularly stress hormones and leukocyte levels, stay within range when the turtle is consuming on live food and being presented with novel items that the turtle may locate at sea (Monreal-Pawlowsky et al, 2017).

# 4.3.1 Novel food items

# 4.3.1.1 Dietary supplements

The majority of studies have applied the use of novel food as an enrichment method, aiming to stimulate appetite, curiosity and movement to forage actively (Bluvias and Eckert, 2010). Similarly, boredom or stereotypical behaviours among individuals can be avoided by changing the presentation of feed and enhancing foraging by scattering or hiding it (Brown, 2009). A variety of food items can be offered, especially easy to catch live preys like crabs for Loggerheads and Ridleys species. However, novel foods should

not be offered to individuals that do not have a stable digestive system and/or are not in preparation for a near release (Bluvias and Eckert, 2010).

# 4.3.1.2 Freezing feed

Bluvias and Eckert (2010) suggested one method, consisting of the introduction of ice blocks filled with shrimp, fish or vegetables (Figure 4). Likewise, this was successfully established and described by the Georgia Sea Turtle Center (2001), where Loggerheads and Green turtles patients grasped their beaks and chased frozen treats, which mimics their natural behaviour towards hard-shelled blue crabs, whelks, and/or horseshoe crabs. Alternatively, some turtles learned to wait for the food to reach the bottom of the tank once the ice melted.



Figure 4.

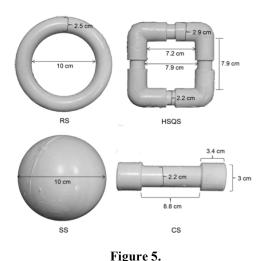
Ice blocks filled with shrimp as an environmental enrichment (Courtesy: https://seaturtleresearch.weebly.com/enrichment.html)

# 4.3.1.3 Drilled pipes and feeding mat

Filled drilled pipes with leafy or sliced vegetables, or feeding mats made of rubber can encourage foraging behaviours in species such as Green turtles that feeds primarily on sea grass and algae (Bluvias and Eckert, 2010). For example, a research showed positive results when applying polyvinyl chloride (PVC) lettuce feeder plastic and water-cooler jug with holes filled with fish and squid for Green turtles and Loggerhead turtles (Therrien et al, 2007)

#### 4.3.2 Enrichment devices

Healthy patients are optimal candidates for the implementation of large, smooth rocks, waterfalls, back scratchers and hiding places for enhancing tactile stimulation and natural exploration (Bluvias and Eckert, 2010) (Figure 6). This was successfully established as described by Therrien et al (2007), where rubbing on the PVC pipes and waterfalls was observed, which is a similar response towards crabs or fish cleaning their shells in their natural habitat. On the other hand, Kanghae et al (2021) studied enrichment devices preferences in Green turtles by using four different shapes such as ring shape, hollow square shape, sphere shape and cylinder shape (Figure 5). Subsequently, the ring shape device was described as one of the most appropriate enrichment devices, since patients interacted more with it, followed by the hollow square shape device. Likewise, the percentage of wounds suffered through biting was significantly reduced in the groups exposed to enrichment devices, notably in the turtles exposed to the sphere shape, followed by the ring shape device.



Morphological appearance of four enrichment devices (Courtesy: Kanghae et al (2021)



Figure 6.
Back scratcher for sea turtles
(Courtesy:
https://marinesavers/com/2020/11/turtle-toys/)

Moreover, a similar behavior between sea turtles and dog was described by Therrien et al (2007) and Lloyd et al, (2012), where sea turtles will habituate to the same enrichment device and eventually lose interest. Therefore, ad libitum access should be limited, and the device should be change regularly and/or rotated for short periods of time to maintain stimulation.

# 4.3.3 Analyzing the effectiveness of enrichment programs

Many parameters are available for assessing the effectiveness of enrichment programs. In particular, the use of enrichment devices as an alternative approach to improve animal welfare. A recent study carried out by Kanghae et al (2021), reported that different forms of enrichment devices do not influence the growth performance and feed utilization parameters of Green turtles. Hence, enrichment devices do not have neither negative nor positive effects on the health status of the turtles. Secondly, Kanghae et al (2021) indicated that hematological parameters vary between enrichment devices used, demonstrating that they have no harmful or beneficial influence on health (Table 11). In the same way, the research proved that elemental composition of the carapace (carbon, calcium, sulphur, and chlorine) is not negatively affected by the introduction of enrichment items.

A large number of sea turtles have been held in rehabilitation centers for several years, based on the premise that they would have a lower chance of survival. However, Monreal-Pawlowsky et al. (2017) conducted a research in which a wounded Loggerhead turtle was safely returned into the wild after losing a limb and being held in captivity for ten years. Enrichment devices were provided based on the reaction to the enrichment offered the previous day (Table 12). The trial was designed in such a manner that whether the animal received regular feed or fasted was determined by the reaction to the device provided.

**Table 11.** Haematological parameters of green turtles exposed to various enrichment devices.

Haematological parameters	Control	Ring shape	Hollow square shape	Sphere shape	Cylinder shape
RBC (×10 <sup>5</sup> cells/μl)	2.83 ± 0.72	2.27 ± 0.64	$2.27 \pm 0.64$	2.33 ± 0.59	$2.40 \pm 0.66$
Hb (g/dl)	6.15 ±2.15	5.77 ± 0.39	$5.50 \pm 0.49$	5.73 ± 1.13	$5.63 \pm 0.33$
Hct (%)	14.53 ± 5.46	17.33 ± 1.20	$16.67 \pm 1.45$	19.67 ± 2.96	17.00 ± 1.00
WBC (( $\times 10^5$ cells/ $\mu$ l)	1.79 ± 0.49	1.40 ± 0.39	$1.21 \pm 0.27$	0.71 ± 0.37	$0.50 \pm 0.24$
Lymphocyte (%)	70.33 ± 8.09	55.33 ± 4.84	$64.33 \pm 7.54$	48.67 ± 8.37	49.67 ± 2.91
Monocyte (%)	13.67 ± 4.67	25.33 ± 5.24	$18.33 \pm 6.06$	20.67 ± 2.19	28.00 ± 4.51
Eosinophil (%)	16.00 ± 4.51	19.33 ± 1.20	$17.33 \pm 2.60$	30.67 ± 6.39	22.33 ± 7.22
BUN (mg/dl)	29.37 ± 1.58	26.70 ± 0.40	$29.30 \pm 0.91$	26.07 ± 1.19	28.90 ± 1.85
Creatinine (mg/dl)	0.35 ± 0.09	0.46 ± 0.07	$0.38 \pm 0.09$	$0.36 \pm 0.04$	$0.41 \pm 0.06$
Uric acid (mg/dl)	5.72 ± 0.49	7.80 ± 0.52	$6.30 \pm 1.45$	6.52 ± 0.62	$8.25 \pm 1.13$
Cholesterol (mg/dl)	50.00 ± 11.85	68.67 ± 5.67	$78.00 \pm 7.55$	66.67 ± 7.22	65.67 ± 2.85
Triglyceride (mg/dl)	185.33 ± 36.68	241.00 ± 30.00	191.00 ± 4.00	196.50 ± 25.50	275.00 ± 16.00
Total protein (g/dl)	7.57 ± 0.07	7.73 ± 0.37	$8.00 \pm 0.90$	7.90 ± 0.21	$7.83 \pm 0.37$

Note: The sampling was done at the end of the ten week experiment. Data are expressed as tank mean  $\pm$  SEM (n = 3 per treatment).

Different superscripts in the same row indicate a significant difference (p < .05).

From: Kanghae H., Thongprajukaew K., Inphrom S., Malawa S., Sandos P., Sotong P., Boonsuk K (2021) Enrichment devices for green Turtles (*Chelonida Mydas*) reared in captivity programs. ZooBiology 40 (5) 407-416

 Table 12. List of all enrichment devices used during the programme

<b>Enrichment type</b>	Enrichment device	Action	Expected action from sea turtle
Nutritional	Live sea urchins	Morning: introduced, if not eaten until 17:00, retrieve all but one.	Interest within 30 min. Intake within 5h.
Nutritional	10 Live crabs	Introduced. Monitoring for two hours.	Interest within 30 min. Intake during the day.
Nutritional	2 kg herring, mackerel, mussels, clams, red mullet.	Introduce it all in one place.	Full intake within a max of 8 h.
Nutritional	Fresh mackerel	Food distributed at different points.	Active swimming. Full intake within 5 h max.
Nutritional	Live crabs and hidden sardines	Hide within live rocks until next day.	Smells the food. Full intake by next day.
Nutritional	5 kg fresh dead fish	Leave it until next day. Water quality needs to be monitored.	Interest within 30 min, intake during afternoon and night.
Nutritional	Live jellyfish	Introduced.	Intake within 3 h max.
Nutritional	10 mussels and 10 fresh	Hided within live rocks until next day.	Interest. Full intake until next day.
Structural	1 meter long boat defence, white, floating on the surface	Introduced for 10 min. max. If it is really stressful.	dive and get away from the defence.
Structural	Two yellow fishing buoys	Introduced for 10 min. max. If it is really stressful.	dive and get away from the buoys.

Structural/Sensory	Wooden log on surface of pool	Introduced for 30 min. max if the animal scratches itself against it or 10 min if it dives away from it, or less if the turtle shows high stress levels and swimms against the walls.	either dive away or scratch itself.
Structural/Sensory	Artificial rain	Created for 30 minutes.	not fear or stress showed.
Structural/Sensory	Live rocks	Introduced	Scratching and moving them around
Sensory	Noise	Expose to noise from a CD recorder mimicking ships.	not show any reaction or dive away from it.

From: Monreal-Pawlowsky T., Marco-Cabedo V., Palencia Membrive G., Sanjose J., Fuentes O., Jimenez E., Manteca X (2017) Environmental enrichment facilitates release and survival of an injured loggerhead sea turtle (*Caretta caretta*) after Ten Years in Captivity. Journal of Zoo and Aquarium Reserch 5 (4) 182-186

Finally, as Watters and Meehan (2007) describes, there are several causes why release programs might fail. Primarily, because the animal is unable to perform foraging in their natural habitat and/or does not know how to successfully respond to predators. Therefore, rescue centres must enrich their environment in variety of ways, rather than relying solely on foraging tactics while disregarding the concept of predators.

#### 4.4 Husbandry

Successful rehabilitation requires more than adequate veterinarian assistance. Any rehabilitation attempt must include adequate housing, appropriate nourishment, and correct handling. In addition, water quality; temperature, flow velocity, facility design, and a high-quality diet are all key elements in facilitating healing processes. The recovery period will be aided by appropriate husbandry and nursing care. However, an absence of appropriate husbandry impedes and complicates the rehabilitation process. According to the guidelines for the initial treatment and care of rescued sea turtles (2021), husbandry should be adapted to each individual, depending on the behaviour

pattern and health status, since requirements for sea turtles in the initial period following rescue differs from requirements for sea turtles in longer-term care.

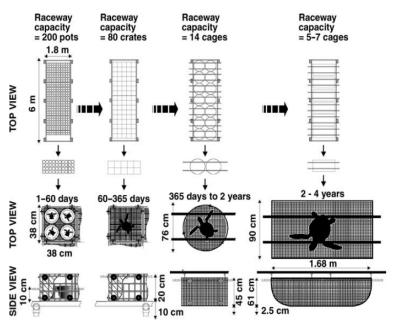


Figure 7.
Progression of rearing container size (Courtesy: Higgins, 2003)

#### 4.4.1 Rehabilitation tanks

Sea turtles require tanks with nonabrasive surfaces (Bluvias and Eckert, 2010). When available, polls with circular or oval shaped are preferred, in order for them to not get stuck (Hall, 2018). On the other hand, once the patients go from intensive care to rehabilitation, appropriate assessment of the turtle's ability to cope in the ocean is necessary. Filter systems or wave pools can generate current and turtles can swim against to build up strength, as well as reducing pathogens and algae growth (Hall, 2018).

Depending on the turtle's size, a minimum adequate tank size will be required. For example, ranging from a 10cm SCL turtle needs a tank with  $\geq$  929 square cm<sup>2</sup> of surface area to a 90cm SCL turtle needs a tank with  $\geq$  114271 square cm<sup>2</sup> of surface area (Hall, 2018) (Figure 7). Other literature provides a helpful calculation for tank size: 10cm SCL turtle needs a tank with  $\geq$  1 square foot of surface area (Bluvias and Eckert, 2010). Finally, water depth of 0.6-1.2 m allows the turtle to fully dive and return to the surface with minimal effort (Bluvias and Eckert, 2010).

#### 4.4.2 Debilitated turtles

Debilitated sea turtles are often extremely weak to be housed in water tanks upon arrival. Instead, these patients are maintained on padded surface waterbeds (Hall, 2018), with water levels below the nostrils according to the guidelines for the initial treatment and care of rescued sea turtles (2021), this helps to keep the animal wet and prevent drowning. Furthermore, sick or injured turtles are often dry-docked on padded surfaces. If the period is prolonged, damp towels over the carapace are required (Guidelines for the initial treatment and care of rescued sea turtles, 2021). Once the turtle is strong enough, they are transferred to a sided tank; therefore, it is imperative that good water quality is maintained.

#### 4.4.3 Water quality

Good water quality is an essential part of housing sea turtles for rehabilitation purposes. After 24 hours of arrival animals should be transitioned into warm clean seawater and be supervised for buoyancy, ability to swim, and strength, according to the guidelines for the initial treatment and care of rescued sea turtles (2021). Alternatively, tap water can be mix with marine salt at a dilution of 7 cups to 50 litres of water to create 30 ppt (Hall, 2018). Salinity should be maintained between 20-35 ppt (Bluvias and Eckert, 2010).

# 4.4.4 Water temperature

Sea turtles are ectothermic, meaning that their body temperature depends on external sources such as water temperature and/or sunlight. The optimum water temperature for treating turtles is 26°C (Hall, 2018). In addition, if attempting to increase the gut motility in order to remove debris or other blocking foreign bodies, the water temperature can be gradually increased to 30°C (Hall, 2018). However, temperatures above 32°C, can promote algae and bacteria populations propagate rapidly (Higgins, 2003). Under normal natural conditions, marine algae establishes and grows in patches on sea turtle shells and provide incidental food for fishes that feeds on ectoparasites. Nevertheless, in the absence of symbiotic grazer species and the presence of algae growth, keratinised shell layers can degrade resulting in significant morbidity. Besides, the presence of dense mats of algae serves to mask signs of injury and disease (Arena, 2014).

In cold stunned turtles, the water temperature upon arrival should be around 21°C and then gradually increments of 1°C per day should be achieved until it reaches 26°C and the desire core body temperature is obtained, in order to lessen physiological stress (Hall, 2018). Moreover, if the water is too cold, immune suppression can be an outcome, apart from delaying healing and depressing appetite. Similarly, hyperthermia can happen if the water temperatures are too high (Hall, 2018). Another important factor is the temperature over the tanks, which should range 29–32°C at night and reduced to 24-26°C during the day. Heaters, fans and cross-flow ventilation can be used depending on the time of the year (Higgins, 2003).

# 4.4.5 Water pH

As waste products accumulates in the tank water quality decreases from the accumulation of waste products; therefore, pH eventually decreases; therefore, pH is considered to be an indicator of water quality (Higgins, 2003). Optimal water pH ranges from 7.5 to 8.5. Besides this, pools must be free of minerals such as lead or copper paints, which can be toxic for the turtles. Similarly, chlorine levels greater than 1.0 ppm can irritate the turtle's eyes (Hall, 2018).

# 4.4.6 Ultra Violet light

Natural diurnal light patterns should be replicated for turtles housed in captivity. According to Keller and Mustin (2017) weight gain and growth can be achieve in Green turtles in outdoors enclosures with direct and indirect sunlight or full-spectrum lighting with a natural photoperiod. Also, turtles prefer shade structures when it is provided as an option. In contrast, excess light and nontherapeutic direct sunlight can promote algae growth, elevated water temperatures and sunburn (Higgins, 2003).

# 4.4.7 Hatchlings

Husbandry of hatchlings, in particular, requires direct sunlight through an open window or exposure to a UV lamp for 30 minutes each day in glassfish aquariums with inbuilt filters and heaters (Hall, 2018). One way to assess hatchling welfare and stress levels is to measure corticosteroids concentrations, growth and mortality as biomarkers to define the most adequate rearing conditions to maintain hatchling welfare. For example, while in captivity, hatchlings are routinely handled, for example, for data collection and cleaning. Hatchlings can acclimate to frequent handling since no significant increase on

corticosteroids is detected. However, increases in corticosteroids levels can be seem in isolated hatchlings. On the other hand, it is suggested that optimal growth can be reached by providing single housing, whereas mortality increases in tanks with three or more hatchlings (Usategui-Martín, 2021)

### 4.5 Health problems in captive sea turtles

Clinical approach of sea turtles in captivity continues to play an essential role. However, it is important to understand the causes of population depletion via epidemiological research, if many of these species are to recover satisfactorily. Recent research have conducted to the idea that sea turtles may operate as sentinel markers of environmental health due to the relationship of specific diseases such as fibropapillomatosis and harmful environmental conditions.

# 4.5.1 Common disorders during captivity

#### 4.5.1.1 Malnutrition

Sea turtles presenting concave plastrons, sunken eyes, and muscle atrophy with reduced weight, are to be considered emaciated. Emaciation is usually related to disorders involving flippers, mouth or eyes, impeding vision or mobility, chronic diseases or parasitism which can cease feeding for long periods of time and waste away, resulting in cachectic myopathies (George, 1996). Mashkour et al (2020) describes a variety of parasites infecting sea turtles, primarily trematodes such as gastrointestinal flukes (*Digeneans*) and cardiovascular flukes (*Spirorchidae*). On the other hand, gastrointestinal nematodes like *Anisakidae* and *Kathlanidae* have been reported to infect Loggerhead turtles. Normal total protein concentrations for reptiles have been reported to be 3–7 g/dL, but in hypoproteinemic turtles ranging 2–3.79 g/dL, this can be an indicator of chronic malnutrition. Likewise, low haematocrit values can also indicate malnutrition (Coleman et al., 2016).

# 4.5.1.2 Bloating-floating

Overfeeding commercially prepared pellet diet in species such as Loggerheads and Kemp's ridleys, can contribute to intestinal blockages, bloating and floating (Bluvias and Eckert, 2010; Higgins, 2003) which can result in carapace–plastron deformities, rendering the turtle unfit for release and/or often leading to death (Higgins, 2003). Therefore, monitoring very carefully and specific feeding formula are required. Higgins

(2003) emphasises the National Marine Fisheries Service, Sea Turtle Facility (NMFS STF) model to describe successful feeding techniques, which uses floating pellets for the first 2 years, and switches to a natural fish-based diet afterwards. Also, the amount of feed is increase or decrease from the formula on the basis of the appearance and activity of the turtles. On the other hand, pre-soaking the feed in water can reduce the abdominal bloating of turtles (Kanghae et al (2017)

#### 4.5.1.3 Stress

Stress plays an important role when dealing with sea turtles in captivity, like other animals, they lose their ability to fight disease when they are stressed. Stressors such as salinity, pollution, temperature, nutritional or physical trauma triggers corticosterone production, which decreases the humoral and/or cell-mediated mechanisms, inhibiting the immune system's capacity to respond to infectious pathogens (Caliani et al., 2019). Opportunistic bacteria species such as Vibrio and Flavobacterium are found naturally in seawater and only become pathogenic when animals are stressed resulting in weak immune system, and disturbed gut microbiota (Higgins, 2003).

Similarly, high water temperatures can induce stress and consequently increases the severity of Gray patch disease lesions. However, an inactivated vaccine has been produced against ChHV-1 (Rao et al, 2020). Finally, the most critical period during rehabilitation is the first two months, while after more than one year the immune values are similar to those of free-ranging turtles, indicating acclimatisation towards captivity (Caliani et al., 2019).

# 4.5.2 Pre-existing conditions

#### 4.5.2.1 Trauma and injuries

Traumatic injuries represent one of the most common reasons for stranding sea turtles. This includes predator bites, by-catch, boat strike, accidents and mating season, leading to infection, fractures on limbs or shell. Also, if the animal is kept in overcrowded facilities, aggressive behaviours can be observed (Mashkour et al., 2020).

# 4.5.2.2 Debilitated Turtle Syndrome (DTS) and Cold Stunning

When water temperature drops below 10°C, turtles become weak and/or inactive triggering immunosuppression. Mashkour et al (2020) strongly suggests that this can

lead in some cases to DTS, which includes emaciation, anaemia, lethargy, hypoglycaemia and additionally coverage by harmless epibiota.

#### 4.5.2.3 Gastrointestinal disorders

More relevant than ever, climate change and macroplastic pollution contributes to gastrointestinal disorders in sea turtle populations. Ingestion of plastic can lead to perforation, obstruction, and obstipation. Even if they survive, growth and reproduction is reduced due to lower nutrient intake. Gut impaction and faecoliths are also observed in stranded individuals without obvious or physical cause. However, climate changes are affecting marine grounds and therefore, their foraging behaviours (Mashkour et al., 2020).

#### 4.5.2.4 Bacteria and antibiotic-resistance

According to Higgins (2003) the most prevalent bacteria species isolated from sea turtles belong to four genera of gram-negative bacteria, such as Vibrio, Aeromonas, Pseudomonas, Cytophaga- Flavobacterium. Moreover other bacteria species including Streptococcus, Salmonella, and Coliform can be found in Green turtles, Hawksbill, Loggerhead, and Kemp's ridleys. Another study conducted with young Green turtles at the Sea Turtle Conservation Centre of Thailand (STCCT) found three extensive bacteria families belonging to Staphylococcaceae (45 isolates; 40.5%), Enterobacteriaceae (40 isolates; 36.0%), and Vibrionaceae (16 isolates; 14.4%) (Chuen-Im et al, 2021).

Early detection, isolation, and treatment with injectable antibiotics can improve effectively the chances of recovery. However, antibiotic resistance is a serious health problem for both humans and animals. For example, research has provided evidence for

Beta-lactam drugs like Ampicillin, to be one of the most common causes of bacterial resistance, followed by Penicillin and Cefazolin, since they are commonly used to treat Gram-negative bacterial infections. On the contrary, a low Aminoglycoside resistance rates were observed in seawater from holding tanks. Nevertheless, within five Aminoglycosides, the most resistance isolates were against Amikacin (Chuen-Im, 2021).

# 4.5.2.5 Herpes virus

Associated with several diseases of marine turtles including gray patch disease (GPD) of Green turtles and fibropapillomatosis of Green turtles, Loggerhead, and Olive ridley

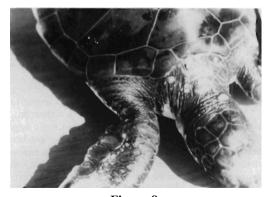


Figure 8.

Green turtle with "pustular" lesions on neck and flippers (Courtesy: Haines, 1978)



Figure 9.

Cutaneous fibropapillomatosis of the left axilla in Green turtles (Courtesy: Flint, 2013)

(Curry et al., 2000). Gray patch disease caused by Chelonid Herpes Virus 1 (ChHV-1), is a critical and prevalent disease in captive sea turtles (Crane, 2013; Rao et al., 2020). Hatchlings die instantly and no mark is typical at this point. On the other hand, juveniles survive and subsequently develop the greyish patchy lesions (Haines & Kleese, 1976). There are two forms of the disease, one is considered benign and includes various non-spreading papules and/or pustules found on the skin of the neck and flippers (Figure 8). On the other hand, a lethal form is manifested rapidly with more extensive greyish patches with raised edges (Figure 9) (Domiciano, 2017; Haines, 1978).

Similarly, fibropapillomatosis (FP) is an infectious disease caused by Chelonid Herpes Virus 5 (ChHV-5) (Crane, 2013, Rao et al., 2020). Single or multiple proliferative masses with a wide variety of gross appearances such as flat plaques, pedunculated, sessile, verrucous, smooth, or polypoid nodules, or a combination of multiple types develop anywhere on the body (Page-Karjian, 2019). It is of greatest concern in species like Green turtles, as it has reached a panzootic status, which is the equivalent to a pandemic in human populations (Jones et al., 2016).

# 4.5.3 Blood biochemistry and haematology

Measurement of blood parameters can be beneficial for indicating overall health status of wild sea turtles, individual health evaluations of stranded turtles, and searching possible causes of mortality. Serum is usually used for biochemical analysis in mammals. However, it is not recommended for reptile blood work because clotting is

unpredictable and allows changes in the chemical composition of the sample (Casal et al, 2009).

A large number of existing studies have examined how a wide variety of factors in sea turtles species can affect blood parameters. For instance, Deem et al (2009) suggested as shown in Table 13 and 14, that there is a number of differences found between foraging, nesting and stranded Loggerhead turtles. Therefore, high protein values in nesting Loggerhead turtles, indicates their current egg laying activity. In contrast, low total protein for stranded turtles can be associated with poor nutrition, chronic parasitic infections and/or protein-losing disease. Additionally, blood values can be influenced by age, size, reproductive status, environmental conditions and nutrition (Rousselet et al, 2013; Casal et al, 2009; Fazio et al, 2021). Therefore, regarding age, there is a significant increase in packed cell volume (PCV) values correlated to the carapace length in adult sea turtles (Casal et al, 2009).

Statistical plasma analysis shows no influence from fibropapillomatosis in free ranging turtles. However, captive sea turtles suffering from fibropapillomatosis shows significantly higher levels of alkaline phosphatase (ALP) and significantly lower levels of lactate compared to healthy captive individuals (Swimmer, 2000). In short, another research conducted by Fazio et al (2021) pertaining to measure blood parameters to indicate illness or good health in one hundred Loggerheads, strongly suggests that turtles with illness and serious illness showed lower total protein and albumin compared to individuals with good health. Also, turtles suffering from serious illness showed lower creatinine concentrations and higher aspartate aminotransferase (AST) activities. Finally, turtles suffering from illness showed lower urea concentrations.

**Table 13.** Hematologic values in Loggerhead sea turtles

Parameter (SI units)	Foraging <sup>a</sup>	Nesting <sup>a</sup>	Stranded a
PCV (1/1)	0.18-0.40	0.23-0.40	0.09-0.36
RBCs $(x10^3/\mu l)$	220,000-1,220,000	250,000-1,110,000	-
WBC $(x10^3/\mu l)$	4,000-17,400	4,000-23,950	7,650-24,450
Heterophils(x10 <sup>3</sup> /μl)	345-7,164	2,400-14,220	200-14,060
Lymphocytes(x10 <sup>3</sup> /µl)	299-4,830	900-3,300	780-33,430
Monocytes (x10 <sup>3</sup> /μl)	64-2,750	0-1,950	0-150
Eosinophils (x10 <sup>3</sup> /μl)	192-3,584	105-2,530	0-380
Basophils (x10 <sup>3</sup> /µl)	-	0-180	6-70

<sup>&</sup>lt;sup>a</sup> Values ranging from minimum to maxium

Packed cell volumen (PCV); Red blood cells (RBCs); White blood cells (WBC)

From: Deem S. L., Norton T. M., Mitchell M., Segars A., Alleman A. R., Cray C., Poppenga R. H., Dodd M., Karesh W. B. (2009) Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (caretta caretta) along the coast of Georgia, U.S.A. Journal of Wildlife diseases 45 (1) 41-56

Table 14. Plasma biochemistry values in Loggerhead sea turtles

Parameter (SI units)	Foraging <sup>a</sup>	Nesting a	Stranded a
TP (g/l)	16-56	46-61	4-39
Albumin (g/l)	8-16	14-19	3-12
Globulin (g/l)	10-40	27-51	1-24
Creatinine (µmol/l)	8.84-44.2	8.84-53.04	8.84-70.72
Urea (mmol/l)	0.357-38.2	1.79-4.64	9.28-33.92
Cholesterol (mmol/l)	1.17-5.18	4.90-8.78	0.13-5.05
Tryglyceride(mmol/l)	0.17-1.38	4.41-4.89	0.11-0.29
Glucose (mmol/l)	3.9-7.6	4.1-6.3	1.72-7.66
Calcium (mmol/l)	1.4-2.08	0.65-3.18	1.08-1.9
ALT (U/l)	0-29	3-30	10-44
AST (U/l)	2-255	116-190	113-1,199
LDH (U/l)	6-1,376	22-1,172	244-3,876

<sup>&</sup>lt;sup>a</sup> Values ranging from minimum to maximum

Total protein (TP); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); lactate dehydrogenase (LDH).

From: Deem S. L., Norton T. M., Mitchell M., Segars A., Alleman A. R., Cray C., Poppenga R. H., Dodd M., Karesh W. B. (2009) Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (caretta caretta) along the coast of Georgia, USA. Journal of Wildlife diseases 45 (1) 41-56

#### 5. Materials and methods

My literature review is based on the collecting data in English and Spanish languages studies, including articles, journals, books and online resources. My major sources for data collection were researchgate, google scholar, pubmed, sciencedirect, vetbooks.ir and finally a variety of articles from sea turtle rescue centres and/or organisations such as: Olive Ridley Project, oceana and Georgia Sea Turtle Centre.

#### 6. Conclusions

Based on the aim and objectives of my literature review to what findings I encountered, it can be concluded that some fields and/or areas of study still need more update and recent bibliographic references. For example, no information is available on the precise nutritional requirement for each species. On the other hand, it becomes evident that little information is available on captive rearing of certain sea turtle species, especially Olive ridley. However, the improvements in medical management and research on physiological parameters in captive turtles have improved significantly over the years; these findings suggest that the largest biological obstacles to rearing sea turtles in captivity are diet and diseases.

Moreover, there is a clear need to unified substantial methodologies, techniques and procedures for the environmental management and feeding optimisation, considering there is a lack of knowledge on how to approach needs of the different types of sea turtle species, besides behavioural mitts and requirements, leading to mismanagement which impacts negatively adaptation and more importantly health.

When understanding diseases of sea turtles, often-complex scenarios can be encounter on understanding their drivers and how to manage them; therefore, collaborations between veterinarians, biologist, environmental scientist and other professions would be ideal. New strategies will be required to develop innovative areas and laboratory tools to robust baseline reference intervals to assess the health of captive sea turtles. In conclusion, a veterinary approach to analyse sea turtle health with a collaborative team of researchers working in complementary areas could increase our general understanding on health and diseases aetiology; in addition to the health of marine environment. Nevertheless, further research on other variables, apart from nutrition; water quality and enrichment would be worth exploring.

#### 7. Summary

The present literature review included previous research about disciplines such as marine biology, ecology and veterinary medicine on marine turtles, extending from the 70's till more recent years. The purpose of this research was to identify effective feeding methods and nutritional parameters use during captivity, besides from rehabilitation management in rescue centres; as well as, emphasising the most common diseases and/or disorders before and during captivity. Therefore, throughout the first two chapters, natural and artificial diets were discussed. Some researchers suggested that carapace anomalies in sea turtles could be due to an insufficient vitamin D supplementation during captivity, and other studies supported the idea that due to a slow breakdown of the large quantity of calcium deposited in Green turtle carapace and bones, turtles can stay asymptomatic for long periods of time. In the same way, more study is needed to determine long term consequences of limited UV exposure or low dietary vitamin D supplementation in Green turtles and Loggerheads, particularly suffering from DTS or trauma. Following this, many diets containing natural ingredients were proposed. However, the impact of feeding commercially available vegetables such as cucumbers, green peppers and lettuce against algae and sea grass could be potentially being studied.

It can be appreciated on the next lines about environmental enrichment methods, the positive impact on changing periodically objects, since it is a main factor influencing their well being and development as living creatures. As a result future exploration into colour and size of enrichment devices could be useful for finding further rehabilitation techniques. Finally, common disorders and/or diseases before and during captivity were discussed, but more importantly how ineffectiveness of rehabilitation therapy in infected turtles could be hampered by the high rates of antibiotic resistance among bacteria. In that case, antibiotic-resistant bacteria should be considered a serious health risk for sea turtles, and monitoring of these bacteria should be incorporated into microbiological water quality studies for sea turtle husbandry. Despite probiotic supplements could be used after antibiotic treatments, there was no evidence of data and/or research on their effect on the gut microbiota of sea turtles.

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