Certainty of the uncertain - a review of age determination methods in sea turtles

Olive Ridley Project technical report number ORP-2025-07

Authors: *Joe Rigby, Lara Kalisch, Jane R. Lloyd, Olivia Forster and Stephanie Köhnk*

Report number: ORP-2025-007

Version: 1.0

Date of publication: 28 July 2025

Abstract.—**Accurate age estimations have important implications for the evaluation of the reproductive output and potential of populations, as well as their existing population structure. Both factors are crucial considerations for the implementation of conservation and management strategies in endangered species. Sea turtles are enigmatic species with life history stages largely evading direct human observation, which poses significant challenges for demographic evaluation and the designation of conservation strategies. In this review different methods for sea turtle age estimation are presented and compared to evaluated accessibility and applicability for life history studies. While the methods vary greatly in complexity, sample requirements and costs involved, no single method with sufficient accuracy is available at the moment.**

Key Words.— DNA Methylation, Radiometric Dating, Skeletochronology, Telomere Length Analysis, Conservation, Green Turtle, Loggerhead, Leatherback

Short Title.— Age determination methods in sea turtles

This technical report was developed as part of a student placement for Joe Rigby, studying Veterinary Medicine at University of Bristol, in cooperation with the Olive Ridley Project.

Table 2 Supplementary data is available upon request from info@oliveridleyproject.org

INTRODUCTION

Demographic models and management plans are often limited by our inability to determine the age of individuals within a population (Avens and Snover 2013). Accurate age determination is a pivotal factor in conservation efforts, especially for species exhibiting slow life history traits and facing the risk of extinction, such as sea turtles (see for example Heppell et al. 1999). The absence of external morphological features that provide age information, combined with the growth variability and the enigmatic 'lost years', during which post-hatchlings develop away from coastlines, pose significant challenges to the accurate estimation of age in individuals as well as across populations (Mansfield et al. 2014). Therefore, when attempting to estimate the age of sea turtles, researchers often resort to indirect methods such as utilizing morphometric measurements, assessing growth rates or employing other approaches such as skeletochronology, various genetic and epigenetic markers, or radiometric dating (Hatase et al. 2008; Plot et al. 2012; Avens et al. 2013; Van Houtan et al. 2016; Mayne et al. 2022).

Skeletochronology has long been thought of as the most reliable and accurate method for determining the age of sea turtles, representing a consensus within the scientific community dedicated to the study of these animals (Avens and Snover 2013; Goshe et al. 2016; Guarino et al. 2020; Usategui-Martin et

al. 2023). The method utilizes the fact that bone growth follows a cyclic pattern with annual periodicity (Bjorndal et al. 1998). During this cycle, bone formation temporarily pauses or slows down before entering a phase of relatively rapid new bone formation (Usategui-Martin et al 2023). Within these skeletal growth marks, there are dark, thin lines known as 'lines of arrested growth' (LAGS) and light, broader sections. The LAGs signify the deceleration or cessation of skeletal growth, whereas the lighter border sections indicate active bone formation at faster growth rates (Zug et al. 1986; Avens and Snover 2013). Together, these two lines form skeletal growth marks (GMs) (Snover and Hohn 2004), which serve as invaluable indicators for estimating age when the annual periodicity of the GM is validated for a particular species. Traditionally, the interpretation that one GM equates to approximately one year of life has been based on the notion that endogenous physiological rhythms synchronize with environmental parameters (Usategui-Martin et al 2023). This is particularly true for reptiles, given that their poikilothermic nature means their metabolism is predominantly regulated by the temperature of their respective environment (Dawson 1975).

More recently, additional methods have been developed to address the challenge of accurately determining the age of sea turtles. One non-genetic method introduced for ageing sea turtles is radiometric dating, which compares isotope levels in biological samples to reference levels. Conrad et al. (2023) used uranium isotopes produced during the period of nuclear testing in the mid-20th century to show sea turtles bioaccumulate uranium, while Van Houtan et al. (2016) used bomb radiocarbon. Genetic approaches have also progressed, offering the significant advantage of applicability to live animals. Measurement of telomere length provides insights into biological age, as telomeres naturally shorten with age and cell division in most animals. This method has been successfully applied in humans (Haussmann et al. 2003) and is increasingly used in animal studies, including sea turtles (Hatase et al. 2008; Plot et al. 2012; Le Clercq et al. 2023;). Additionally, epigenetic clocks analyze DNA methylation patterns, which are known to change with age. Methylation-based age clocks have been developed for various species, including whales and dolphins (Le Clercq et al. 2023). Recently, Mayne et al. (2020) introduced the first epigenetic clock for sea turtles, presenting a promising tool to explore the age structure of sea turtle populations.

In the context of sea turtle conservation, evaluating accurate methods for determining age is crucial for effective management strategies. This literature review aims to assess the history and current status of diverse approaches in age determination, offering a comprehensive overview of current methodologies applicable to sea turtles including prerequisites, cost, accuracy, effectiveness, sample types required, and limitations. By synthesizing up-to-date research findings, this review aims to provide researchers with valuable insights into the strengths and limitations of each method and how they can be applied to different sea turtle species across various locations.

METHODS

Database Development.—We performed searches on Google Scholar for the following search terms: "sea turtle ageing", "sea turtle epigenetics", "sea turtle ageing + skeletochronology", "sea turtle skeletochronology", "skeletochronology", as well as "skeletochronology + each of the sea turtle species", "limitations of skeletochronology", and "limitations of skeletochronology in sea turtles", "Sea turtle radiocarbon ageing", "Sea turtle radio dating age", "Sea Turtle telomere length + age" ,"Animals whose telomeres do not shorten with age", "Stress signs in sea turtles". Additionally, Avens and Snover (2013) and references therein are included in the study. We used the artificial intelligence literature mapping tool 'Research Rabbit' to find and review relevant papers based on the initial search results obtained from Google Scholar. Search results were manually screened for relevance and applicability to sea turtles specifically, before inclusion in this review. The open

searches resulted in four main methods of age determination, namely skeletochronology, DNA methylation, telomere length analysis and radiometric dating. For further analysis, all papers were clustered under one of these topics.

Method evaluation.—We examined the different methods for published prerequisites, estimated costs, accuracy, effectiveness, potential sample type and listed limitations to create a comprehensive overview and allow for the comparison of all currently established methodologies (Table 1).

Table 1. Comparison of established methodologies, according to prerequisites, costs, accuracy, effectiveness, sample type & limitations

	Skeletal Chronology	**DNA Methylation**	**Telomere Length**	**Radiometric Dating**
Prerequisites	• Assumption that a growth mark must represent a cycle of known duration and one growth mark must be laid down each cycle. • If growth marks are destroyed by remodelling, a reliable method for estimates must be available. • Validation studies crucial for verifying annual growth rings and for interpreting abnormal LAGs. • Correction factor protocol must be employed to estimate number of lost LAGs. • If standard back calculations of body size at earlier ages are made based on increments recorded in hard structure, there must be a constant proportional relationship between growth increment in body size and growth increments in hard structure. • Vast amount of equipment required.	• DNA extraction Kit i.e. (DNeasy Blood and Tissue Kit (Qiagen) & Ethanol • Laboratory facilities that allow for Reduced Representation Bisulfite Sequencing (RRBS), PCR and sequencing of libraries. • Trained personnel for data analysis	• DNA extraction Kit i.e. (DNeasy Blood and Tissue Kit (Qiagen) & Ethanol • Laboratory facilities that allow for Bisulfite sequencing, PCR and sequencing of libraries. • Trained personnel for data analysis	• The successful application of bomb carbon-14 requires a cross section of samples from the period of time in which the particularly diagnostic bomb carbon-14 occurred. • Scutes must be without external wear or damage
Cost	• Cost to collection of samples varies • Time taken to collect enough samples • Transportation of samples • Permits for transportation • Lab tech cost if applicable with the ability to interpret samples	• After initial construction of an epigenetic clock, cost effective analysis at larger scales	• qPCR relatively low cost (Lin et al. 2019) • Trap qPCR with reduced cost and steps in the assay developed by Pinto et al. (2021): 2 USD per Sample	• (299USD for human analysis of biological clock home kit)) • DNeasy Blood and Tissue Kit (Qiagen); Cost; 207 EUR for 50 spin columns = 4.14EUR per column • Bisulfite Conversion using common Kit i.e. Bisulfite Kit from Qiagen; Cost: 390.60Euro for 50 spin columns= 7.8EUR per spin column). Processing time under 7 hours • Methyl-Seq DNA Library Prep Kit • Sequencing of Libraries requires specialized equipment such as an accelerator mass spectrometer and a milling device, as well as the use of isotopic fractionation correction and other sample preparation procedures. These equipment and

				procedures can be costly research may require collaboration with various institutions and archives to amass sufficient specimens
Accuracy	• Considered reliable as an indirect method for estimating age and growth rates of marine turtles (for a review see Avens and Snover 2013; Goshe et al. 2016; Avens et al. 2017)	• highly accurate with a median absolute error of 2.1 years (4.7% of maximum age in data set) for green turtles • DNA methylation might surpass telomere length in terms of both accuracy and cross-taxa portability	• Accurate age determination in many animals including birds (Clercq et al. 2023). • No significant correlation between telomere length and age has been established for sea turtles.	
Effectiveness	• Assumptions about; deposition rates, recognition of layers, interpretation of layers and loss of layers	• Easy to obtain samples • Potential for high-throughput analysis of samples • Can be applied on live specimen • Requires skilled personnel for data analysis • Requires well equipped laboratory facilities	• Requires trained professionals to collect and analyse data • Measurements of telomerase activity can be affected by experimental variation. • Handling needs to be careful to avoid contamination with enzyme inhibitors, foreign DNA/RNA and external proteins.	• Potential to validate age estimates from skeletochronology (Steward et al. 2006)
Sample Type	• Bone sample collected from deceased individual • Left humerus most commonly used • Ossicles (leatherback)	• Skin biopsy samples	• Blood or Epidermis • Blood has shown no correlation between age and telomere length (Hastase et al. (2008) and Plot et al. (2012))	Post marginal scute samples collected from deceased individuals
Limitations	• Complete life history records are often preclude by the loss of early growth layers due to inner bone reabsorption • Compression of LAGs at the periphery of the bone, due to decreased growth and the loss of early GMs by endosteal resorption and the expansion of the medullary cavity, making accuracy decrease with age • Deposition of annual growth marks in tropical marine environments could be highly variable • Interpretation of growth marks can be difficult even for trained individuals as it can be hard to distinguish between true LAGs and false LAGs. • Validation of GMs is necessary	• Impaired health and environmental factors can influence methylation patterns • Biological sex can confound analysis • Not tested on all species of sea turtles • Needs to be verified for older individuals	• Factors including oxidative persistent inflammation or environmental toxins can affect telomere length permanently • Telomere length can be heritable • No significant correlation between telomere shortening and age in sea turtles established till the present day. • Only investigated for Loggerhead and Leatherback turtles • Not all species exhibit telomere shortening with age. Species like sea turtles with fast early growth and continuous growth have high telomerase activity in somatic tissues which allows for teleport length to be regenerated. • Location of samples can influence findings	• Deceased animals • Likely only applicable for hawksbill turtles as PMs are large and may contain a near-complete chronology. Although the methods we developed in Van Houtan

			• Reproduction/ Fasting can increase oxidative stress and impact telomere length	

RESULTS

The Google scholar search revealed a total of 37 peer reviewed papers and books that conducted age estimation studies in sea turtles. An additional 12 papers were excluded from this review due to species relevance, literature review, and non-capture mark recapture studies. The most successful search term was "sea turtle ageing - skeletochronology" yielding 25 relevant results, confirming that the most frequently applied method is skeletochronology (Supplementary Table 2). The areas of research with the fewest published ageing methods in sea turtles are radiometric dating and DNA methylation.

Sea turtle age estimation studies have been conducted at 21 locations in all major ocean basins, with the highest number of studies concentrated in the Northern Atlantic and Northern Pacific area (Fig. 1). The Mediterranean Sea has been distinguished from the broad North Atlantic category for mapping purposes to highlight the region's turtle ageing research efforts. Hawaii has the most consistent application of studies over time with publications from 2002 - 2023. While the USA Atlantic coast and Gulf of Mexico account for 12 papers published from 1986 - 2015. Overall, study efforts focusing on sea turtle estimation have been relatively stable since the initial study utilizing skeletochronology was published in 1986. Every year, only one to three studies investigating the question have been published, with studied individuals rarely exceeding 150 specimens (Fig. 2).

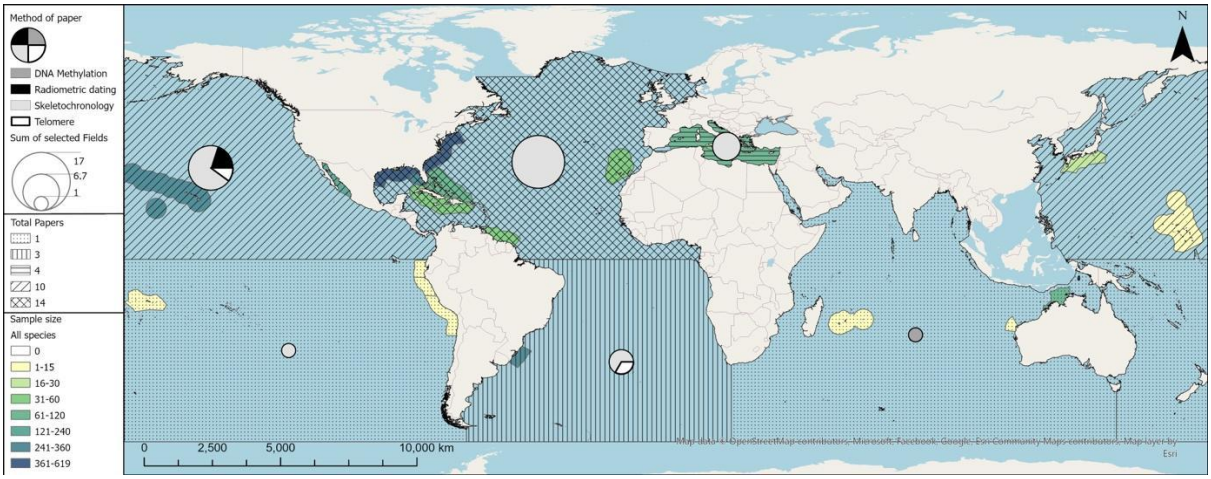

Figure 1. Global map displaying pie charts representing the types of aging research conducted on sea turtles, with chart sizes scaled to the total number of research methods applied in each oceanographic region (Flanders Marine Institute 2018). Since a single paper may cover multiple regions, counts are cumulative (see Supplementary Table 2 for details). The world's oceans and the Mediterranean Sea are shaded using graduated symbology to indicate the total number of aging studies per region. Specific water body subregions (Flanders Marine Institute 2020) are highlighted with graduated colours showing the sample sizes where individual studies have been conducted. Map produced in ArcGIS Pro with Base layer OpenStreetMap.

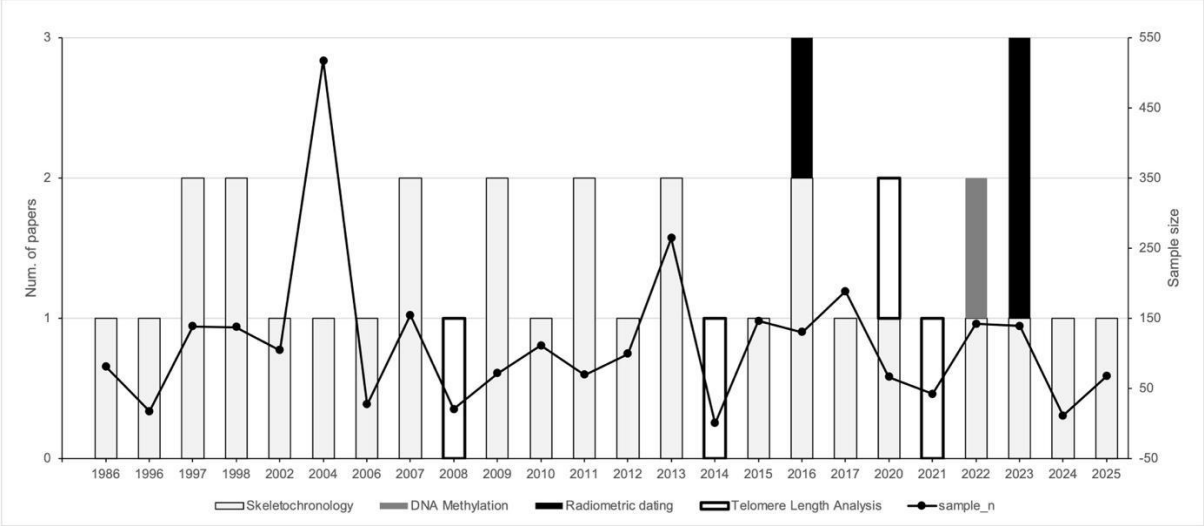

Figure 2. A stacked bar chart displays the number of published sea turtle ageing papers per year (x-axis), broken down by research method. Each method is color-coded to match the legend in Figure 1. Overlaid on the same plot is a line graph showing the total number of samples used each year. The left y-axis (primary axis) indicates the number of papers, while the right y-axis (secondary axis) shows the sample count.

The majority of papers investigate only one species, with only five studies addressing multiple species (Supplementary Material Table 2). The most commonly studied species was the Loggerhead Turtle (N=14), followed by the Green Turtle (N=13). While sample sizes varied greatly between individual studies, the overall highest number of individuals were studied using skeletochronology, with Loggerheads (N=1113) and Green Turtles (N=721) representing the largest cohorts in total (Fig. 3).

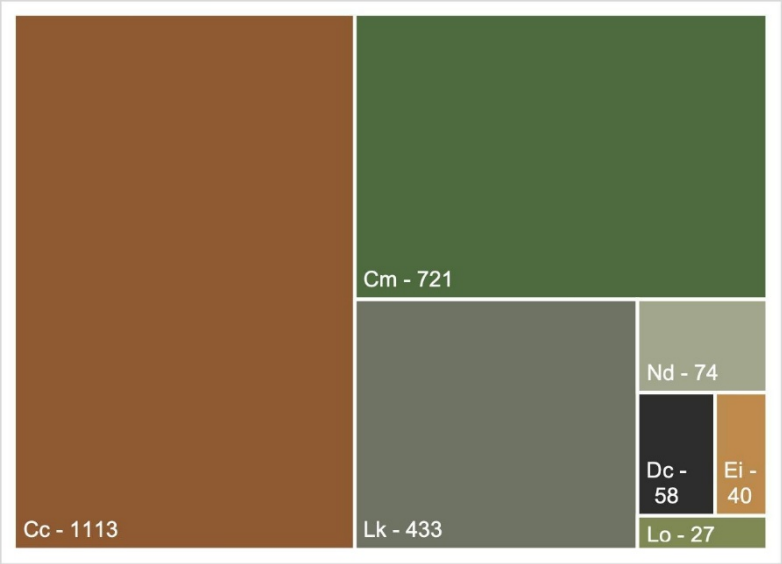

Figure 3 Treemap showing the total number of skeletochronology research samples by sea turtle species. Each tile is labeled with the species code and corresponding sample size. Species codes follow this convention: Cc = Caretta caretta (Loggerhead), Cm = Chelonia mydas (Green Turtle), Dc = Dermochelys coriacea (Leatherback), Ei = Eretmochelys imbricata (Hawksbill), Lk = Lepidochelys kempii (Kemp's Ridley), Lo = Lepidochelys olivacea (Olive Ridley), Nd = Natator depressus (Flatback).

Skeletochronology.—Skeletochronology has long been the most popular use of age determination in sea turtles, 28 papers were reviewed that included the use of skeletochronology. These papers have been published consistently between 1986 and 2023 with peaks in 2007 and 2013 with a bias to loggerhead and green sea turtle species (Fig. 3, Supplementary Table 2). It is the only age determination method that has been tested on all 7 species of sea turtles.

Conducting age estimations using skeletochronology requires a vast amount of specialized equipment, including saws with diamond blades that have the ability to cut ultra thin samples (around 25µm), chemicals for staining and decalcification, and a microscope with imaging capabilities.

Skeletochronology has varying methods that have been used since the mid 1900s, some simply inspect an untreated sample, while others use advanced techniques involving electron microscopes or microradiography (Brothers et al. 1976; Hohn 1980; Avens and Snover 2013). In sea turtles, the humerus bone is used most commonly as a sample as Zug et al. (1986) found that it showed the least amount or reabsorption of GMs compared to other bones. The humerus is surgically removed from dead sea turtles very carefully, if the bone is cut accidently then the sample is compromised and may give inaccurate results. Once the humerus is removed from the body, the tissues surrounding it must be stripped away, this can be done gently with a knife or by cooking the bone at moderate temperature in water, then dried in the sun for several weeks (Avens and Snover 2013). A cross section of the bone is then cut at the distal end, perpendicular to the long axis using a low-speed saw with a diamond blade under water (Snover and Hohn 2004; Avens and Snover 2013). Parham and Zug (1997) stated that untreated bone should be cut into cross-sections 0.5-0.8mm and immersed in a 4:6 solution glycerin:ethanol which can then be examined under a microscope (Avens and Snover 2013). In cross-sections that are to be decalcified, the sample should be cut to 2-3mm then decalcified using a solution of formaldehyde and a dilute acid (Snover and Hohn 2004; Goshe et al. 2009; Avens and Snover 2013). Once the sample is decalcified, a microtome is used to cut 25µm sections which are stained in hematoxylin. This highlights the LAGs within the sample when under a microscope using a glycerin solution on the bone cross-section. The process of decalcification is demonstrated as providing more a reliable result therefore it is the recommended method of choice for skeletochronology age determination (Goshe et al. 2009). The LAGs are then counted and can be used to determine an estimated age of the sea turtle the sample came from.

Sandik et al. (2024) went on to find that rotary microtomes provided more accurate results than the cheaper and quicker cryostat microtomes due to the cryostat having wider variations and readability of LAGs from histologically prepared samples. This indicates the use of rotary microtomes will increase skeletochronology accuracy in future studies.

Comparison is often made to known age sea turtles or ones captured and injected with oxytetracycline which at specific high doses will incorporate into growing bones. This will then light up under ultraviolet light when examined after death. This gives researchers a known date from when the injections were given to examination of the samples to assess levels of reabsorption and to confirm if GMs are laid down annually (Snover et al. 2011).

DNA Methylation.— Mayne et al. (2022) provide the only study up to this date that has investigated the use of DNA methylation as a method for determining the age of sea turtles. Aging is accompanied by cellular changes that typically vary across different types of tissues. However, cytosine methylation has shown a strong correlation with ageing across all tissues, and is therefore a useful method to determine age in a variety of mammals (Lu et al. 2023). The molecular process involves the addition of a methyl group to the C-5 position of the cytosine ring in DNA. In vertebrates, this addition is most commonly found at CpG sites (cytosine-phosphate-guanine sites). Mayne et al. (2022) have introduced the first-ever epigenetic clock designed for reptiles by utilizing skin samples obtained from green turtles with known ages. To ensure the applicability of their findings to other sea turtle species, the researchers focused exclusively on CpG sites that are shared among all marine turtles.

Skin samples were collected from green turtles with known ages representing two distinct populations, along with an additional sample from each of the seven sea turtle species, except for

Kemp's Ridley. These samples were used to identify the CpG sites that are universally present across species.

The epigenetic age prediction model was developed using Reduced Representation Bisulfite Sequencing (RRBS), using an Illumina platform for library preparation and sequencing. The sequencing reads were aligned to the green turtle genome only, due to the lack of fully annotated genomes for other sea turtle species. Alignment followed quality control of raw sequencing data, and included methylation calling (methylKit package in R). A machine learning model was applied to develop the epigenetic clock based on methylation patterns at the selected CpG sites. The researcher found a mean absolute error of 2.1 years for green turtles, with an increase in older ages. Therefore, the clock does not provide precise determination of the absolute age of individual sea turtles, but allows for categorization in size classes.

To create a more cost-effective alternative to RRBS, a multiplex PCR assay targeting 18 age-related CpG sites was designed for green turtles. The PCR assay was validated using green turtle samples of known age, and the methylation levels at the targeted CpG sites were analyzed through sequencing on an Illumina platform.

Telomere length analysis.—The use of telomere length analysis has not been a common use of age determination in sea turtles with only four published articles being analyzed for this study. Telomeres are specialized DNA structures located at the ends of chromosomes, playing a crucial role in preventing chromosome tangling or fusion (Aubert and Lansdorp, 2008). During cell replication, telomeres naturally shorten, but this process is typically counteracted by the enzyme telomerase, which repairs and maintains the telomeres. Telomeres and telomerase are vital for proper animal development as they safeguard the integrity of DNA (Aubert and Lansdorp, 2008). In early stages of life, organisms possess abundant amounts of telomerase, allowing for efficient repair of telomeres. However, as individuals age, telomerase activity diminishes, resulting in progressive telomere shortening with each cell division of 40-200 bp (base-pairs) in humans (Andrews et al. 2010). Unfortunately, very limited research is available on the rate at which reptiles' telomeres shorten with each cell division or if they even shorten at all.

Plot et al. (2012) described an adapted method of measuring telomere length in sea turtles originally described by Criscuolo et al. (2009) for the use in avian species. The method requires a 5 µL blood sample, from which DNA is extracted using a commercial kit (Plot et al., 2012), and followed by a quantitative Polymerase Chain Reaction (qPCR) assay to determine the telomere length which is compared to a reference DNA to determine the difference in length. Plot et al. (2012) opted to test two control genes, RNA fingerprint protein 35 and 18S rRNA, to examine the relationship between blood telomere length and age in 42 Leatherback Turtles. Their findings revealed no significant correlation between telomere length and age in these turtles. The authors state that the lack of blood telomere shortening with age in sea turtles is likely due to absence of telomere-based senescence in many species of chelonians. Moreover, it is proposed that species like sea turtles with fast early growth and continuous growth have high telomerase activity in somatic tissues (Plot et al 2012). The same study also highlighted the importance that telomere studies can have in investigating the reproductive health of sea turtle populations in the future.

Hatase et al. (2008) studied 16 captive loggerhead turtles with known age and found older individuals had shorter telomeres in samples taken from the epidermis. Results were not significant, but revealed that older turtles have smaller relative T/S ratios in the epidermis. The study shows the potential of telomere shortening in specific tissues in determining sea turtle age but results of this study may be limited due to the small sampling size.

Telomere studies used to determine age in reptiles are inconclusive till the present day and oxidative damage caused by stress, persistent inflammation or environmental toxins can permanently alter telomere length (Bartlett 2015; Dupoué et al. 2022; Le Clercq et al. 2023). We must also consider how the selection of samples like blood or epidermis will affect results in long-lived reptiles. Barlian and Riani (2020) also suggest that fibroblast skin cell culture is a valuable model for studying aging in Green Turtles, as telomere length and telomerase activity may be tissue- and species-specific in this species.

Radiometric Dating.—To date, only two studies have employed radiometric dating on sea turtles, focusing on Hawksbill and Green Turtles (Van Houtan et al. 2016; Conrad et al. 2023). Van Houtan et al. (2016) collected scute samples from stranded turtles and individuals raised in captivity across all life stages, including hatchlings, while Conrad at el. (2023) identified uranium deposits in chelonian scutes that matched known past nuclear events.

The keratinized carapace, similar to the humerus bone, forms growth rings, enabling the calculation of growth rates. Van Houtan et al. (2016) used growth rings and bomb radiocarbon in post marginal scutes of Hawksbill Turtles in Hawaii, whose thick post marginal scutes provided a reliable source of samples, to estimate sea turtle age and age at first maturity. Growth rings were analyzed on polished cross sections, to avoid data loss due to abrasion and tissue loss. The scutes were cut using a slow electric saw with diamond blade and analyzed using a standard microscope and an accelerator mass spectrometry was used to quantify bomb C-14. The scutes were tested for carbon-14, and the values were compared to background rates from massive *Porites* coral in Hawaii.

The date-referenced material from Hawksbill scutes displayed a weakened Δ14C signal compared to corals. The study found that Hawaiian Hawksbill Turtles deposit a mean of eight scutal growth rings annually and calculated age at maturity was 29, which is higher than estimates from other studies (see for example Hawks et al. 2014, Van Houtan et al., 2014).

Conrad et al. (2023) obtained five samples from various chelonians, including a Green Turtle, to test for anthropogenic uranium (^{235}U and ^{236}U) signatures in scutes. They identified contamination that matched known nuclear histories in the areas the animals were found in, showing that sea turtles bioaccumulate radionuclides over time. This method could be used for age determination in sea turtles in areas with known past nuclear events.

DISCUSSION

As of today, no cost-efficient and accurate method to determine the age of sea turtles has been developed. All methods found during this review process have been shown to be impacted by significant confounding factors and lack confirmed validation or cross-checking with other methods.

Skeletochronology.—Even though many research studies have estimated the age of sea turtles under the assumption that growth rings are laid down annually, there still remains some debate as to whether free-living sea turtles in less seasonal environments, for example the tropics, have a more variable GM pattern. Despite Klinger and Musick (1992) finding annual deposition of GM in one life stage of Loggerhead Turtles in temperate environments and Snover et al. (2011) finding annual GMs in seven Hawaiian Green Turtles, Bjorndal et al. (1998) found that in the 25 Green Turtles studied, no visible growth marks from humeral biopsies after 1.3 and 2.4 year periods could be found. This is not a

surprise as tropical marine environments are expected to support continual growth and bone deposition in immature animals (Bjorndal et al.1998). This study highlights a significant limitation for the use of skeletochronology in all species of sea turtle. The majority of immature sea turtles will undergo extensive migrations which may take them to tropical environments therefore significantly affecting GM deposition and rates of growth, resulting in poor estimates of age and back calculations of growth at age.

Similarly, LAG deposition correlated to external temperature might not apply in Leatherback Turtles, which are capable of maintaining a relatively constant body temperature (James et al. 2006; Avens et al. 2009), while still exhibiting growth marks similar to that of other sea turtle species (Avens et al. 2009), prompting questions about if temperature changes do indeed cause GMs, and what degree of temperature change is responsible or what other factors may have an influence.

Additionally, Bjorndal et al. (1998) also went on to state that the use of skeletochronology does not, and cannot account for remodeling of the humerus during growth. As well as remodeling during growth, some sea turtles will unfortunately sustain damage to their bones from other animals or anthropogenic causes which could cause them to have inconsistent growth rings visible during analysis (Avens and Snover, 2013).

Interpreting growth marks can be difficult even for trained specialists, as it can be hard to distinguish between true LAGs and false LAGs without knowing the individual's history (Avens et al. 2009; Goshe et al. 2010). Non cyclic GMs can occur as a result of a cessation of growth due to changes in food availability, injury or disease (Sinsch et al. 2007; Klevezal 2017). Therefore, validation studies or reliable reference samples are crucial not just for verifying the annual nature of a growth mark but also when recognizing and interpreting abnormal LAGs (Avens et al. 2009). Anomalous LAGs including double, splitting and supplemental lines, pose common challenges in skeletochronological studies in reptiles (Snover and Hohn 2004).

Moreover, two additional difficulties are frequently encountered; the compression of LAGs at the periphery of the bone, due to decreased growth and the loss of early GMs by endosteal resorption and the expansion of the medullary cavity. As a result, the precision of skeletochronology diminishes with age in sea turtles (Klinger and Musick 1992; Snover and Hohn 2004). This issue is notably pronounced in skeletochronological studies of Loggerheads (Klinger and Musick 1992; Parham and Zug 1997), green (Zug and Glor 1998; Zug et al. 2002) and Kemp's Ridley Turtles (Zug et al. 2002). In order to overcome this problem, protocols have been employed to establish a correlation between bone dimension and body size. This correlation is thought to help estimate the numbers of layers lost, however it requires the GMs annual development to be tested for each species and population. Sandik et al. (2025) also found that resorption rates are significantly different when comparing humeral and phalanx samples from Green Turtles but are not significantly different when comparing the same samples in Loggerheads. This shows there are clear differences between species that must be understood to accurately use skeletochronology for sea turtle age estimations.

Even with the wealth of research conducted using skeletochronology for age determination, Wilson et al. (2003), after reviewing 145 published articles stated that, "only four case studies had sufficient data to indicate that a consistent number of rings was added each year." This shows a clear lack in rigorous and accurate research into how effective the method really is at determining the age of sea turtles. Morales-Merida et al. (2024) also stated that the original designers of the method made it clear it was not suitable for use of age determination of individual sea turtles, posing clear concerns over its reliability.

Skeletochronology varies greatly in cost. Costs can include the collection of samples which can come from all parts of the world, the time it takes to collect enough samples to conduct a reliable study, transportation of specimens including the time it takes to obtain permits if necessary, and the use of a

lab that has the appropriate equipment needed. If a lab is being set up for such a project the cost would increase dramatically due to the nature of the equipment stated in the prerequisites. If the individual conducting the research does not have experience in processing the samples, additional cost will be incurred to employ someone that can do so and then interpret the samples which is a specialist skill. All of these limitations make skeletochronology less accessible in remote or economically challenged areas.

DNA Methylation.—The development of a sea turtle epigenetic clock represents a significant advancement in sea turtle biology. Epigenetic clocks serve as robust tools for achieving accurate age estimates and have already been proven successful in several animal species including birds, fishes and whales (Le Clercq et al. 2023). The advantage of conducting analyses on live samples further contributes to the applicability and importance of this approach for sea turtle conservation.

In the context of sea turtles, the ease of collecting skin biopsy samples from nesting turtles or within rehabilitation facilities enhances the accessibility of the method. The collection process is straightforward, requiring only minimal training. An additional practical aspect is that sea turtle skin samples do not necessitate cold temperature storage (Meyer et al. 2023), making this method widely applicable and adaptable to diverse settings.

Once an epigenetic clock has been successfully developed for all sea turtle species, it holds the potential for high-throughput analysis of samples, as highlighted by Mayne et al. (2022). In the absence of laboratory facilities that allow for sequencing of samples, these can be shipped to specialized laboratories for processing and analysis. Resulting cost will vary with facility and sample number with a decrease in per sample cost with an increase in sample number.

However, use of DNA methylation to determine age has certain limitations. Firstly, biological sex can lead to differing methylation patterns, with females ageing slower (Paolo-Iseppi et al. 2017; Le Clercq et al. 2023; Mayne et al. 2023), potentially causing confounding results. It is therefore important to either remove the sex chromosome or have an equal sex ratio in the analysis (Mayne et al. 2022). Secondly, impaired health, such as age-related diseases, can influence methylation patterns and mimic age-related changes (Ito et al. 2017). Research on ageing in sea turtles through DNA methylation has primarily focused on captive specimens (Mayne et al. 2022). However, wild populations frequently encounter health challenges, such as exposure to environmental toxins, which can affect methylation patterns and potentially introduce biases into the data. Population and environmental factors can also affect methylation patterns. For instance, Martín-del-Campo et al. (2019) found a positive correlation between mercury concentrations and DNA methylation in embryos with Schistosomus reflexus syndrome, a rare malformation. Caracappa et al. (2016) further documented reduced methylation levels in loggerhead turtles with non modal scutes. Choosing CpG sites minimally affected by environmental or genetic factors is crucial to reflect age-related changes only (Ito et al. 2017).

Future studies should further develop the clock and include a minimum sampling number of 134 to create an epigenetic clock with high statistical accuracy and include samples of older individuals (Mayne et al. 2022). The successful modification and testing of the clock require samples of all species with known ages. To address these limitations and refine the clock's accuracy, the authors recommend further research utilizing recapture time intervals instead of relying on absolute known ages. This approach could provide a more robust assessment of the clock's performance, particularly for older individuals.

Additionally, testing the clock's applicability to other turtle species would broaden its potential applications as current results are only valid for Green Turtles. Researchers looking to expand on this work must have expertise in molecular biology, access to molecular facilities, and bioinformatics

skills. Expanding the model also requires genomic data from all relevant species. For green turtles, the multiplex PCR assay developed by the authors can be used to estimate age, but it should first be validated with known-age samples. With continued development, the epigenetic clock has the potential to become a valuable tool for sea turtle conservation and management.

Emerging technologies, such as Oxford Nanopore Technology (ONT), are a promising tool to enhance this methodology. This technology allows for direct, real time detection of methylation without the need for bisulfite sequencing and hence reduces cost and sample degradation. Nanopore sequencing enables genome-wide methylation analysis across multiple sea turtle species using minimal DNA input, making it a valuable tool to consider for future research in this field (Jain et al. 2016; Simpson et al. 2017; Sigurpalsdottir et al. 2024).

Telomere Length Analysis.—Although telomere length loss has been the accepted rule for ageing animals, many do not exhibit this shortening, including Magellanic Penguins (Cerchiara et al. 2017), Leach's Storm Petrel (Haussmann et al. 2003), and the Water Python (Ujvari and Madsen 2009). Both Water Pythons and Leach's Storm Petrels have in fact been identified as lengthening their telomeres as they age, showing there are clearly outliers to the accepted rule. Gomes et al. (2010) also states that reptiles, invertebrates, and amphibians have persistent telomerase activity throughout their lives in somatic tissues which allows telomere length to be maintained and regenerated. This alone could make telomere length analysis for ageing invalid in reptile species.

Telomere length analysis comes with many other limitations. Methodological differences, such as the use of various DNA extraction kits, can significantly influence telomere length measurements (Lin et al. 2018). Additionally, the source tissue of the telomere sample plays a crucial role. Both studies from Hatase et al. (2008) and Plot et al. (2012) found no correlation between blood telomere length and age in Loggerhead and Leatherback Turtles respectively. Hatase et al. (2008) however, found that epididymis telomere length did tend to be shorter in older Loggerhead Turtles compared to younger, but the results were not significant, showing further work into what sample is most reliable for ageing is required.

Another limitation of telomere length analysis is that there are multiple factors that can influence telomere length including basal levels of corticosterone (Chaloupka et al. 2015) which can increase due to chronic stress (Kim et al. 2013). It would be very challenging to assess if samples taken from sea turtles in the wild were highly stressed or not. Even in captivity, sea turtles exhibit stress in many different forms. Stress can also be triggered by a number of factors including oxidative stress which due to the rising levels of pollution in the world's oceans, is a real problem for sea turtles, especially hatchlings experiencing accelerated growth (Plot et al. 2012; Morao et al. 2022). It is well known that female sea turtles invest a lot of energy into reproduction and will fast for long periods of time as they migrate to their nesting beaches. Plot et al. (2012) found that these actions can increase oxidative stress, further impacting telomere length.

Telomere length has also been linked to the heritage of an individual in birds and mammals. Reichert et al. (2015) found that birds (King Penguins) inherit their telomere length from the maternal side while Le Clercq et al. (2023) stated in mammals it is predominantly inherited from the paternal side, however there is a lack of research in the heritability of telomere length in reptiles. Furthermore, there has only been research into telomere length analysis for Loggerhead, Green and Leatherback Turtles at present day (Plot et al. 2012; Hatase et al. 2008; Barlian and Riani 2020). Barlian and Riani (2020) found that Green Turtles not only had increased telomerase activity as they aged but also longer telomeres, demonstrating that sea turtles do not age as humans do and telomere length analysis may not be a valid tool for age determination. However, Barlian and Riani (2020) only used one two-year old Green Turtle as their sample so more research needs to be done to confirm these findings.

Radiometric Dating.—Using growth rings from post marginal keratinized carapace scutes is a promising and cost efficient method to estimate the age of sea turtles, however this method requires scutes without significant damage to receive near complete chronology and is most likely only applicable for hawksbill turtles, as they have most keratin deposits. This methodology can only be applied on deceased animals, limiting its applicability in conservation. Working with bomb carbon-14 further requires reference records, which are not available for all regions and deposition rates are likely to be influenced by factors such as climate (Van Houtan et al. 2016). The accuracy of the obtained data has not yet been confirmed.

Conrad et al. (2023) did however find that sea turtles bioaccumulate uranium signatures in their scutes which could be used to determine an estimate of age of sea turtles in areas of dated past nuclear events. They determined an age estimate for an Eastern Box Turtle down to a seven year window using this technique along with electron microscopy and energy dispersive X-ray spectroscopy. Unfortunately, sea turtles migrate long distances and may move in and out of contaminated areas. More research is required to determine if this method can be an effective way to determine age estimations but Conrad et al. (2023) did state that this method could be useful in monitoring the environmental impacts past, present, and future nuclear events can have on chelonian species which in turn would aid conservation efforts.

CONCLUSION

As shown in this review, determining the age of sea turtles is not an easy task. All methods have limitations as well as advantages. Based on our findings telomere length analysis cannot be deemed a reliable method of age determination in sea turtles. Skeletochronology is a well researched area but requires large amounts of expensive equipment, highly trained professionals, and deceased individuals. Radiometric dating is still relatively understudied but has shown some promise in Hawksbill Turtles due to the high levels of keratin in their scutes. It may however be limited in its application to other species of sea turtles. The use of epigenetics has not been used extensively in sea turtle age determination but does show great promise. It is non-lethal and relatively cost effective, but does not have the ability to determine definitive age, however can be used to identify age classes. Therefore, currently no fully reliable and cost effective method is available which provides reliable, definitive age determination of sea turtles. More research, including joint application of different age determination methods on the same specimens, is needed to further the development of accurate and cost effective methods to aid in the revelation of demographics and age structures in sea turtle populations, thus allowing for more effective and impactful management and conservation efforts around the world.

Acknowledgments.– The ORP team would like to thank all donors and partners for their continued support of the charity and all aspects of its work.

LITERATURE CITED

Andrews, N.P., H. Fujii, J.J. Goronzy, and C.M. Weyand. 2010. Telomeres and immunological diseases of aging. Gerontology 56:390–403.

Aubert, G. and P.M. Lansdorp. 2008. Telomeres and aging. Physiological reviews 88:557–579.

Avens, L., J.C. Taylor, L.R. Goshe, T.T. Jones, and M. Hasting. 2009. Use of skeletochronological analysis to estimate the age of leatherback sea turtles *Dermochelys coriacea* in the western North Atlantic. Endangered Species Research 8:165–177.

Avens, L., L.R. Goshe, M. Pajuelo, K.A. Bjorndal, B.D. MacDonald, G.E. Lemons, A.B. Bolten, and J.A. Seminoff. 2013. Complementary skeletochronology and stable isotope analyses offer new insight into juvenile loggerhead sea turtle oceanic stage duration and growth dynamics. Marine Ecology Progress Series 491:235–251.

Avens, L., and M.L. Snover. 2013. Age and age estimation in sea turtles. Pp. 97–134 *In* The Biology of Sea Turtles. Wyneken, J., K.J. Lohmann, and J.A. Musick (Eds.). CRC Press, Boca Raton, Florida, USA.

Barlian, A., and Y.D. Riani. 2020. Aging process in dermal fibroblast cell culture of Green Turtle (*Chelonia mydas*). BIO Journal of Biological Science, Technology and Management 2:11–17.

Bartlett, Z. 2015. Telemores and Telomerase in cellular aging (Senescence). *In* Arizona State University, School of Life Sciences, Centre for Biology and Society, Embryo Encyclopedia. Turriziani Colonna (F. (Ed.). Arizona Board of Regents. https://keep.lib.asu.edu/items/172898 Accessed on 20 May 2025.

Bjorndal, K.A., A.B. Bolten, R.A. Bennett, E.R. Jacobson, T.J. Wronski, J.J. Valeski, and P.J. Eliazar. 1998. Age and growth in sea turtles: limitations of skeletochronology for demographic studies. Copeia:23–30.

Brothers, E.B., C.P. Mathews, and R. Lasker. 1976. Daily growth increments in otoliths from larval and adult fishes. Fishery Bulletin 74:1–8.

Caracappa, S., A. Pisciotta, M.F. Persichetti, G. Caracappa, R. Alduina, and M. Arculeo. 2016. Nonmodal scutes patterns in the Loggerhead Sea Turtle (*Caretta caretta*): a possible epigenetic effect?. Canadian Journal of Zoology 94:379–383.

Cerchiara, J.A., R.A. Risques, D. Prunkard, J.R. Smith, O.J. Kane, and P.D. Boersma. 2017. Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. Ecology and Evolution 7:5682–5691.

Chaloupka, M. and C. Limpus. 2005. Estimates of sex-and age-class-specific survival probabilities for a southern Great Barrier Reef green sea turtle population. Marine Biology, 146:1251–1261.

Criscuolo, F., P. Bize, L. Nasir, N.B. Metcalfe, C.G. Foote, K. Griffiths, E.A. Gault, and P. Monaghan. 2009. Real-time quantitative PCR assay for measurement of avian telomeres. Journal of Avian Biology 40:342–347.

Conrad, C., J. Inglis, A. Wende, M. Sanborn, N. Mukundan, A. Price, T. Tenner, K. Wurth, B. Naes, J. Fair et al. 2023. Anthropogenic uranium signatures in turtles, tortoises and sea turtes from nuclear sites. PNAS Nexus 2:1–10. https://academic.oup.com/pnasnexus/article/2/8/pgad241/7244772

Dawson, W.R. 1975. On the Physiological Significance of the Preferred Body Temperatures of Reptiles. Pp. 443–473 *In* Gates, D.M., and R.B. Schmerl (Eds.). Perspectives of Biophysical Ecology. Ecological Studies, vol 12. Springer, Berlin, Heidelberg.

Dupoué, A., P. Blaimont, F. Angelier, C. Ribout, D. Rozen-Rechels, M. Richard, D. Miles, P. de Villemereuil, A. Rutschmann, A. Badiane et al. 2022. Lizards from warm and declining populations

are born with extremely short telomeres. Proceedings of the National Academie of Sciences 119:1–7. https://www.pnas.org/doi/pdf/10.1073/pnas.2201371119

Flanders Marine Institute. 2018. IHO Sea Areas, version 3. Available online at https://www.marineregions.org/ : https://doi.org/10.14284/323 Accessed on 20 May 2025

Flanders Marine Institute. 2020. The intersect of the Exclusive Economic Zones and IHO sea areas, version 4. Available online at https://www.marineregions.org/ : https://doi.org/10.14284/402 Accessed on 20 May 2025.

Gomes, N.M., J.W. Shay, and W.E. Wright, 2010. Telomere biology in Metazoa. FEBS Letters 584:3741–3751.

Goshe, L.R., L. Avens, J. Bybee, and A.A. Hohn. 2009. An evaluation of histological techniques used in skeletochronological age estimation of sea turtles. Chelonian Conservation and Biology 8:217–222.

Goshe, L.R., L. Avens, F.S. Scharf, and A.L. Southwood. 2010. Estimation of age at maturation and growth of Atlantic green turtles (*Chelonia mydas*) using skeletochronology. Marine Biology 157:1725–1740.

Goshe, L.R., M.L. Snover, A.A. Hohn, and G.H. Balazs. 2016. Validation of back-calculated body lengths and timing of growth mark deposition in Hawaiian green sea turtles. Ecology and Evolution 6:3208–3215.

Guarino, F.M., F. Di Nocera, F. Pollaro, G. Galiero, D. Iaccarino, D. Iovino, M. Mezzasalma, A. Petraccioli, G. Odierna, and N. Maio. 2020. Skeletochronology, age at maturity and cause of mortality of loggerhead sea turtles *Caretta caretta* stranded along the beaches of Campania (south-western Italy, western Mediterranean Sea). Herpetozoa 33:39–51.

Hatase, H., R. Sudo, K.K. Watanabe, T. Kasugai, T. Saito, H. Okamoto, I. Uchida, and K. Tsukamoto. 2008. Shorter telomere length with age in the loggerhead turtle: a new hope for live sea turtle age estimation. Genes & Genetic Systems 83:423–426.

Haussmann, M.F., D.W. Winkler, K.M. O'Reilly, C.E. Huntington, I.C. Nisbet, and C.M. Vleck. 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short–lived ones. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270:1387–1392.

Heppell, S.S., Crowder, L.B., Menzel, T.R. and Musick, J.A., 1999. Life table analysis of long-lived marine species with implications for conservation and management. In American Fisheries Society Symposium 23:137–148.

Hohn, A.A., 1980. Analysis of growth layers in the teeth of *Tursiops truncatus* using light microscopy, microradiography, and SEM. Report of the International Whaling Commission, 3, pp. 155–160.

Ito, G., K. Yoshimura, and Y. Momoi. 2017. Analysis of DNA methylation of potential age-related methylation sites in canine peripheral blood leukocytes. Journal of Veterinary Medical Science 79:745–750.

Jain, M., H.E. Olsen, B. Paten, and M. Akeson. 2016. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biology 17:1–11.

James, M.C., S.A. Sherrill-Mix, K. Martin, and R.A. Myers. 2006. Canadian waters provide critical foraging habitat for leatherback sea turtles. Biological Conservation 133:347–357.

Klinger, R., and J. Musick. 1992. Annular growth layers in juvenile loggerhead turtles (*Caretta caretta.*) Bulletin of Marine Science 51:224–230.

Kim, J.G., H.S. Jung, K.J. Kim, S.S. Min, and B.J. Yoon. 2013. Basal blood corticosterone level is correlated with susceptibility to chronic restraint stress in mice. Neuroscience Letters 555:137–142.

Klevezal, G.A. 2017. Recording structures of mammals. Routledge, London, UK

Le Clercq, L.-S., A. Kotzé, J.P. Grobler, and D.L. Dalton. 2023. Biological clocks as age estimation markers in animals: a systematic review and meta-analysis. Biological Reviews 98:1972–2011.

Lin, J., J. Sun, S. Wang, J.M. Milush, C.A.R. Baker, M. Coccia, R.B. Effros, E. Puterman, E. Blackburn, A.A. Prather, and E. Epel. 2018. *In vitro* Proinflammatory Gene Expression Predicts in vivo Telomere Shortening: A Preliminary Study. Psychoneuroendocrinology 96:179–187

Lu, A.T., Z. Fei, A. Haghani, T.R. Robeck, J.A. Zoller, C.Z. Li, R. Lowe, Q. Yan, J. Zhang, H. Vu et al. 2023. Universal DNA methylation age across mammalian tissues. Nature Aging 3:1144–1166.

Mansfield, K.L., J. Wyneken, W.P. Porter, and J. Luo. 2014. First satellite tracks of neonate sea turtles redefine the 'lost years' oceanic niche. Proceedings of the Royal Society B: Biological Sciences 281:1–9. https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.2013.3039

Martín-del-Campo, R., A. Bárcenas-Ibarra, G. Lund, D. Rodríguez-Ríos, L. Yong-Villalobos, J. García-Hernández, and A. García-Gasca. 2019. Mercury concentration, DNA methylation, and mitochondrial DNA damage in Olive Ridley Sea turtle embryos with Schistosomus Reflexus syndrome. Veterinary Pathology 56:940–949.

Mayne, B., A.D. Tucker, O. Berry, and S Jarman. 2020. Lifespan estimation in marine turtles using genomic promoter CpG density. PLoS ONE 15:1–8. https://doi.org/10.1371/journal.pone.0236888

Mayne, B., W. Mustin, V. Baboolal, F. Casella, K. Ballorain, M. Barret, M.A. Vanderklift, A.D. Tucker, D. Korbie, S. Jarman, and O. Berry. 2022. Age prediction of green turtles with an epigenetic clock. Molecular Ecology Resources 22:2275–2284.

Mayne, B., W. Mustin, V. Baboolal, F. Casella, K. Ballorain, M. Barret, M.A. Vanderklift, A.D. Tucker, and O. Berry 2023. Differential methylation between sex in adult green sea turtle skin biopsies. Frontiers in Marine Science 10:1–6. https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2023.1169808/full

Meyer, O.S., M. Meyer Andersen, C. Børsting, N. Morling, H.C. Wulf, P. Alshede Philipsen, C.M. Lerche, and J. Dyrberg Andersen. 2023. Comparison of global DNA methylation analysis by whole genome bisulfite sequencing and the Infinium Mouse Methylation BeadChip using fresh and fresh-frozen mouse epidermis. Epigenetics 18:1–11.
https://www.tandfonline.com/doi/pdf/10.1080/15592294.2022.2144574

Morales-Mérida, B. A., N.J. Pilcher, and M. Girondot. 2024. How Old Is a Turtle? Challenges in Interpreting Age Information in Sea Turtles. Ecologies 5:502–511.

Morao, I.F., M.F. Lemos, R. Felix, S. Vieira, C. Barata, and S.C. Novais. 2022. Stress response markers in the blood of São Tomé green sea turtles (*Chelonia mydas*) and their relation with accumulated metal levels. Environmental Pollution 293:1–12.
https://digital.csic.es/bitstream/10261/309983/1/1-s2.0-S0269749121020728-main.pdf

Paoli-Iseppi, D., B.E. Deagle, C.R. McMahon, M.A. Hindell, J.L. Dickinson, and S.N. Jarman. 2017. Measuring animal age with DNA methylation: From humans to wild animals. Frontiers in Genetics 8:106.

Parham, J.F., and G.R. Zug. 1997. Age and growth of loggerhead sea turtles (*Caretta caretta*) of coastal Georgia: an assessment of skeletochronological age-estimates. Bulletin of Marine Science 61:287–304.

Plot, V., F. Criscuolo, S. Zahn, and J.Y. Georges. 2012. Telomeres, age and reproduction in a long-lived reptile. PloS ONE 7:1–6. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0040855

Reichert, S., E.R. Rojas, S. Zahn, J.P. Robin, F. Criscuolo, and S. Massemin. 2015. Maternal telomere length inheritance in the king penguin. Heredity 114:10–16.

Sandık, E., B. Sönmez, and Ş.Y. Özdilek. 2024. Comparison of rotary and cryostat microtomes in the skeletochronology on green turtle humerus bones. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 29:823–829.

Sandık, E., B. Sönmez, and Ş.Y. Özdilek. 2025. Discrepancies in the number of lines of arrested growth (LAG) in the tissues of the humerus and phalanx of sea turtles. The Science of Nature 112:11. https://doi.org/10.1007/s00114-025-01963-7

Sigurpalsdottir, B.D., O.A. Stefansson, G. Holley, D. Beyter, F. Zink, M. Þ. Hardarson, S.Þ. Sverrisson, N. Kristinsdottir, D.N. Magnusdottir, O.Þ. Magnusson, and D.F. Gudbjartsson. 2024. A comparison of methods for detecting DNA methylation from long-read sequencing of human genomes. Genome Biology 25:69.

Simpson, J.T., R.E. Workman, P.C. Zuzarte, M. David, L.J. Dursi, and W. Timp. 2017. Detecting DNA cytosine methylation using nanopore sequencing. Nature Methods 14:407–410.

Sinsch, U., N. Oromi, and D. Sanuy. 2007. Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. The Herpetological Journal 17:129–137.

Snover, M.L. and A.A. Hohn. 2004. Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. Fisheries Bulletin 102:682–693.

Snover, M.L., A.A. Hohn, L.R. Goshe, and G.H. Balazs. 2011. Validation of annual skeletal marks in green sea turtles *Chelonia mydas* using tetracycline labeling. Aquatic Biology 12:197–204.

Ujvari, B. and T. Madsen. 2009. Short telomeres in hatchling snakes: erythrocyte telomere dynamics and longevity in tropical pythons. PloS ONE 4:1–5. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0007493

Usategui-Martín, A., R.A. Valverde, P. Ostiategui-Francia, A. Fariñas-Bermejo, Y. Paz-Sánchez, and A. Liria-Loza. 2023. First skeletochronological analysis on loggerhead yearlings (*Caretta caretta*) in the Canary Islands. Marine Biology 170:95.

Van Houtan, K.S., A.H. Andrews, T.T. Jones, S.K. Murakawa, and M.E. Hagemann, M. 2016. Time in tortoiseshell: a bomb radiocarbon-validated chronology in sea turtle scutes. Proceedings of the Royal Society B: Biological Sciences 283:1–8. https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.2015.2220

Wilson, D.S., C.R. Tracy, and C.R. Tracy. 2003. Estimating Age of Turtles from Growth Rings: A Critical Evaluation of the Technique. Herpetologica 59:178–94.

Zug, G.R., G.H. Balazs, J.A. Wetherall, D.M. Parker, K. Shawn, and K. Murakavua. 2002. Age and growth of Hawaiian green seaturtles (*Chelonia mydas*): an analysis based on skeletochronology. Fisheries Bulletin 100:117–127.

Zug, G.R., and R.E. Glor. 1998. Estimates of age and growth in a population of green sea turtles (*Chelonia mydas*) from the Indian River lagoon system, Florida: a skeletochronological analysis. Canadian Journal of Zoology 76:1497–1506.

Zug, G.R., A.H. Wynn, and C. Ruckdeschel. 1986. Age Determination of Loggerhead Sea Turtles, *Caretta caretta*, by Incremental Growth Marks in the Skeleton. Smithsonian Institution Press, Washington, USA.