Life cycle and ecology of the loggerhead turtle (Caretta caretta, Linnaeus, 1758)

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Life cycle and ecology of the loggerhead turtle (*Caretta caretta*, Linnaeus, 1758):

Development and application of the Dynamic Energy Budget model

Nina Marn

Exploring the effects of plastic ingestion
on the energy budget

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ŽIVOTNI CIKLUS I EKOLOGIJA GLAVATE ŽELVE (*Caretta caretta*, Linnaeus, 1758): RAZVOJ I PRIMJENA MODELA DINAMIČKOG ENERGIJSKOG PRORAČUNA

LIFE CYCLE AND ECOLOGY OF THE LOGGERHEAD TURTLE (*Caretta caretta*, Linnaeus, 1758): DEVELOPMENT AND APPLICATION OF THE DYNAMIC ENERGY BUDGET MODEL

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Contents

Chapter 1

General introduction

Being able to predict the metabolic responses of individuals or populations to a changing environment is a powerful tool for understanding a species, and ensuring the appropriate protection of biodiversity amidst the occurring global environmental changes. An increasingly worrying environmental pressure is the vast amount of anthropogenic debris present in the marine habitat, most of it plastic [243, 46, 7, 119]. With 275 million metric tons of plastic waste generated solely in 2010, 4.8 to 12.7 million metric tons of that entering the ocean [95], and "only" 35 thousand tons swirling in the giant garbage patches in the oceans [46], one cannot help but wonder where did the rest of the plastic go, and where will the remaining plastic end up. Plastic items floating, sinking, or being washed up on beaches can cause tremendous harm to the marine environment. Interaction with plastics, either in the form of entanglement or ingestion, has been documented for more than 267 marine species [117], especially sea turtles, seabirds, cetaceans, fish, and whales [117, 260, 198, 199, 152, 19]. Incidence of plastic ingestion has been increasing [229, 194], could be larger than previously thought [46], and, as the plastic debris continues to fragment into smaller particles [7], it will probably increase even more. Studying the effects of plastic ingestion in more detail is therefore a necessity.

Species such as loggerhead sea turtles, requiring as much as 20-40 years to reach puberty [264, 209, 5], are especially vulnerable to environmental pressures occurring too rapidly for them to adapt to [41]. Due to high natural and anthropogenic mortality, only few of the individuals survive long enough to reach puberty and reproduce [143]. Anthropogenic debris has been found in the digestive systems of sea turtles from all oceans [243], and could have a substantial impact on the quantity and digestibility of ingested food. Plastic ingestion has been shown to reduce the amount of ingested food [146, 199], thus prolonging the period needed for obtaining puberty and lowering the energy available for reproduction. Plastic ingestion could therefore have drastic long-term effects on populations of loggerhead turtles, but the cumulative sublethal effects have not yet been quantified.

As plastic ingestion affects the ingested energy, a full life cycle model based on an energy budget would allow quantification of the effects on processes such as energy acquisition and expenditure (for growth, maintenance, maturation, and reproduction). Developing such a model, however, requires an in depth knowledge about the species, several types of data, and a consistent underlying theory.

The loggerhead turtles are a critically endangered (IUCN) globally distributed migratory species protected by the Environmental Species Act, CITES, Barcelona and Bern convention, as well as European habitat directive [105], but despite the protection many populations of loggerhead turtles are still declining. Loggerhead turtles add not only to the environmental biodiversity and tourist appeal of an area, but they also connect the marine and land ecosystems, and are at the top of the food chain as they feed on jellyfish, molluscs, crabs, and fish, making them extremely valuable for the balance of the ecosystems.

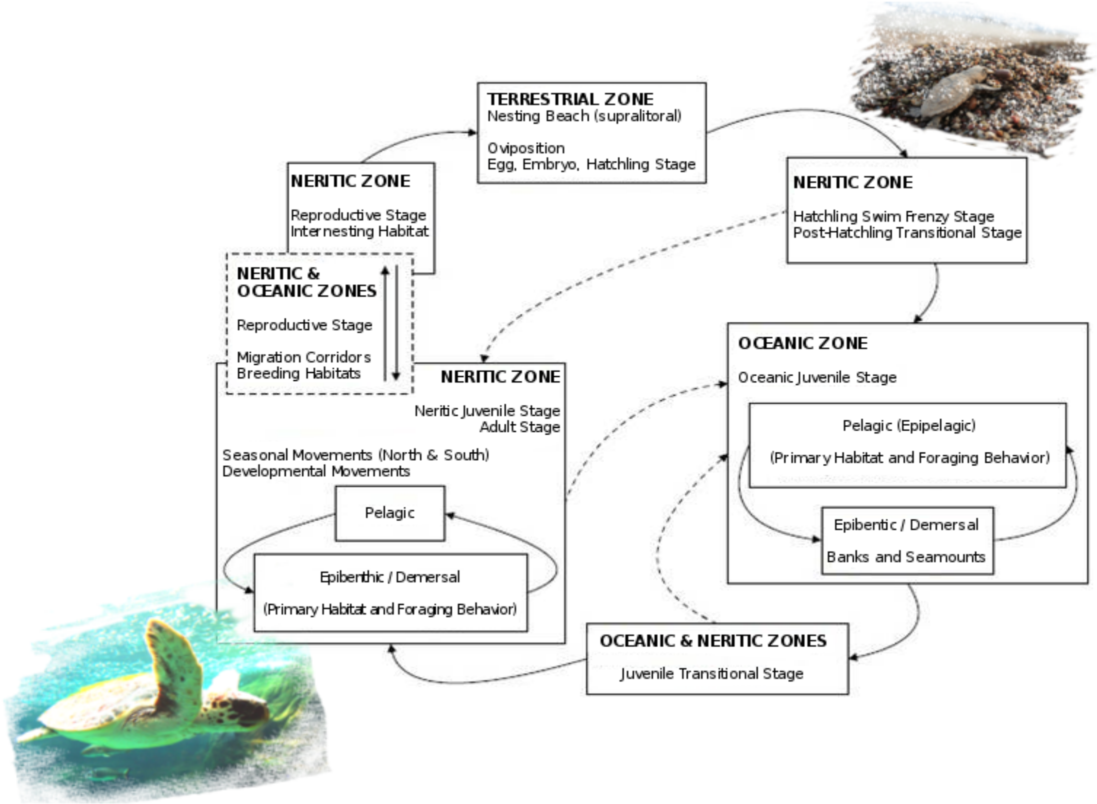

Figure 1.1: In general, loggerhead turtles use three types of habitats during their life cycle: terrestrial habitat for depositing nests and embryonic development, oceanic habitat for feeding and migrating, and neritic habitat for feeding and mating. Because of the large areal and various habitats which they use during the life cycle, loggerhead turtles are extremely vulnerable to anthropogenic pressures and climate change [107]. Life history diagram (from Bolten [59], used with author's permission) includes life stages and corresponding ecoystems (represented with boxes) and movements between life stages and ecosystems (solid lines); dotted lines are speculative .

Understanding of the species ecology and life cycle is an essential prerequisite for effective protection, but existing data is limited by scope, is mostly related to a specific population or life stage, and the results of some studies suggest seemingly conflicting conclusions. Mathematical models help to unify the available literature data and understand the conclusions, and are increasingly utilized for the research and protection of sea turtles (e.g. [143, 209, 92, 201]). The development of new and improvement of existing methods (satellite telemetry, skeletochronology, genetic analysis, etc.), have lead to a better understanding of loggerhead turtles, and with it awareness about variability between individuals and populations, as well as the need to adapt existing methods of protection [250].

Despite advances in methodology, some important factors for population models and protection planning, such as growth rates and the exact age at sexual maturity, still lack reliable data (cf. [209, 264]). Empirical growth curves currently applied in various analyses have limited usability, because they require a large data base, and can only predict growth in a known environment. These problems can be bypassed by applying mechanistic models such as those based on the Dynamic Energy Budget (DEB) theory [109].

Completing energy budgets for sea turtles over ecologically relevant timescales has been identified as one of the key research areas almost a decade ago [87], but the complete energy budget model of the loggerhead turtle is still lacking. The DEB theory [109, 217, 218, 162] is one of the most complete and consistent universal ecological theories. It defines the processes of acquiring and using energy for maturation, growth, and reproduction in a way consistent with the physical laws of thermodynamics, biochemistry, and clearly stated underlying assumptions, thus making the results and conclusions stronger and more comprehensive compared to other types of models. Because of its universality, models based on DEB theory have already been developed for more than 400 species from all major groups of invertebrates and vertebrates, results of which can be accessed online in the Add_my_pet library [110].

In order to construct a full life cycle model of loggerhead turtles, as much data of as many types as possible is desirable, as the dynamics of the processes quantified by a DEB model are determined by all types of data that are simultaneously used as model input [126]. The life cycle of loggerhead turtles has long been a mystery due to the migratory way of life, slow growth and maturation, and difficulties in rearing in captivity [23, 219]. The first data for growth rates of captive reared loggerhead turtles were reported by Parker [174], indicating a significantly faster growth and sexual maturation than was previously thought. However, growth rates differed among the individuals reared in captivity [175], and were different compared to the values calculated for wild populations (e.g., [181, 38, 63]). Most of the methods used for growth calculations convert the measured carapace length into age of the individuals and/ or growth rate, relying on the assumption that the carapace length is an adequate indicator of the size of the individual. In addition, measuring the carapace is not standardized, and it is common practice to convert different types of measures, e.g., straight and curved carapace length, using conversion formulae not always specific to a geographical area or life stage. The accuracy of any model depends on the accuracy of the assumptions and formulae: significant differences between populations and/ or life stages cause errors when applying the model, which has important consequences for the understanding of the ecology, and the protection of loggerhead turtles. Differences were observed between populations of different geographical areas [181, 28, 223], and a change in shape (allometric growth) was noticed during the first few weeks of sea turtles [202], but the implications of these studies have never been thoroughly investigated.

Genetically and geographically distinct populations (e.g., North Atlantic and Mediterranean) differ not only in growth rates [181], but also in the average size of individuals and eggs [136, 23]. The conditions in the Mediterranean basin are different to that in the North Atlantic, with relatively small environmental oscillations [166], small productivity [130, 263], and higher salinity and sea surface temperature [226, 172, 133, 166, 263]. The environmental conditions can have strong effects on the size of hatchlings, and the growth and reproduction of sea turtles [201, 203, 92], so different environmental conditions (e.g., primary productivity, temperature, and salinity) could be causing the interpopulation variability. Other possible causes are locally-specific selection pressures, genetic features, and behavior adaptations [181]. It is possible that differences between populations reflect the evolutionary trends, since the populations are geographically and genetically distinct.

Age at reaching sexual maturity was noted for several captive reared loggerhead turtles (e.g., [210]), however it is difficult to compare these values with estimates for wild populations. The differences in estimates for some populations [181, 38] may be due to the choice of method (cf., [34, 38]), interpopulation variability [264], or other factors. Since the adults are the chief determinant of population growth [143], it is important to understand the extent to which conditions in the environment and lifestyle of individuals affect the rate of growth, maturation and reproduction of individuals [250]. In addition, poylphasic growth suggested by Chaloupka [40], would imply a different age-at-length than that estimated based on the von Bertalanffy growth curve. The correlation between global climate fluctuations and local temperature changes with the duration of certain life cycle phases of sea turtles [92, 119] could be much more significant because they have a cumulative effect throughout the sea turtle's life cycle, and requires special attention.

The overall aim of the research conducted as part of the thesis was to test the effect of the anthropogenic and environmental pressures (with emphasis on ingestion of plastic debris), and provide new insights into the processes (growth, maturation, and reproduction) important for the life cycle and ecology, and thus the protection of the loggerhead sea turtle, using the collected literature and experimental data, and a life cycle model based on the DEB theory. Developing and applying the model made it possible to explore the variability of individuals within populations and among populations, comparing multiple populations at the level of individuals and the environment, and testing the influence of selected global and local pressures on this protected species.

The thesis is structured as follows:

After the general introduction presented in Chapter 1, *Chapter 2* tackles the problem of disjointed and conflicting data, and explores to what extent do loggerhead turtle populations and life stages differ in morphology. In order to take into account the possible geographic and life stage variability, I study two neighboring populations and all post-embryonic life stages by comparing the ratio of carapace length, width and height of sea turtles. I conduct a detailed analysis of empirical models (growth curves, conversion formulae). One of the aims is to answer a somewhat technical question whether or not can the growth of loggerhead turtles be considered isomorphic. Considerable deviations from isomorphy would require additional steps when defining through out the life the acquisition (or use) of energy in relation to the surface area-volume ratio.

The focus of *Chapter 3* is on developing a full life cycle model of loggerhead turtles. Due to substantial variability present in data related to loggerhead turtles living in different sea basins, I decided to focus on a geographically defined population rather than the whole species. In this chapter the *North Atlantic* population of loggerhead turtles is analyzed as it has one of the largest nesting aggregations of loggerhead turtles [228]. After estimating the parameter values using the covariation method [126] of the package DEBtool [112] implemented in Matlab, I compare model predictions to observations, and discuss the implications of the results.

In *Chapter 4* another population of loggerhead turtles, the *Mediterranean* population, is the main focus, together with the comparison between individuals belonging to the Mediterranean, and individuals belonging to the North Atlantic population. Individuals belonging to the two populations are first compared based solely on their morphology (length, weight, and the ratio of the two) at two life events: hatching and nesting. The average egg size reported for each population is taken into account, as it has been generally reported to account for most of the variation in hatchling sizes. As the next step, I develop a DEB model for the individuals of the Mediterranean population, analyze the model predictions, and discuss the implications of the results. Then I compare the model parameters between the populations, and suggest a physiological (maturity based) explanation for the adults having such markedly different sizes at nesting. In addition, posthatchling growth is analyzed in more detail, expanding the results of the previous chapter which suggested faster growth of posthatchlings than predicted by the model. Lastly, I reproduce a pattern of biphasic growth by modifying the food availability during the first part of the life cycle.

Chapter 5 showcases the applications of the DEB model to study the effects of temperature and food availability, and the effects of plastic ingestion on the energy budget and life cycle of the loggerhead turtle. I simulate a realistic range of temperatures and food densities to explore their effect on the energy budget, i.e. observable quantities such as size and reproduction output. I present a mechanism for the effects of plastic ingestion on the energy budget, applicable to any species for which the DEB parameters are known. I simulate a range of observed amounts of ingested debris, and study their effects on the processes of growth, maturation, and reproduction while assuming the plastic has (a) the same, and (b) several times longer gut residence time compared to that of food.

Finally, in *Chapter 6* I discuss my results in a broader context, and present an outlook on future studies, applications, and possible expansions of the developed model.

Chapter 2

Size scaling in western North Atlantic loggerhead turtles

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Abstract

Sea turtles face threats globally and are protected by national and international laws. Allometry and scaling models greatly aid sea turtle conservation and research, and help to better understand the biology of sea turtles. Scaling, however, may differ between regions and/or life stages. We analyze differences between (i) two different regional subsets and (ii) three different life stage subsets of the western North Atlantic loggerhead turtles by comparing the relative growth of body width and depth in relation to body length, and discuss the implications.

Results suggest that the differences between scaling relationships of different regional subsets are negligible, and models fitted on data from one region of the western North Atlantic can safely be used on data for the same life stage from another North Atlantic region. On the other hand, using models fitted on data for one life stage to describe other life stages is not recommended if accuracy is of paramount importance. In particular, young loggerhead turtles that have not recruited to neritic habitats should be studied and modeled separately whenever practical, while neritic juveniles and adults can be modeled together as one group. Even though morphometric scaling varies among life stages, a common model for all life stages can be used as a general description of scaling, and assuming isometric growth as a simplification is justified. In addition to linear models traditionally used for scaling on log-log axes, we test the performance of a saturating (curvilinear) model. The saturating model is statistically preferred in some cases, but the accuracy gained by the saturating model is marginal.

2.1 Introduction

Sea turtles face threats globally, and most species are listed by the IUCN (International Union for Conservation of Nature) as threatened, endangered, or critically endangered (see [236] for details), and are protected by national and international laws, such as CITES Convention (Appendix I), Endangered Species Act (ESA, PL93-205), and the Bern convention (CETS No. 104).

The conservation and research of sea turtles are greatly aided by allometry: the study of the relationship of body size to shape, anatomy, physiology, and behavior. Applications of allometry include relating metabolic rate, dive depth and duration, or reproductive output either to body mass [91, 247], or to carapace length [26, 82]. Carapace length is the measurement most often used to report growth rate (in $cm.yr^{-1}$), or construct growth models [14, 209], and age-at-length or life history stage duration models for sea turtles [129, 13, 25, 38]. When using carapace length as a measurement of body size, isometric growth is indirectly assumed. Isometric growth implies that ratios of length, width, and body depth are preserved, while size changes during ontogeny or evolution [205], i.e., growth appears to be accompanied with no change in shape.

Allometric scaling is also applied in morphometrics, when noting relative growth rates of different components of the organism [116]. For example, scaling equations are used to convert known measures of sea turtle size to those needed for a specific application (curved carapace length to straight carapace length, carapace length to carapace width etc.). Important application of the conversion equations is in the conservation of sea turtles: for example, dimensions of turtle excluder devices (TEDs) depend on projected turtle carapace width and height. TEDs are openings in fishing nets implemented to reduce the by-catch of sea turtles in shrimp trawls operating inshore and offshore in west Atlantic and east Pacific ocean [57, 197, 99]. Turtles inhabiting those areas differ in sizes and life stages, and measuring all dimensions of all life stages in all habitats is impractical, if not impossible. Because in most cases only carapace length is reported, it is important to know relationships between the length and other dimensions of the carapace, and whether or not these relationships differ between areas and/or life stages.

Wallace et al. [247] found that scaling of metabolic rates with mass in three species of sea turtles is allometric, and differs depending on the life stage of the individuals. Differences related to size and/or developmental stage may also be possible in morphology: tail elongation in maturing sea turtle males is a well known example of change in morphological scaling used as an indication of maturation. Sea turtles inhabit a wide range of habitats during their life cycle, so changes in morphology could be related not only to the reproductive functionality (function of tail during copulation), but also as a response to morphological functionality: avoiding predators [265, 202], or adapting shape to new hydrodynamic conditions when changing habitats.

Another source of variability in morphometry, and consequently scaling relations, might be the geographical region. For example, loggerhead turtles in the Mediterranean sea are generally smaller than loggerhead turtles of the same life stage in the North Atlantic ocean [136, 165]. Even though there are cases when the turtles from these regions are grouped together for analysis (e.g. [143, 244]), they are usually studied separately. Geographic subsets of these populations (loggerheads caught in the same area), on the other hand, are sometimes considered morphologically similar and analyzed together. Groups are often arbitrarily defined ([186], but see [228] for list of subpopulation designators), extrapolations are made from one geographic subset to the whole population [57], and scaling models are reported for a population rather than a subset of that population [4, 178, 244]. This approach is practical because identifying the exact region of origin can be done only by genetic analysis [6] as individuals from the same subpopulation may be encountered in different geographical areas [186], and individuals from different subpopulations use the same geographical area [228]. However, the reported (inconclusive) regional differences in scaling relationships between two geographic subsets of adults inhabiting North Atlantic [28], if confirmed, might imply that arbitrary grouping and extrapolations between stages and regions might not be appropriate.

If the understanding of morphology is considerably wrong, the scaling models and management decisions based on such models will be wrong. Differences between geographic subsets would require adaptations in the scaling models and decisions dependent upon size and shape (e.g., TED regulations). Differences between life stages would require adaptations in those decisions based on population models indirectly assuming isometric growth and using only one measure of size (i.e., length) as a proxy for growth or the age of sea turtles. In some cases, specific scaling relationships are not available, and general models must be used for legislation, conservation, or research purposes. If there are considerable errors that arise from utilizing a non-specific relationship, the errors have to be identified and taken into account. Caution has been suggested when extrapolating scaling models to sea turtles of different sizes or from different regions [64, 28]. However, we are not aware of a publication that points to all of the implications, specifically reports morphological scaling relationships, and systematically compares them across regions and life stages.

We focused on the western North Atlantic population of loggerhead turtles. We studied morphometric scaling relationships within two geographic subsets and three life stages of this population. Data collection and types of analyses are described in the first and second part of the methods section. Analyses of two geographic subsets of populations and of three life stage subsets are described in the subsections of results. In the analysis of life stage subsets, we additionally tested the performance of a saturating model described in the section Models and statistical analysis. Results suggest that for a single life stage, data from different geographic regions of western North Atlantic can be modeled together. Young loggerhead turtles that have not yet recruited to neritic habitats (posthatchlings and oceanic juveniles) should be modeled separately from neritic juveniles and adults if accuracy is important. Regardless of differences between life stages, one set of scaling models described the whole size span (multiple life stages) satisfactorily, and produced a good fit when a linear model was fitted on log-log axes. Implications of the results are discussed in the last section of the paper.

2.2 Methods

2.2.1 Data

We surveyed literature reporting morphometric relationships and/or paired measurements of straight carapace length (SCL), straight carapace width (SCW), and body depth (BD) of North Atlantic loggerhead turtles. We chose to work with straight carapace length (SCL) because measurements of SCL exhibit less variability than those of curved carapace length [64, 21]. Using available resources [54, 56, 28, 223]), we gathered data for western North Atlantic loggerhead turtles ranging in SCL from 3.4 cm to 109 cm. Based on the size span of loggerhead turtles [209], and the size range of the data, all post-embryonic life stages were represented. A total of $N = 17731$ data points were obtained, but not all data points were used in the analysis (Table 2.1).

For the first analysis, we grouped the data into two subsets, based on the geographic region where turtles were encountered and measured: northern subset ('north') consisting of sea turtles that hatched or were found nesting in South Carolina, and southern subset ('south') consisting of sea turtles that hatched or were found nesting in the area around Florida peninsula. Data triplets (Table 2.1) were raw data for captive reared posthatchlings (up to 10 weeks old), and data pairs were data points digitized from graphed logarithmic relationships of SCW to SCL, and BD to SCL for wild nesting adults. For the purposes of this analysis, we assumed that the relationship of carapace length and carapace width is not affected by captive rearing conditions, only the rate at which the turtles reach a certain size. However, considering other factors (life stage, type of data), we decided to analyze posthatchlings and nesting adults separately. In the posthatchling group, there was almost three times as much data for the 'south' than for the 'north' subset, so data from the 'south' were censored (randomly selected 1300 triplets) to match the number of data triplets of the 'north' subset. Consequently, we used a total of 2567 data triplets for posthatchlings (analyzed as SCW to SCL, BD to SCL, and BD to SCW data pairs), and 227 SCW to SCL data pairs for adults (Table 2.1).

.

Table 2.1: Data overview. We studied straight carapace length (SCL), straight carapace width (SCW), and body depth (BD). We used *(SCL, SCW)*, *(SCL, BD)*, and *(SCW, BD)* data pairs for the analysis, meaning that one data triplet yielded 3 data pairs. See text for details. Life stage subsets: 'I' - posthatchlings and oceanic juveniles, 'II' - neritic juveniles, and 'III' - nesting adults. Range of SCL or SCW values is also reported (in centimeters).

Type of data	*All data points*		*Analysis: Regions* 'north'	'south'	*Analysis: Life stages* 'I'	'II'	'III'
(*SCL*, *SCW*)	*N*=	371* (1,2,3)	112 (3)	105 (2)	48 (1)	71 (1)	252 (1,2,3)
pair	SCL	8.1 - 109	80.7 - 107.4	81 - 109	8.1 - 41.3	42.1 - 80.6	80.7 -109
(*SCL*,*BD*)	*N*=	280* (1,3)	-	-	55 (1)	71 (1)	154 (1,2,3)
pair	SCL	8.1 - 109			8.1 - 40.9	41.7 - 80.6	81.4 - 109
(*SCW*,*BD*)	*N*=	253** (1,3)	-	-	47 (1)	59 (1)	147 (1,2,3)
pair	SCW	6.8 - 98.5			6.8 - 33.1 §	33.8 - 61 §	61.5 - 98.5
(*SCL*,*SCW*,*BD*)	*N*=	5609 (4)	1267	1300 †	1065 †	-	-
triplet	SCL	3.4 - 10.1	3.4 - 10	4.1 - 10	3.4 - 10		
Total number of data pairs	*N*=	17731	2646	2705	3345	201	553

Data sources: [1] Epperly and Teas, [56], Figs 1 and 2 from Appendix 1; [2] Ehrhart and Yoder, [54], Fig 3; [3] Byrd et al., [28], Fig 2 panels c and d; [4] this study

Digitalization software PlotReader (version 1.55.0.0) was used for data import. Overlaping datapoints could not be differentiated.

** Data pairs reconstructed by relating (log(*SCL*), log(*SCW*)), and (log(*SCW*), log(*BD*)) data pairs, by using common values of log(*SCL*). In cases where there was more than one value from one relationship mapping to the single value of the other (due to overlap of the data points), the average of the values was paired with the common measurement.

§ For this relationship, the SCW to SCL relationship was used for dividing data into subsets: smallest SCW from subset 'II' was used as SCW at recruitment, and smallest SCW from subset 'III' was used as SCW at nesting

† data censored to perform a more balanced analysis: 1300 data triplets were randomly chosen from 4342 data triplets available for that population subset, to match the number of data triplets for the other subset. Later, 1056 data triples were randomly chosen from the 2567 triplets, so that the percentage of (SCL, SCW) data pairs of subset 'I' matches the percentage of the total size span occupied by this subset.

For the second analysis, we divided the data into three subsets: 'I', 'II', and 'III', based on the length of individuals. Each of the three subsets represented a different life stage: 'I' - young loggerhead turtles that have not recruited to neritic habitat (posthatchlings and oceanic juveniles), 'II' - neritic juveniles, and 'III' - nesting adults. We used 41.5 cm SCL as a size at recruitment to neritic habitat, and 80.7 cm SCL as a size at onset of nesting (becoming an adult). Carapace length of a sea turtle at the time of recruitment is between 41.5 and 58.2 cm SCL (converted from 46 and 64 cm CCL reported in [13]). Although Snover [215] report a narrower range (48.5-51.5 cm SCL), we conservatively used the lower end of the wider range reported in [13]. Size at onset of nesting was determined as the minimum reported for nesting females [54, 28]. Using the lower end was a conservative estimate to ensure that sea turtles that have already recruited to neritic habitats, or have started nesting, are not grouped and analyzed with those who have not. Subset 'I' initially had 89% of the data points, even though it covers only 38% of the total SCL range in the data. To avoid giving too much weight to the subset, and achieve a more uniform distribution of datapoints across the size span, we sub-sampled the subset 'I' in such a way that the relative number of data pairs in subset 'I' of the SCW to SCL relationship reflected the 38% calculated for the SCL span: 2567 data triplets used in the previous analysis were additionally censored to 1065, and then used to construct SCW to SCL, BD to SCL, and BD to SCW data pairs. Next, data from other sources were added. Type, number and sources of data points are given in Table 2.1.

In addition to data sets listed in Table 2.1, we created a dataset 'both' for the analysis of combined geographic subsets, and datasets 'I+II', 'II+III', and 'I+II+III' for the analysis of combined life stage subsets. The additional data sets were merged combinations of the censored subsets.

2.2.2 Models and statistical analysis

To test specificity of allometric scaling relationships of western North Atlantic loggerhead turtles, we performed two analyses: (i) analysis of geographic subsets ('north' and 'south'), and (ii) analysis of life stage subsets ('I', 'II', 'III'). Each analysis consisted of two steps.

In the first step, for each subset we calculated pairwise ratios r_1, r_2, and r_3 between the three variables (straight carapace length - SCL, straight carapace width - SCW, and body depth -BD): $r_1 = SCW/SCL, r_2 = BD/SCL, r_3 = BD/SCW$. In the analysis of geographic subsets ('north' and 'south'), we could calculate ratios r_1, r_2, and r_3 for posthatchlings, and only ratio r_1 for adults in each subset. In the analysis of life stage subsets ('I', 'II', 'III'), we could calculate ratios r_1, r_2, and r_3 for each of the three subsets.

We compared the ratios by plotting their distributions, and calculating standard descriptive statistics (median, interquartile range, min, max). By analyzing the ratios, we obtained a first glance at the differences and/or similarities among the compared groups. For example, the distribution of the ratios between two regions should stay the same if animals from those regions have similar shapes. Additionally, the analysis of the ratio distributions between life stages highlighted the extent to which loggerhead turtles deviate from the assumption of isomorphism.

In the second step, the power law was used to scale SCW to SCL, BD to SCL, and BD to SCW. The power law,

$$y = A \cdot x^b,$$

where A is the conversion factor from one characteristic to another, and b defines the nature of the scaling (isometric if $b = 1$, allometric otherwise), was found to describe a multitude of correlations between size and metabolic activity or behavior, and was also applied in morphometrics [205, 116].

We first $\log_e$ transformed the data to reduce the effect of outliers, stabilize variance, and linearize the relationship for least squared-error regression [266]. The log transformation of the power law resulted in three linear models, one for each scaling relationship:

$$\log(SCW) = a + b \cdot \log(SCL), \tag{2.1}$$

$$\log(BD) = a + b \cdot \log(SCL), \tag{2.2}$$

$$\log(BD) = a + b \cdot \log(SCW), \tag{2.3}$$

where a is $\log_e(A)$, the intercept on the y-axis, and b is the slope of the line on the log-log plot. The linear models (equations 2.1- 2.3) were fitted to $\log_e$ transformed data described in section Data using least squared-error linear regression (fit function implemented in MATLAB R2011b). We evaluated the goodness of fit (coefficient of determination R^2), and used analysis of covariance (ANOCOVA) models and multiple comparison procedures to compare the model slopes with ANCOVA and Tukey-Kramer test ($p < 0.05$, `aoctool` and `multcompare` functions implemented in MATLAB R2011b).

In the analysis of regional data sets ('north', 'south', and 'both'), we fitted models (2.1) to (2.3) to each data set within the posthatchling group, and model (2.1) to data sets within the adult group. Results are given in the section Analysis of regional subsets 'north' and 'south'.

In the analysis of life stage data sets ('I', 'II', 'III', 'I+II', 'II+III', and 'I+II+III'), we fitted models (2.1) to (2.3) to each data set. While comparing life stage subsets, we could not test for differences between regional subsets within each life stage. Therefore, data obtained from different geographic regions were pooled for analysis. The pooling is further justified by the observed uniformity of carapace length and width among nesting loggerheads from different western North Atlantic subpopulations ([224], and references within). The uniform distribution of data points (achieved by censoring the subset 'I' prior to the analysis) made it possible to estimate model parameters on merged groups, without attributing too much weight to any of the stages. For the models fitted on all available data (the combined dataset 'I+II+III'), we tested whether the growth of sea turtles is isometric, by testing whether the parameter b is significantly different from 1. We calculated what would the covariant variables ($\log(SCW)$ and $\log(BD)$) be for a given value of $\log(SCL)$ or $\log(SCW)$ if growth is isometric, using as input the average SCL and SCW values at hatching, recruitment, and nesting. We compared the predictions by the isometric model ($b = 1$) to the predictions by the allometric model (b regressed by model fitting), calculated the error, and compared the prediction intervals of the models. Results are given in the section Analysis of life stage subsets 'I' , 'II' , and 'III'.

The preliminary results suggested a non-linear relationship of the data on the log-log axes. Therefore, we also investigated whether a curvilinear model would perform significantly better. We chose a type II functional form of the saturating relationship because it had the same number of parameters as the linear model. Other models, for example those suggested by [104], [251], might have been equally appropriate, but they would either introduce new parameters that do not add to the mechanistic explanation [104], or would require fitting using untransformed data, thereby obstructing direct comparison of parameter values [251]. The type II functional form of the saturating relationship was:

$$y = \frac{A \cdot x}{b + x}.$$

After log transformation, we got:

$$\log(SCW) = a + \log(SCL) - \log(SCL + b), \tag{2.4}$$

$$\log(BD) = a + \log(SCL) - \log(SCL + b), \tag{2.5}$$

$$\log(BD) = a + \log(SCW) - \log(SCW + b). \tag{2.6}$$

We compared the performance of linear models (equations 2.1- 2.3, marked with 'M1'), and non-linear models (equations 2.4- 2.6, marked with 'M2') using goodness of fit statistics (R^2 and RMSE), and Akaike Weights [245]. The goodness of fit statistics evaluate model performance independently: higher R^2, and lower RMSE (Root Mean Square Error) indicate better performance. The Akaike weight for a certain model is a probability that the particular model is the best model of those investigated, given the particular data [245]. Akaike weights, therefore, cross-compare the performance of the models by evaluating the probability with which one model should be chosen over the other. Results are given in the section Performance of saturating models 'M2'. All calculations were done in MATLAB R2011b.

2.3 Results

Coefficients of scaling relationships between length, width, and depth of western North Atlantic loggerhead turtles did not significantly differ between two regional subsets, but were significantly different between life stage subsets. When accuracy is not of paramount importance, the common model for all life stages can be used, and isometric growth can be assumed. The tested non-linear models, although statistically preferred over the linear class of models in some cases, did not yield considerably different results.

2.3.1 Analysis of regional subsets 'north' and 'south'

Each regional subset ('north' and 'south') of western North Atlantic loggerheads consisted of a posthatchling and an adult group, which were analyzed separately (see subsection Data for details). The standard descriptive statistics of ratios r_1 (SCW/SCL), r_2 (BD/SCL), and r_3 (BD/SCW) for posthatchlings, and r_1 for adults were similar between different regions for all ratios (Table 2.2). The null hypothesis that ratios come from the same distribution was not rejected for two cases: posthatchling r_3 and adult r_1 (Mann-Whitney U test, $p > 0.05$). The differences between medians of the ratios posthatchling r_1 and posthatchling r_2 were statistically significant (Mann-Whitney U test, $p < 0.05$), but they were small (1.7% and 1.4% for r_1 and r_2, respectively). Similar descriptive statistics values for ratios of size measurements (Table 2.2) suggest that the individuals encountered in these two geographic subsets have similar morphology (i.e., shape).

Table 2.2: Descriptive statistics: number of data points (N), median, interquartile range (IQR), minimum, and maximum of ratios, for posthatchlings and adults of regional subsets 'north' and 'south'.

posthatchlings	*SCW/SCL*	**N**	**median**	**IQR**	**min**	**max**
	'south'	1300	0.8308	0.0345	0.5162	0.9784
	'north'	1267	0.8141	0.0356	0.6345	1.0199
	BD/SCL	**N**	**median**	**IQR.**	**min**	**max**
	'south'	1300	0.4457	0.0274	0.3398	0.5819
	'north'	1267	0.4378	0.0280	0.3071	0.5727
	BD/SCW	**N**	**median**	**IQR**	**min**	**max**
	'south'	1300	0.5374	0.0419	0.4406	0.8834
	'north'	1267	0.5395	0.0429	0.3766	0.6450
adults	*SCW/SCL*	**N**	**median**	**IQR**	**min**	**max**
	'south'	105	0.7638	0.0378	0.6823	0.9268
	'north'	112	0.7577	0.0414	0.6805	0.9618

Fitting linear scaling models (equations 2.1- 2.3) to the datasets 'north', 'south', and 'both' produced three predictive regression equations (henceforth referred to as 'm_{north}', 'm_{south}', and 'm_{both}') for each model, differing only in parameter values. R^2 values of all regression equations within the *posthatchling group* were high (0.97 for SCWvsSCL, and 0.94 for the other two relations, Table 2.3). Predictive regression equations for one posthatchling dataset showed a small difference in goodness of fit ($\triangle R^2 \leqslant 0.01$) when used to describe the other posthatchling dataset (e.g. $'m_{north}'$ models used for the 'south' dataset). Likewise, when the general $'m_{both}'$ regression equation was used for describing regional posthatchling data sets ('south' or 'north'), goodness of fit was similar to that of the regionally specific regression equation ($\triangle R^2 \leqslant 0.01$). Although slopes (parameter b) of some regression equations were statistically different (ANCOVA, $p < 0.05$, Table 2.3), the width of 95% prediction confidence intervals of $'m_{both}'$ overlaps with that of the subset-specific regression equations (Fig 2.1 for $\log(SCW)$ to $\log(SCL)$ relationship, other relationships not shown but having similar trends). Results within the *adult group* for the $\log(SCW)$ to $\log(SCL)$ relationship corroborated the similarity between scaling relationships of different regions. There were again only small differences ($\triangle R^2 \leqslant 0.01$) when predictive regression equations for one adult dataset were used for describing the other adult dataset, and there was practically no difference between the R^2 values of the subset-specific ($'m_{north}'$, $'m_{south}'$) and general ($'m_{both}'$) regression equations. No two regression slopes within the adult group were significantly different (ANCOVA, $p < 0.05$, Table 2.3). Considering that we did not find sufficient evidence to support the hypothesis that differences in scaling between 'north' and 'south' regional subsets are large, we suggest that any analysis can be simplified by grouping the regional subsets.

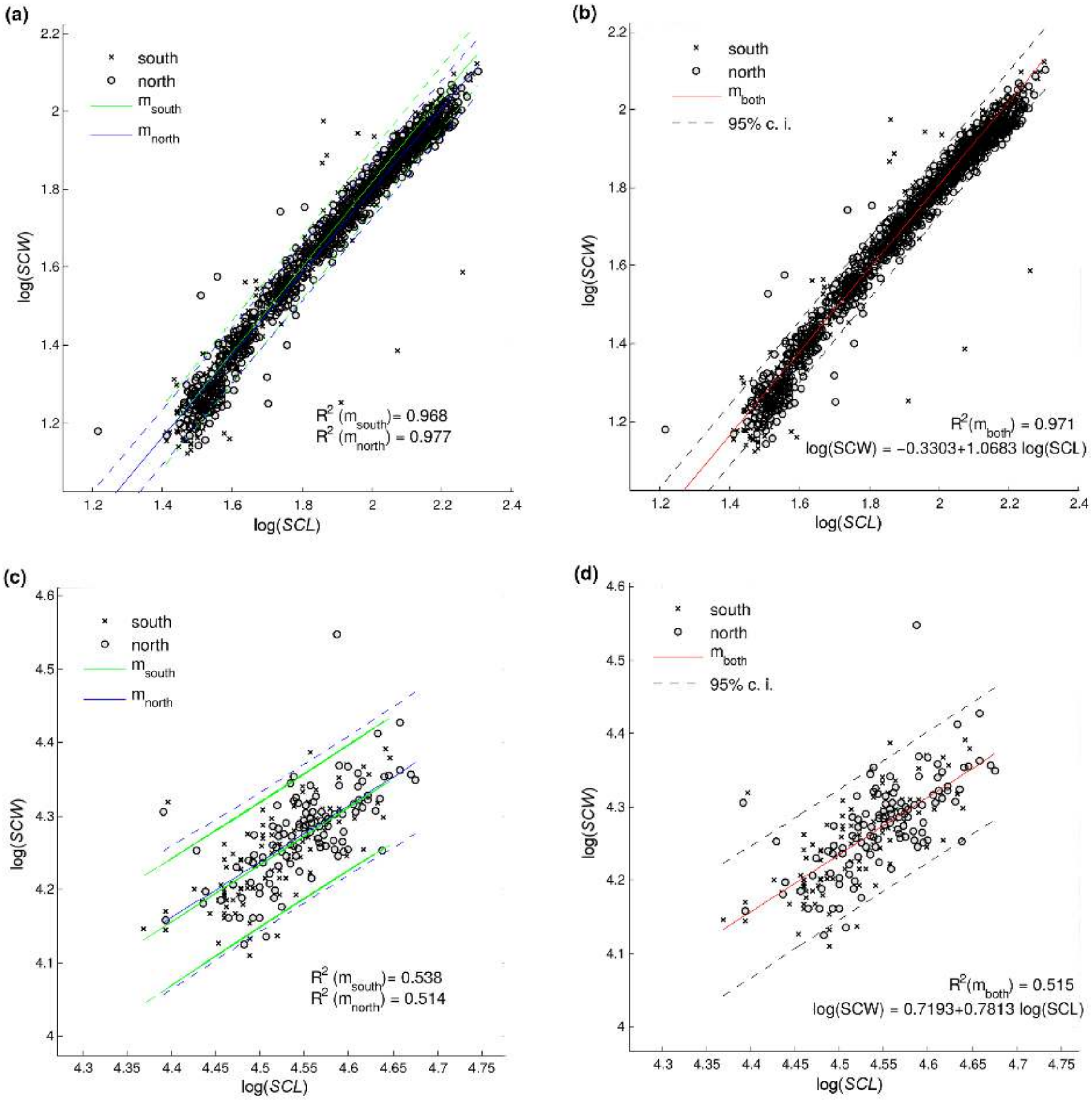

Figure 2.1: Predictions of $\log(SCW)$ from $\log(SCL)$ by regression equations $'m_{north}'$, $'m_{south}'$, and $'m_{both}'$ specific for regional subsets 'north', 'south', and 'both'. Panels (a) and (b): data from the posthatchling group. Panels (c) and (d): data for the adult group. The recommended regression equations are displayed in the plot, while the parameters for remaining equations are provided in Table 2.3. Dashed lines mark the 95% confidence intervals of the predictions.

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Table 2.3: Analysis of linear scaling models for regional subsets 'north' and 'south'. For each dataset (listed under 'datasets') we analysed the performance of three predictive regression equations, differing only in the values of model parameters. Parameter values are given under the name of the dataset used for regression. R^2 value describes the goodness of fit of the regression equation listed in the column to the dataset listed in the row. 'Slope diff' indicates whether or not the slopes of two regression equations are significantly different (Tukey-Kramer test, $p < 0.05$), where one regression equation is specific for the dataset listed in the row, and the other for the dataset listed in the column. All regression equations are in the form of $y = a + b \cdot x$ (equations 2.1- 2.3 in 'Methods'). We analysed separately data from posthatchlings and adults, see subsection Data for details.

POSTHATCHLINGS

Scaling	Analysis				
SCW vs SCL	**dataset used for regression →**		**'south'**	**'north'**	**'both'**
	datasets ↓		a = -0.3623	a = -0.3090	a = -0.3303
			b = 1.0899	b = 1.0523	b = 1.0683
	'south'	R^2	0.9677	0.9607	0.9659
		Slope diff.	-	**Yes**	**Yes**
	'north'	R^2	0.9699	0.9769	0.9753
		Slope diff.	**Yes**	-	**Yes**
	'both'	R^2	0.9689	0.9691	0.9707
		Slope diff.	**Yes**	**Yes**	-
BD vs SCL	**dataset used for regression →**		**'south'**	**'north'**	**'both'**
	datasets ↓		a = -0.8124	a = -0.7764	a = -0.7898
			b = 1.0028	b = 0.9746	b = 0.9863
	'south'	R^2	0.9420	0.9349	0.9401
		Slope diff.	-	**Yes**	No
	'north'	R^2	0.9413	0.9481	0.9464
		Slope diff.	**Yes**	-	No
	'both'	R^2	0.9416	0.9417	0.9434
		Slope diff.	No	No	-
BD vs SCW	**dataset used for regression →**		**'south'**	**'north'**	**'both'**
	datasets ↓		a = -0.4509	a = -0.4616	a = -0.4564
			b = 0.9034	b = 0.9092	b = 0.9064
	'south'	R^2	0.9384	0.9383	0.9384
		Slope diff.	-	No	No
	'north'	R^2	0.9353	0.9354	0.9353
		Slope diff.	No	-	No
	'both'	R^2	0.9368	0.9368	0.9368
		Slope diff.	No	No	-

ADULTS

Scaling	Analysis				
SCW vs SCL	**dataset used for regression →**		**'south'**	**'north'**	**'both'**
	datasets ↓		a = 0.7305	a = 0.7810	a = 0.7193
			b = 0.7785	b = 0.7681	b = 0.7813
	'south'	R^2	0.5382	0.5343	0.5374
		Slope diff.	-	No	No
	'north'	R^2	0.4500	0.4531	0.4523
		Slope diff.	No	-	No
	'both'	R^2	0.5143	0.5141	0.5151
		Slope diff.	No	No	-

2.3.2 Analysis of life stage subsets 'I' , 'II' , and 'III'

The standard descriptive statistics of ratios r_1 (SCW/SCL), r_2 (BD/SCL), and r_3(BD/SCW) suggest that morphology of loggerhead turtles differs between life stages. Interquartile range (IQR) of the ratios related to subset 'I' was smaller than IQR of the ratios related to subsets 'II' and 'III' (Table 2.4), indicating that variability of data in subsets 'II' and 'III' is larger, even though there are fewer data points than in subset 'I'. The null-hypothesis that all samples of the same ratio (e.g. r_1^I, r_1^{II}, and r_1^{III}) come from the same distribution was rejected (Kruskall Wallis test, $p < 0.05$), however pairwise ratio analysis couldn't reject the hypothesis that ratios related to subset 'II' come from the same distribution as those related to subset 'III'(e.g. r_1^{II}, and r_1^{III}) (Mann-Whitney U test, $p > 0.05$). This was the case for all three ratios, suggesting that subsets 'II' and 'III' could be merged into a single dataset when applying morphometric scaling, but subset 'I' should be considered separately.

Table 2.4: Descriptive statistics: Number of data points (N), median, interquartile range (IQR), minimum, and maximum of ratios, for life stage subsets 'I', 'II', and 'III'.

SCW/SCL	**N**	**median**	**IQR**	**min**	**max**
'I'	1113	0.823	0.0385	0.510	1.020
'II'	71	0.819	0.0537	0.741	0.914
'III'	252	0.761	0.0438	0.680	0.980
BD/SCL	**N**	**median**	**IQR**	**min**	**max**
'I'	1120	0.442	0.0279	0.288	0.521
'II'	71	0.407	0.0407	0.262	0.485
'III'	154	0.364	0.0390	0.301	0.549
BD/SCW	**N**	**median**	**IQR**	**min**	**max**
'I'	1112	0.537	0.0409	0.377	0.695
'II'	59	0.492	0.0583	0.313	0.586
'III'	147	0.477	0.0531	0.330	0.770

Fitting linear scaling models (equations 2.1- 2.3) to the datasets 'I', 'II', and 'III' produced three predictive regression equations (henceforth referred to as $'m_I'$, $'m_{II}'$, and $'m_{III}'$) for each model, differing only in parameter values. For models describing the scaling relationships of carapace width to carapace length (equation 2.1), and body depth to carapace length (equation 2.2), the slopes (parameter b) of $'m_I'$, $'m_{II}'$, and $'m_{III}'$ were not significantly different when datasets describing sequential life stages were used for model fitting (Tukey-Kramer test, $p > 0.05$, Table 2.5 and Fig 2.2). The relationship of body depth to carapace width (equation 2.3) showed a different trend, with the slopes of $'m_{III}'$ significantly different from other slopes (Tukey-Kramer test, $p < 0.05$, Table 2.5 and Fig 2.2). In general, $'m_{III}'$ regression equations had the lowest R^2 values (Table 2.5), and the widest 95% confidence intervals of parameters and predictions (Fig 2.2).

Table 2.5: Analysis of linear scaling models for life stage datasets. For each dataset (listed under 'datasets') we analyzed the performance of six predictive regression equations, differing only in the values of model parameters. Parameter values are given under the name of the dataset used for regression. R^2 value describes the goodness of fit of the regression equation listed in the column to the dataset listed in the row. We marked for readability R^2 values when the regression equation was used for the dataset it was fitted on. 'Slope diff' indicates whether or not the slopes of two regression equations are significantly different (Tukey-Kramer test, $p < 0.05$), where one regression equation is specific for the dataset listed in the row, and the other for the dataset listed in the column. All regression equations are in the form of $y = a + b \cdot x$ (equation 2.1 - 2.3 in Models and statistical analysis). See subsection Data for definitions.

	dataset for regression →		**'I'**	**II'**	**'III'**	**'I+II'**	**'II+III'**	**'I+II+III'**
SCW	**datasets** ↓		a = -0.2456	a = 0.1072	a = 0.5041	a = -0.2193	a = 0.4014	a = -0.1658
vs			b = 1.0233	b = 0.9253	b = 0.8293	b = 1.0094	b = 0.8521	b = 0.9816
SCL	**'I'**	R^2	0.9902	0.8148	0.0760	0.990	0.3377	0.9886
		Slope diff.	-	No	**Yes**	No	**Yes**	**Yes**
	'II'	R^2	0.7815	0.8931	0.8805	0.8750	0.8872	0.7885
		Slope diff.	No	-	No	No	No	No
	'III'	R^2	N/A†	0.2053	0.5059	N/A†	0.5054	0.4046
		Slope diff.	**Yes**	No	-	**Yes**	No	**Yes**
	'I+II'	R^2	0.9957	0.9313	0.6587	0.9960	0.7553	0.9952
		Slope diff.	No	No	**Yes**	-	**Yes**	**Yes**
	'II+III'	R^2	0.5550	0.9021	0.9328	0.7435	0.9335	0.9110
		Slope diff.	**Yes**	No	No	**Yes**	-	**Yes**
	'I+II+III'	R^2	0.9958	0.9798	0.9023	0.9971	0.9299	0.9982
		Slope diff.	**Yes**	No	**Yes**	**Yes**	**Yes**	-
	dataset for regression →		**'I'**	**II'**	**'III'**	**'I+II'**	**'II+III'**	**'I+II+III'**
BD	**datasets** ↓		a = -0.7415	a = -0.3128	a = -0.2475	a = -0.7365	a = -0.2660	a = -0.7075
vs			b = 0.9599	b = 0.8525	b = 0.8367	b = 0.9572	b = 0.8408	b = 0.9422
SCL	**'I'**	R^2	0.9822	0.6550	0.5443	0.9822	0.5792	0.9819
		Slope diff.	-	No	No	No	**Yes**	No
	'II'	R^2	0.6860	0.6992	0.6989	0.6885	0.6990	0.6576
		Slope diff.	No	-	No	No	No	No
	'III'	R^2	N/A†	0.1910	0.1942	N/A†	0.1942	0.1646
		Slope diff.	No	No	-	No	No	No
	'I+II'	R^2	0.9918	0.8662	0.8237	0.9918	0.8371	0.9915
		Slope diff.	No	No	No	-	**Yes**	No
	'II+III'	R2	0.7512	0.8088	0.8093	0.7626	0.8093	0.7974
		Slope diff.	**Yes**	No	No	**Yes**	-	**Yes**
	'I+II+III'	R^2	0.9949	0.9476	0.9315	0.9950	0.9366	0.9953
		Slope diff.	No	No	No	No	**Yes**	-
	dataset for regression →		**'I'**	**II'**	**'III'**	**'I+II'**	**'II+III'**	**'I+II+III'**
BD	**datasets** ↓		a = -0.5072	a = 0.1245	a = 1.9431	a = -0.5234	a = -0.3780	a = -0.5397
vs			b = 0.9356	b = 0.7809	b = 0.3772	b = 0.9453	b = 0.9177	b = 0.9549
SCW	**'I'**	R^2	0.9776	0.0068	N/A†	0.9775	0.9076	0.9771
		Slope diff.	-	No	**Yes**	No	No	No
	'II'	R^2	0.4931	0.5755	N/A†	0.5397	0.5310	0.5455
		Slope diff.	No	-	**Yes**	No	No	**Yes**
	'III'	R^2	N/A†	N/A†	0.0596	N/A†	N/A†	N/A†
		Slope diff.	**Yes**	**Yes**	-	**Yes**	**Yes**	**Yes**
	'I+II'	R^2	0.9892	0.5963	N/A†	0.9893	0.9610	0.9892
		Slope diff.	No	No	**Yes**	-	No	No
	'II+III'	R^2	0.6931	0.6521	0.3818	0.7322	0.7512	0.7489
		Slope diff.	No	No	**Yes**	No	-	No
	'I+II+III'	R^2	0.9938	0.8562	N/A†	0.9941	0.9845	0.9943
		Slope diff.	No	**Yes**	**Yes**	No	No	-

† The linear model $y=a+bx$ underperforms relative to the null-model with $b=0$.

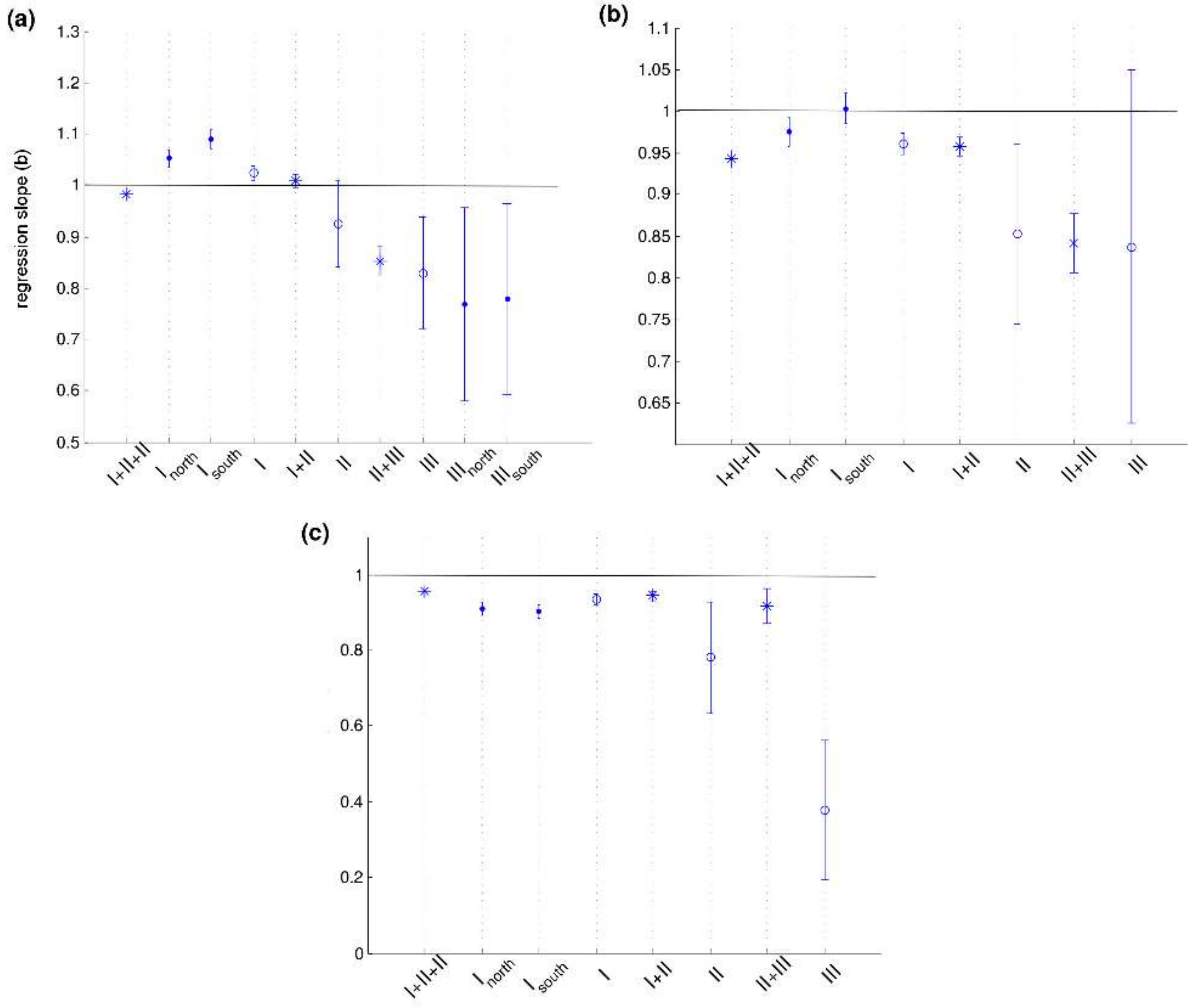

Figure 2.2: Model slopes with 95% confidence intervals. For scaling relationships of SCW to SCL (panel (a)), BD to SCL (panel (b)), and BD to SCW (panel (c)). In addition to slopes of regression equations specific for life stage subsets ('I', 'II', and 'III', marked with circles), and combined data sets ('I+II', 'II+III', and 'I+II+III', marked with asterisks), we show slopes of regression equations specific for regional subsets, which describe either exclusively posthatchlings ($'I_{north}'$, $'I_{south}'$) or nesting adults ($'III_{north}'$, $'III_{south}'$), all marked with dots. Analysis of regional subsets is described in section Analysis of regional subsets 'north' and 'south'. Horizontal full line represents the slope of an isometric model ($b = 1$).

We then used predictive regression equations specific to one subset, to predict values for the other two subsets. Results are given in Table 2.5 for all three scaling relationships, and in Fig 2.3 for the relationship of carapace width to carapace length (equation 2.1). Predictions for the relationship of body depth to carapace length (equation 2.2) were similar to the ones shown in the figure: only $'m_I'$ was suitable for subset 'I', and only $'m_{II}'$

and 'm_{III}' described subsets 'II' and 'III' with satisfactory accuracy. For the relationship of body depth to carapace width (equation 2.3), again only 'm_I' was suitable for subset 'I', however the slopes (Tukey-Kramer test, $p < 0.05$) and consequently predictions of 'm_{II}' and 'm_{III}' were significantly different. This might have been a consequence of data scatter in subset 'III', and relatively low correlation of $\log BD$ to $\log SCW$ (R^2=0.06) (Table 2.5).

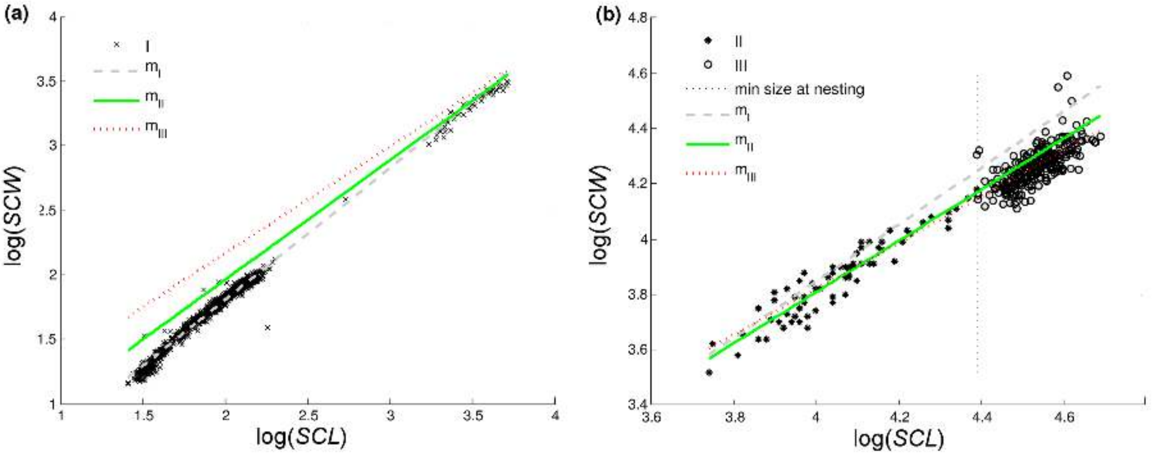

Figure 2.3: Predictions of $\log(SCW)$ by regression equations 'm_I', 'm_{II}', and 'm_{III}'. Regression equations are specific for life stage subsets 'I', 'II', and 'III' (respectively). Panel (a): subset 'I', panel (b): subsets 'II' and 'III'. Parameters for the equations are provided in Table 2.5.

When we analyzed the performance of models fitted on combined datasets ('I+II', and 'II+III'), the slopes of predictive regression equations 'm_{I+II}' and 'm_{II+III}' were not significantly different from the slopes of predictive equations for the subsets merged into the combined datasets (Tukey-Kramer test, $p > 0.05$, Table 2.5). However, 'm_{I+II}' slightly overestimated $\log(SCW)$ and $\log(BD)$ for larger individuals in subset 'II', and the relatively narrow 95% prediction confidence interval of 'm_{I+II}' could not account for the increase of data scatter in subset 'II'. This suggests that 'm_{I+II}' is not suitable for modeling large neritic juveniles. By contrast, 95\% prediction confidence intervals of 'm_{II+III}' are very similar to those of 'm_{II}' and 'm_{III}', giving a wide enough range for predictions, and R^2 value of 'm_{II+III}' for subsets 'II' and 'III' was practically the same as R^2 values of 'm_{II}' and 'm_{III}' for those subsets (Table 2.5). Model analysis, therefore, supports the idea that life stage subsets 'II' and 'III' (representing neritic juveniles and adults) can be merged. Description and predictions of models that we suggest should be used for loggerhead turtles are given in Fig 2.4, panels (a), (c), and (e).

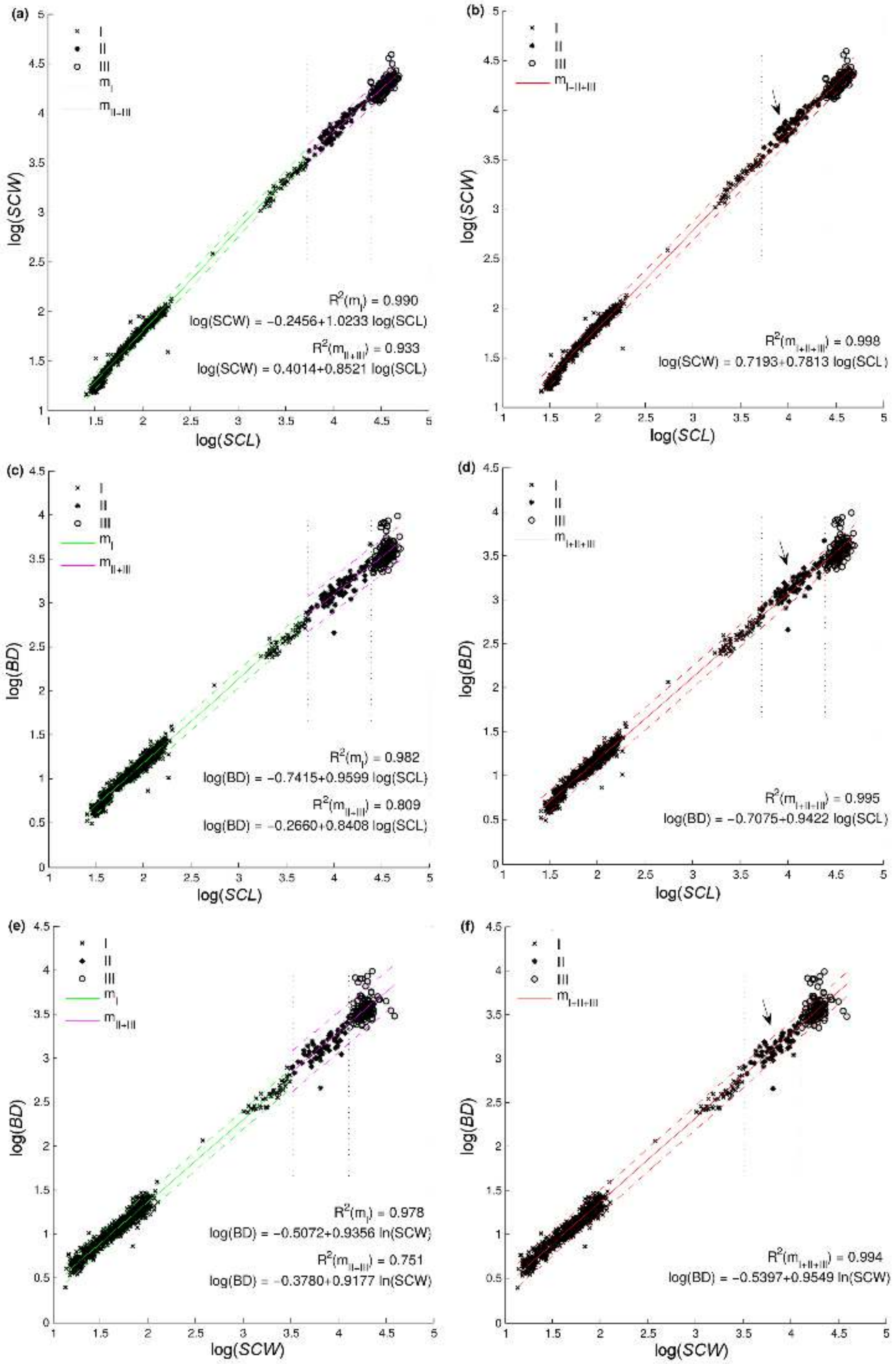

Figure 2.4: Fit of suggested subset-specific ('m_I', 'm_{II+III}', panels (a), (c), (e)), and non-specific ('$m_{I+II+III}$', panels (b), (d), (f)) linear scaling models to data. The relationship of log(*SCW*) to log(*SCL*) is shown in panels (a) and (b), the relationship of log(*BD*) to log(*SCL*) in panels (c) and (d), and the relationship of log(*BD*) to log(*SCW*) in panels (e) and (f). The recommended regression equations are displayed in the plot, while parameters for remaining equations are provided in Table 2.5. Dashed lines mark the 95% confidence intervals of the predictions. Black arrows in panels (b), (d), and (f) point to the size range in which predictions are underestimated.

Next, we fitted models (2.1-2.3) to the dataset combining all three subsets ('I+II+III'), and obtained predictive regression equations '$m_{I+II+III}$'. Description and predictions of '$m_{I+II+III}$' are given in Fig 2.4, panels (b),(d), and (f). Although predictions were satisfactory over the whole size span, some values for juveniles in the subset 'II' were underestimated, which is consistent with the gradual change (decrease) in model slope in later life stages. Generally however, R^2 values of '$m_{I+II+III}$' for subsets 'I', 'II', and 'III' were as high as those of 'm_I', 'm_{II}', and 'm_{III}' (Table 2.5), suggesting that this model can be applied for describing the whole size span of loggerhead turtles.

Finally, we analyzed whether the growth of loggerhead turtles can be considered isometric. The slope coefficients of '$m_{I+II+III}$' were significantly different from 1 (Tukey-Kramer test, $p < 0.05$) for all studied relationships (Fig 2.2), suggesting allometric growth. However, the differences between values calculated by the allometric linear model (regressed value of b) and the isometric linear model ($b = 1$) for the same relationship were less than 5%. Furthermore, the predictions of the isometric model were within the range of predictions of the allometric model for the same relationship (Table 2.6).

Table 2.6: Comparison of the allometric model (value of b regressed by model fitting) to the isometric model ($b = 1$). As the allometric model we used the predictive regression equation '$m_{I+II+III}$'. As size at the event of interest, we used average values at hatching SCL = 4.5 cm [223], recruitment SCL = 48 cm [13], and nesting SCL = 93 cm [54, 28] for the relationships of carapace width and body depth to carapace length. For the relationship of body depth to carapace width we calculated SCW values that would correspond to average carapace lengths at hatching, recruitment, and nesting, using '$m_{I+II+III}$'. Error was calculated for $\log_e$ transformed data as [100(value predicted by isometric model - value predicted by allometric model)/ value predicted by allometric model].

event of interest	relationship	error (%)	predictions range (cm)	
			allometric model	*isometric model*
hatching	*SCW vs SCL*	-1.41	3.39 - 4.06	3.63 - 3.65
	BD vs SCL	-7.05	1.79 - 2.30	1.92 - 1.94
	BD vs SCW	-5.17	1.78 - 2.33	1.95 - 1.97
recruitment	*SCW vs SCL*	0.69	34.60 - 41.45	38.73 - 38.93
	BD vs SCL	2.95	16.69 - 21.43	20.53 - 20.72
	BD vs SCW	2.32	16.37 - 21.45	19.97 - 20.14
nesting	*SCW vs SCL*	0.87	66.23 - 79.33	75.04 - 75.43
	BD vs SCL	3.51	31.12 - 39.98	39.78 - 40.15
	BD vs SCW	2.74	30.42 - 39.87	38.22 - 38.55

2.3.3 Performance of saturating models 'M2'

Saturating (curvilinear) models,'M2', (equations 2.4- 2.6) did not perform markedly better than the commonly used linear models, 'M1', (equations 2.1- 2.3). We tested all models on data sets 'I', 'II+III', and 'I+II+III' that, based on the previous analysis, need to be taken into the account for morphometric scaling. Models 'M1' and 'M2' have satisfactory and almost identical goodness of fit (R^2 values, RMSE) for all relationships of all data sets taken into account (Table 2.7, Fig 2.5). Even though Akaike weights in some cases point with a 100% certainty to a certain model, predictions between the linear and nonlinear class of the same model differ 0.0153 - 4.5093% for SCW and 0.0029 - 4.6456% for BD.

Table 2.7: Comparison of the linear (lin.) and saturating (sat.) type of models $'m_I'$, $'m_{II+III}'$, and $'m_{I+II+III}'$ for the three studied relationships. Performance of models was tested on datasets 'I', 'II+III', and 'I+II+III', and evaluated by goodness of fit statistics (R^2, Root Mean Square Error (RMSE)), and Akaike weights).

dataset/ *model*	**type of model**	**log(*SCW*) vs log(*SCL*)**			**log(*BD*) vs log(*SCL*)**			**log(*BD*) vs log(*SCW*)**		
		R^2	*RMSE*	*Akaike weight*	R^2	*RMSE*	*Akaike weight*	R^2	*RMSE*	*Akaike weight*
'I'/	lin.	0.9902	0.0391	1.0000	0.9822	0.0519	0.0000	0.9776	0.0557	1.0000
$'m_I'$	sat.	0.9897	0.0401	0.0000	0.9828	0.0510	1.0000	0.9763	0.0572	0.0000
'II+III'/	lin.	0.9335	0.0492	0.2856	0.8093	0.1003	0.5525	0.7512	0.1149	0.3647
$'m_{II+III}'$	sat.	0.9339	0.0491	0.7144	0.8090	0.1004	0.4475	0.7525	0.1146	0.6353
'I+II+III'/	lin.	0.9982	0.0460	0.0000	0.9953	0.0637	0.0000	0.9943	0.0688	1.0000
$'m_{I+II+III}'$	sat.	0.9983	0.0439	1.0000	0.9955	0.0623	1.0000	0.9940	0.0704	0.0000

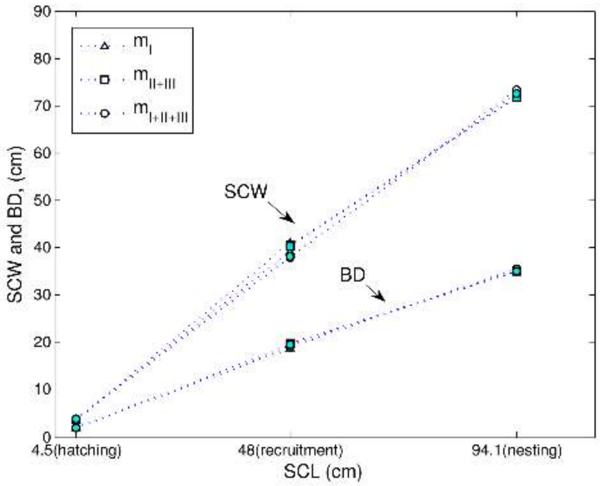

Figure 2.5: Predictions of SCW and BD by two types (M1 - linear, and M2 - saturating) of models $'m_I'$, $'m_{II+III}'$, and $'m_{I+II+III}'$. Predictions are given for average sizes at specific events (hatching, recruitment, nesting). Symbols are coded based on the model (each symbol corresponds to one model), and type (full or empty symbol).

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2.4 Discussion

We analyzed morphometric scaling relationships for straight carapace width (SCW), straight carapace length (SCL), and body depth (BD) of loggerhead turtles using all available data for the western North Atlantic population. The analysis included the scaling relationships of two regional ('north', 'south'), and three life stage ('I', 'II', 'III') subsets, as well as two types of the scaling models: linear and saturating.

Our results suggest that the following models can be used to reasonably well describe scaling relationships of all western North Atlantic loggerhead turtles (all $R^2 > 0.99$), natural logarithm was used:

$$\log(SCW) = -0.1658 + 0.9816 \cdot \log(SCL),$$
$$\log(BD) = -0.7075 + 0.9422 \cdot \log(SCL), \text{ and}$$
$$\log(BD) = -0.5397 + 0.9549 \cdot \log(SCW).$$

Earlier analysis [57] gave similar relationships, especially for the relationship of carapace width and carapace length ($\log SCW = -0.0225 + 0.9507 \cdot \log SCL$, and $\log BD = -0.5682 + 0.9100 \cdot \log SCL$). Our analysis is (i) based on considerably more (>6x) data, and (ii) represents a broader size range (>120x more data for turtles with SCL<20cm and SCL>100cm). Data for sea turtles at the lower end of the size range are mostly obtained from captive reared turtles (here, and in [57]). The scaling relationships of width to length are presumed not to be affected by rearing conditions, but this does present a potential source of error in our data. Pooling captive-reared and wild data, as well as individual variability, may increase data scatter and model uncertainty. Having data from various sources, with potential differences in measurement accuracy and precision add additional sources of scatter. Individual variation has been reported for body depth [223, 224], and dependence of BD measurements on sea turtle's inhalation/exhalation adds accuracy and precision error. In most cases captive reared turtles are fed *ad libitum* to maximize growth, and BD will be greater in these captive data in relation to their wild counterparts. Consequently, the largest scatter was found in the BD data.

Our results suggest that analyzing different life stages of loggerhead turtles separately is appropriate when accuracy is important. For young loggerhead turtles that have not recruited to neritic habitats (posthatchlings and oceanic juveniles smaller than 41.5 cm SCL [13]), predictive regression equations are:

$$\log(SCW) = -0.2456 + 1.0233 \cdot \log(SCL), R^2 = 0.9902,$$
$$\log(BD) = -0.7415 + 0.9599 \cdot \log(SCL), R^2 = 0.9822, \text{ and}$$

$$\log(BD) = -0.5072 + 0.9356 \cdot \log(SCW), R^2 = 0.9776.$$

For neritic juveniles and nesting adults (loggerhead turtles larger than 41.5 cm SCL), we recommend:

$$\log(SCW) = 0.4014 + 0.8521 \cdot \log(SCL), R^2 = 0.9335,$$
$$\log(BD) = -0.2660 + 0.8408 \cdot \log(SCL), R^2 = 0.8093, \text{ and}$$
$$\log(BD) = -0.3780 + 0.9177 \cdot \log(SCW), R^2 = 0.7512.$$

Special attention should be given when using relationships to estimate BD for adults: correlation of BD to SCW and SCL for adults is extremely weak (R^2=0.06, and 0.19 respectively). This is presumably a consequence of large scatter in BD data, more pronounced for large individuals.

2.4.1 Regional subsets

When studying the scaling relationships of SCW and BD to SCL, our results suggest that regional models ('m_{north}' and 'm_{south}') fitted to one regional subset can be used to describe the other, and the models fitted on both subsets ('m_{both}') describe each of the regional subsets with satisfactory accuracy (negligible differences in R^2 value, overlapping 95% prediction confidence intervals). Statistical differences between the model slope of the 'm_{both}', and slopes of the regional models were found only for the $\log(SCW)$ to $\log(SCL)$ relationship within the posthatchling group. This might be a consequence of regionally-specific growth rates in the first couple of weeks, or different rearing conditions: water temperature and food availability [223]. Because of the large sample size ($N > 1500$, this might be the case when "highly significant statistics do not signify equally high biological significance" [205].

Although previous research suggested that regional differences in scaling could be important between the same regions as analyzed here [28, 224], we claim that the differences are negligible. The results of our analysis were not considerably different regardless of whether the subsets were analyzed together or separately, and we suggest that any analysis can be simplified by grouping regional subsets (sea turtles belonging to the same life stage encountered in the specific geographic area) of the western North Atlantic. Byrd et al. [28] computed scaling relationships of carapace width and body depth to carapace length for 130 loggerhead turtles nesting in South Carolina, and found significant differences between the scaling relationship of SCW to SCL and the same relationship published for Florida turtles in Epperly and Teas [56]. Byrd et al. [28] suggested that the differences in scaling relationships could be caused by regional conformational differences, but noted that other causes (e.g. sampling differences and/or allometric growth) could be responsible. Our analysis, comparing individuals of similar sizes and life stages, did not detect considerable differences between regions. We therefore suggest that the differences observed by Byrd et al. [28] are not regional, but instead should be attributed to other causes. This is supported by the findings that SCL and SCW are uniform across the western North Atlantic for nesting females [224]. The latter analysis, however, found a decrease in BD along the north-to-south gradient, not mentioned in Byrd et al. [28]. Taking into account the uniform SCL and SCW reported in the same publication [224], the gradual change in BD should be reflected in regionally-specific scaling relationships that include BD (relationships of body depth to carapace length and to carapace width), with a steeper slope of the model fitted to the northern subset. Due to lack of data, we could not test for differences of scaling relationships that include BD within the adult group, but we could analyze the relationships within the posthatchling group. Surprisingly, the analysis suggested that, for the relationship of body depth to carapace length, it is more likely that the slope of the model specific for the **south** subset will be steeper, suggesting an increase of body depth along the north-to-south gradient. However, as mentioned earlier, scatter in BD data is high. Different measurement techniques and precision, as well as different conditions sea turtles were exposed to prior to the measurements, certainly introduced a substantial error and uncertainty. Body depth may vary by season, as water temperatures and prey quality and availability fluctuate. Additionally, BD may fluctuate from the beginning to the end of the nesting season, as females often don't feed during the nesting period [89]. So, comparing a BD measurement taken at the beginning of the season in one region, to one taken at the end of the season in another region might produce a misleading result. More standardized measurements should be obtained for any relevant conclusions.

2.4.2 Life stage subsets

The analysis among the three life stages revealed significant differences in growth of width and depth relative to body length. Differences between *sequential* life stages were not significant for any of the studied relationships, suggesting a gradual, cumulative life-long allometric growth. Differences between subsets 'I' (posthatchlings and oceanic juveniles) and 'III' (nesting adults) were significant for scaling of SCW and BD to SCL; the analysis of the differences in BD relative to SCW were inconclusive because of a large scatter in BD measurements for adults. The decrease in slope of models (equations. 2.1, 2.2) for SCW and BD as a function of increasing SCL (Fig 2.2) is consistent with such a gradual process. Allometric growth changes throughout the life: initially, SCW and BD out-grow SCL; later in life, SCL grows faster than either SCW or BD. Therefore, extrapolating scaling from one life stage onto another should be avoided. Caution with such extrapolations was suggested before [64], but was not studied in more detail. To study the change in allometric growth, more data on loggerhead turtles in the critical range (between 10 and 25 cm SCL) is necessary. When extrapolated, predictive regression equations for subset 'I' overestimate carapace widths and body depths for subsets 'II' (neritic juveniles) and 'III' (Fig 2.3). When describing scaling in turtles, we recommend using one set of regression equations (models $'m_I'$) for posthatchlings and oceanic juveniles (young sea turtles before recruitment, at SCL<41.5cm, [13]), and another set of equations ($'m_{II+III}'$) for neritic juveniles and adults. Special attention is needed when predicting values for BD of adults. Correlations of BD to SCW and SCL are weak (R^2 of 0.06 and 0.19, respectively). The weak correlation is in large part because of high scatter of available BD data, possibly due to regional gradients in BD observed previously [224]. Additional sources of scatter of BD measurements could be individual variability, different environmental conditions that influence growth, such as temperature and food availability [241], and lack of standardized measurement techniques (e.g. unambiguous reference points on the turtle carapace) that could be applied when measuring body depth [21, 241]. Consequently, certainty of predictive regression equations is low, and using scaling relationships to predict BD in adults should be avoided whenever possible. Grouping neritic juveniles and adults reduces variability and increases confidence in predictions; the grouped model describes SCW well ($R^2 > 0.93$), and yields acceptable predictions for BD ($R^2 > 0.8$). Predictions of the allometric and isometric models were not considerably different, suggesting that the growth of sea turtles is close to being isometric.

2.4.3 Saturating models

Based on the results of data exploration, we tested the performance of saturating scaling models. The tested models did not perform considerably better than linear, and predictions of the linear and saturating model for the same scaling relationship differed less than 4.7%. Surprisingly, for some data that showed the most pronounced curvilinear trend when plotted on log-log axes, the linear model was statistically preferred: e.g. the SCW to SCL relationship for posthatchlings in subset 'I' (see Fig 2.1,panels (a,b) and Table 2.7). In general, the results suggest that it is much more important to use the appropriate model (e.g. $'m_{II+III}'$ for neritic juveniles and/or adults), than it is to use the statistically preferred class of the model (linear or saturating). The linear models account for >90% data variance (Table 2.5), so the result is not surprising. The additional complexity of the saturating models is not justified from practical aspect, and their use will probably be limited. However, even though the linear models may be sufficiently accurate for most applications, the significant difference between morphometric scaling of different life stages found here might benefit from further research [116]. Curvilinear models, such as the saturating models tested here or the models suggested by other authors [251, 104], could prove to be more appropriate than the linear model when accuracy is of utmost importance.

2.4.4 Implications

Scaling relations do not describe the mechanisms or causes of correlation of the observed variables, but they are helpful for discovering patterns, and obtaining predictive regression equations.

Similarity of morphological scaling relationships among different regional subsets of western North Atlantic loggerheads justifies the use of the same equation set for more than one specific subset, or for a whole population. For a theoretical example, if we assume that juveniles and adults in a neritic area are affected by shrimp trawls, we can use the $'m_{II+III}'$ model to estimate the lower boundary of the TED opening size. For the sake of illustration, if we assume a uniform size distribution (which we know to be biased because an actual population would generally have a declining density distribution with size), we can look at the 97-percentile of the size range because the targeted 97% TED efficiency in part depends on the size structure of a population in an area. Using $SCL_{min} = 41.5\,\text{cm}$ (minimum size at recruitment, [13]), and $SCL_{max} = 130\,\text{cm}$ (largest known nesting female, [65]), the 97-percentile of this theoretical size range is 127 cm. This corresponds to a SCW of 93.3 cm, and BD of 45.3 cm (predicted by regression equations $'m_{II+III}'$ specific for neritic juveniles and adults). TED opening - from the size considerations alone - should therefore be a minumum of 93.3 x 45.3 cm. However, although such theoretical size estimates are important for evaluating TED opening dimensions, these are based on just one species (loggerhead turtles), and do not take into account the causes of entanglement for reasons not related to size. When evaluating TED design and dimensions, National Marine Fisheries Service (NMFS) targets 97% efficiency in excluding sea turtles during experimental TED testing, accounting for factors such as: angle of installation, debris, fouling, and other issues, and required dimensions account also for the largest possible individuals, including large green and leatherback turtles ([58], Vol 68, No 35). Current regulations of design and dimensions of TED openings in North Atlantic [164] appear conservative enough to exclude loggerhead turtles based on our analysis. Predictive regression equations reported in this study for loggerhead turtles may be informative in future evaluations. Scaling relations of a single species' size measurements describe the current shape of the animals, and should not be used for explaining evolutionary or other processes [116]. However, scaling is a result of physical principles and various ecological/biological factors [68], and can be a valid starting point for exploring the underlying processes. For example, difference in scaling relationships between posthatchlings and other (later) life stages could reflect constraints faced by the sea turtles in different life stages. Sea turtles could be initially growing preferentially in width to avoid predators [202], and in length later in life to increase in size and improve hydrodynamics, chances of survival [202, 265], and fecundity [88]. Alternatively, smaller oceanic turtles become large enough to capably exploit neritic habitats, and undergo an ontogenetic shift to coastal waters at a threshold carapace size [82]. However, different regression routines should be applied for further analysis of the (non-significant) decrease of the slope throughout the life cycle, and interspecific morphometric analysis should be conducted in order to make conclusions involving evolutionary preferred solutions (see [116] for overview of analyses in scaling). We can use the slope of a scaling relationship not only to compare one relationship to another, but also to infer whether or not the organism's growth is isometric. As mentioned before, the differences between scaling relationships of sequential life stage subsets were not significant, and one set of scaling relationships (marked as '$m_{I+II+III}$') described well the whole analyzed size span of loggerhead turtles, for all three analyzed relationships (SCW to SCL, BD to SCL, and BD to SCW). The comparison of predictions by the current (allometric) models to the predictions by the isometric models (with b=*1*), suggests that the growth of loggerhead turtles can be considered isometric without losing much accuracy. Additionally, the changes in scaling may not affect the growth of total body volume; for example, losses of volume due to slower growth in SCW could be offset by faster growth in SCL. This is supported by the reported scaling of mass to length to the power close to 3 [244] (mass scaling to cubed length would be isometric scaling). In case of isometric growth, i.e., when proportional relationships are preserved while size changes during ontogeny or evolution, a set of implied properties can be applied: all volume-based properties scale proportionally to volume (often expressed as body mass), all surface area-based properties scale with volume to the power 2/3, and all length-based properties scale with volume to the power 1/3 [205, 213]. Further simplification can be made by assuming that the physical length is always the same fraction of the volumetric length (volume to the power 1/3), relating length-based properties directly to physical length. The slight offset from isometric scaling that has been detected needs to be kept in mind, but the (mostly) isometric growth opens the door to simplifications in a variety of modeling applications.

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Chapter 3

Dynamic Energy Budget model of the North Atlantic loggerhead turtles

Abstract

During their life, the average loggerhead turtle experiences an almost 25-fold increase in length, with a carapace at hatching of 4-5 cm straight length, and adult females ranging from 90-130 cm straight carapace length. The average female reproduces every 2-3 years, laying 4-5 clutches of over a hundred eggs per clutch, on the same beach she hatched on 15-30 years ago. Growth, maturation and reproduction are influenced by genetics, but also the environmental conditions (temperature and available food), which constrain the acquisition and use of available energy. Classic (static) growth and reproduction models have limited capacity to account for the environmental factors, and mostly give no insight into the physiology of the studied species, and the interaction between the physiological processes.

Completing energy budgets and constructing energy-based models has been recently identified as one of the key research areas for sea turtles. In this chapter, the Dynamic Energy Budget (DEB) theory is introduced and then used to construct a DEB model of North Atlantic loggerhead turtles. Data was obtained from published and unpublished sources, and all suitable data was used to estimate the model parameters. The estimated parameter values are realistic when compared to parameter values of other sea turtles, and the resulting DEB model describes the life cycle and predicts the life history traits well. The results are discussed with respect to observed and estimated values reported in the literature, and deviations of model predictions from data are discussed with respect to physiological and ecological implications.

3.1 Introduction

The life cycle of the loggerhead turtle can be divided into three stages: embryonic, juvenile, and adult.

Embryonic development, the duration of which is inversely proportional to the incubation temperature [61, 142, 223, 187], lasts 50-60 days with the sex of the embryos determined by temperature in the last third of the embryonic development [156, 265].

Within the juvenile period, further distinction can be made between (post-)hatchlings up to one year of age and 15 cm straight carapace length (SCL), exclusively oceanic juveniles, and juveniles that occasionally feed in neritic areas [228, 23]. After hatching, turtles having an average length of 4.5 cm and a mass of 20 g [174, 23] intensely swim towards the open sea, which is called the swimming frenzy, and then stay in the oceanic habitat feeding mostly opportunistically on a variety of oceanic and pelagic organisms, including jellyfish, molluscs, and oceanic crab species [66]. Upon reaching a certain length (between 41 cm and 63 cm SCL, [228, 13]) and undergoing some (physiological) changes resulting in, e.g., deeper and longer dives [91] and new prey items becoming manageable [138], juveniles start feeding in the neritic zone on larger and nutrient richer pray (larger crabs, molluscs, and fish) [80, 242]. This ontogenetic habitat shift is called *recruitment to neritic habitat*, and is a gradual process. Some loggerhead turtles continue to use both habitat types (oceanic and neritic) even in the adult stage [186, 147]. The average duration of the oceanic juvenile stage was estimated as 8.2 [13] or 14 [215] years, and the average duration of the neritic juvenile stage as 10 [215], 11 [173], 16 [14] or 17.4-20 [25] years.

Within the adult period, a more detailed classification can be made with regards to the type of habitat (oceanic, neritic) the adults are using, and to the exact phase of the nesting season (feeding, migrating, mating) [23]. Loggerhead turtles are considered to reach adulthood (become sexually mature) in the North Atlantic at lengths between 75 and 90 cm SCL [25, 209, 173, 204]. The average age at maturity was estimated to be between 15 and 29 years [173], with estimates of mean age at maturity as late as 45 years [209], and observations of maturity as early as 10 years of age [210]. Nesting occurs every 2-3 years (the period between nesting seasons is called the *remigration interval*), with a reproduction output of 4-5 clutches per nesting season, and 110-140 eggs per clutch [237, 232, 26, 204].

Because the exact moment when loggerheads turtles are sexually mature is hard to observe, in most cases the simplification is made that the event of first nesting denotes that sexual maturity has been reached. The most accurate way to determine age at first nesting would be captive rearing and direct observation of sea turtles. However, the obtained data cannot be directly applied to the wild populations, because sea turtles reared in captive or semi-natural conditions grow much faster and reach maturity at much younger ages, compared to those in the wild [16, 148]. For individuals that have been encountered in the wild nesting for the first time, even the simplification of age at nesting being equal to age at maturity does not remove the uncertainty of age at maturity, because (i) they could have been nesting previously without being encountered [237], and (ii) their age is unknown unless they have been released and tagged from a head-starting program and have not lost their tags while roaming the oceans [13]. Indirect methods of obtaining the age at maturity include assuming certain size at maturity (mostly taken as the smallest nesting female in the area), and estimating how long it would take for sea turtles to reach that size. Estimation methods utilize growth models and skeletochronology (determining age based on the growth marks on the bone), capture-mark-recapture methods, or length-frequency analysis [214, 209, 13, 25]. Part of the variation in estimations can thus be explained by the difference in methodologies (different data collection and/or data analysis). Significant sources of variation are also inter-individual differences present even among the individuals of the same population [175, 16, 223, 25], and differences in environmental conditions the individuals are experiencing, primarily with respect to temperature and food availability [25, 16].

Besides estimating age at maturity, the mentioned methods are used for calculating growth rates and estimating the duration of specific life stages [13, 215, 25, 264]. Growth rates have been shown to vary with respect to the geographical region [148, 25, 223], hatching season [223], and climate oscillations [41]. In addition to growth and maturation, the reproduction is also influenced by the local [144, 145] and global [92, 201, 203] climate oscillations, and the preferred habitat type [80].

Standard growth and reproduction models (e.g. [173, 13, 14, 88]) are constrained by the type of data they require (reliable growth data for growth models, and reproduction data for reproduction predictions), and often cannot account for the environmental factors such as food availability and/or temperature: e.g. the von Bertalanffy growth model assumes constant food and temperature, and therefore can be used for describing and predicting growth only under those (constant) conditions for which the data was obtained. The standard (static) models focus on the available data rather than the processes that connect the data, and therefore cannot be used for determining causal relationships. Process models (e.g., [97, 80, 92]), on the other hand, use different types of data as input and study the underlying processes. The processes of growth, maturation, and reproduction are all influenced by the available energy the organism can acquire and then allocate.

The Dynamic Energy Budget (DEB) theory [218, 217, 162, 109] is one of the most complete and consistent universal ecological theories, and it was therefore chosen as the best suited framework for an energy budget model. We decided to focus on the North Atlantic population of loggerhead turtles because it has one of the largest nesting aggregations of loggerhead turtles [228].

The aim of this chapter is to: (i) give an introduction to DEB theory, and a formal DEB model description; (ii) discuss the collected and evaluated available data for the North Atlantic loggerhead turtle population; (iii) show which data was used to estimate the parameter values for a DEB model of a North Atlantic loggerhead turtle; (iv) present and discuss how well the model can describe the data ; (v) discuss the implications of the obtained results.

3.2 Methods

3.2.1 The DEB model

The basic assumption made for this research was that the loggerhead turtle follows the energetic principles defined by the laws of physics, thermodynamics and biochemistry applied in the standard Dynamic Energy Budget (DEB) theory, and that the turtle can therefore be described well by a standard DEB model. In the (abstract) world of the DEB theory, any organism can be represented with three state variables (compartments): structure, reserve, and maturity (Figure 3.1, Table 3.1). *Structure, V*, is defined as the part of the body that requires (somatic) maintenance and has constant chemical composition. It can be quantified in terms of energy of mass, but DEB theory frequently quantifies it as volume (hence the symbol V). *Reserve, E*, is defines as the part of the body that does not require (somatic) maintenance. It also has a constant chemical composition, receives input in the form of assimilated energy, and is mobilized for metabolic purposes. It can be quantified as volume or mass, but DEB theory frequently quantifies it as energy (hence the symbol E). It serves as a buffer between the environment (with fluctuating food availability), and the organism (with constant energy needs). Energy flows in the body depend on the values of its state variables: the amounts of structure and reserve, and the level of maturity. The amount of reserve per volume of structure is called the energy density, $[E]$, and is a good indicator of the individual's condition because better fed individuals will have a higher reserve density. The structure and the reserve are abstract variables, but can be linked to the "real" (measurable) world via length or weight. Length of a turtle, e.g. straight carapace length (SCL), L_{SCL}, can be converted to the structural length, L, by the shape coefficient, δ_{SCL} , and then cubed to get the volume of structure:

$$L = L_{\text{SCL}} \cdot \delta_{\text{SCL}} = V^{1/3}. \tag{3.1}$$

Weight has contributions from both reserve and structure, which are mostly assumed to have the same specific density ($d_V = d_E$). Food availability is typically quantified by the scaled functional response, f, which is the feeding rate as a fraction of the maximum feeding rate of an individual. The scaled functional response can also be written as

$f = X/(X+K)$, where X is the food abundance, and K the half-saturation coefficient. This quantifier (f) only compares amounts, not quality. Variations in quality can cause f to be larger than 1, if a particular food quality is taken as a reference. When f and the contribution of reserve to weight, ω, are known, weight can be calculated as:

$$W = L^3(1 + f\omega). \tag{3.2}$$

The weight of adult (female) loggerhead turtles will also have a contribution from the reproduction buffer [94]. Dynamics of the reproduction buffer were not explicitly modeled at this stage, but several (reproduction) buffer handling rules had been specified by DEB theory ([109], see also R1 in Table 3.1) and can be included.

The third state variable, *maturity*, E_H, has no physical volume, mass, or energy: its formal status is information, with an increase in maturity translating into an increase in complexity. Maturation requires maturity maintenance (proportional to the level of maturity), and maturation no longer increases after puberty. Maturity is tracked by energy that is cumulatively invested into the process of maturation (increase in maturity), or reproduction after the maximum maturity level - at puberty - is reached. The flow that was previously used for maturation is then used to build up the reproductive buffer. When certain levels of maturity (thresholds) are achieved, the organism transitions from one developmental stage to the other: a switch from an embryo (does not feed) to juvenile (feeds, but does not reproduce) is considered as ’birth’ at E_H^b, and from a juvenile (does not reproduce) to an adult (reproduces) as ’puberty’ at E_H^p.

Consequently, the life cycle of the loggerhead turtle can be described by following the three state variables (structure, reserve, and maturity) which together give information about the size (length and weight), and the life stage (embryo, juvenile, adult) of the individuals. There are certain relations between the state variables, that always hold true: at the start of development the energy density, $[E]$, is infinitesimally large because the amount of structure is approximately zero. During the development the amount of reserve (E) decreases while the amount of structure (L^3) increases, resulting in an energy density at birth ($[E]_b$) equal to that of the mother at the moment of laying the egg ($[E]_b = [E]_{mother}$). This represents the “maternal effect” [111]. Also, the level of maturity (E_H), i.e. the energy invested into the process of maturation is taken as zero at the start of development ($E_H^0 = 0$), and can only increase ($E_H^0 < E_H^b < E_H^p$) .

The changes in state variables result from the underlying processes. The standard DEB model describes the processes of acquisition and use of energy by following specific energy fluxes: assimilation, p_A, mobilization, p_C, maintenance, p_S and p_J, growth, p_G, and maturation or reproduction, p_R (Table 3.1).

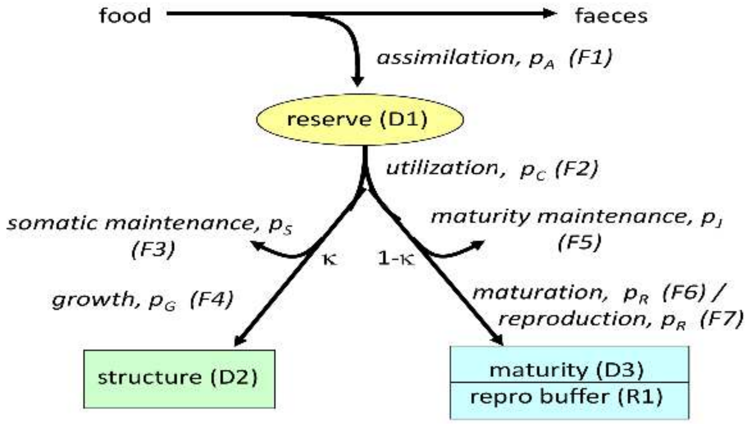

Figure 3.1: The standard DEB model, presentation modified from Kooijman [109]. Marks (D1-D3, F1-F7, R1) correspond to the descriptions in Table 3.1.

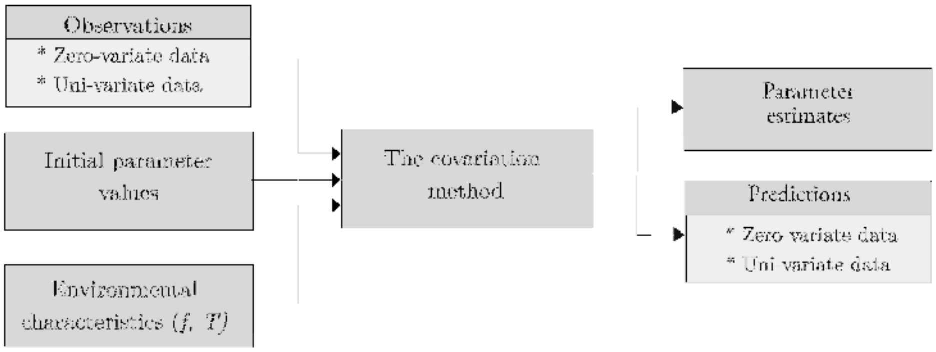

Figure 3.2: A scheme of the estimation process: the covariation method uses all available data (zero- and uni-variate), and environmental characteristics (scaled food availability, f, and temperature, T) to iteratively estimate parameter values, with initial parameter values used as the starting point. In the covariation method, the Nelder-Mead method was used to set and test different parameter values. Next, the predictions obtained with different parameter values for zero- and uni-variate data were evaluated using the weighted least squares criterion. This process was repeated until the set of parameter values which produced the statistically preferred zero- and uni-variate predictions was identified. The output of the covariation method is the final set of parameter estimates and zero- and uni-variate predictions.

The dynamics of the processes are quantified by a set of differential equations with parameters that can be grouped into three main categories: core parameters, auxiliary parameters, and other parameters (Table 3.2). The *core DEB parameters* relate directly to processes controlling state variable dynamics, including the effects of temperature and food availability, and are directly linked to the underlying assumptions of DEB theory. The *auxiliary parameters* are part of the auxiliary theory that links the abstract world of DEB state variables to quantities that can be measured directly, such as length, weight, egg output etc [126]. *Other parameters* include environmental characteristics, such as temperature, or food availability.

The covariation method [126] was used to determine (estimate) the parameter values from data (Figure 3.2). The method simultaneously uses all available information passed to the estimation routines, making the process and the end result independent of any particular sequence in estimating parameter values [126]. Additionally, the quality of the data sets was taken into account by assigning different weight to a specific data point (or data set), with higher quality data having more weight during parameter estimation. For the estimation procedure the `nmregr` function of the DEBtool package ([112], Matlab version 7.3.0) was used, using Nelder-Mead simplex method to find the estimates from initial guesses, and the minimum weighted sum of squared deviations of predictions compared to data as the estimation criterion. DEBtool routines for the covariation method had three types of data as input: zero-variate data, uni-variate data, and pseudo-data. Each type of data and their corresponding values are described in the Section 3.2.2. All data organization and parameter estimation was done using the add_my_pet scripts available on 05/11/2015 [110], implemented in Matlab R2011b.

To obtain model predictions such as growth curves and reproduction output, changes of DEB state variables (structure, reserve, and maturity) with time needed to be computed by solving the relevant ordinary differential equation (ODE) of the model (D1-D3, Figure 3.1, Table 3.1) throughout the specified time span. Matlab function (`ode45`) was used to solve the ODEs for the change in the structural length, and in the reserve and the maturity scaled with the surface area specific assimilation rate, $\{p_{Am}\}$. The specific growth rate was calculated using the scaled energy density, $e = vE/(\{p_{Am}\}L^3)$, and energy investment ratio, $g = [E_G]/(\kappa[E_m])$, as:

$$r = v(e/L - 1/L_m)/(e + g). \tag{3.3}$$

The reproduction output was calculated by the Matlab function `reprod_rate.m` implemented in the DEBtool package [112], where the reproduction output is the function of length, food availability, temperature, maturity levels at birth and puberty, and a set of parameters (κ, κ_R, g, k_J, k_M, and v). For the description of parameters see Table 3.2.

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Table 3.1: The standard DEB model: state variables with corresponding dynamic equations (D1-D3), auxiliary state variables for reproduction, with corresponding equations (R1-R2), and processes with corresponding energy fluxes (F1-F7). Equations and descriptions adapted from Refs. [97, 109]

State variable	Eq.	Dynamic equation	Description
Reserve, E (J)	D1	$\frac{dE}{dt} = p_A - p_C$	Physical part of the organism that quantifies metabolic memory, i.e. serves as an energy buffer between the environment and the organism. Does not require maintenance, and can be readily mobilised for processes.
Structural length, $L = V^{1/3}$(cm)	D2	$\frac{dL}{dt} = \frac{1}{3L^2} \frac{p_G}{[E_G]} = \frac{rL}{3}$	Physical part of the organism that requires energy for maintenance; with $r = \frac{Ev/L - p_S/\kappa}{E + E_G/\kappa}$.
Maturity, E_H (J)	D3	$\frac{dE_H}{dt} = \frac{dE_R}{dt} = p_R$	Information (no physical volume) that requires maintenance, and controls metabolic switching. Maturity increases while $E_H < E_H^p$, with energy allocated to reproduction otherwise.
*Reproduction buffer, E_R (J)	R1	$E_R = \int p_R(E_H \geq E_H^p) dt$	Energy in the reproduction buffer between reproduction events. Before puberty is reached, $E_R = 0$.

Process	Eq.	Energy flux	Description
Assimilation	F1	$p_A = \kappa_X p_X =$ $= \{p_{Am}\} f L^2$	Fraction of the ingestion flux that gets fixed into the reserve, with the food availability given as $f = \frac{X}{K_X + X}$. It is related to the surface are of the structure via a compound parameter $\{p_{Am}\} = z[p_M]/\kappa$, and therefore depends on the size of the organism. For parameter descriptions see next table.
Utilization (Mobilization)	F2	$p_C = E(v/L - r)$	The utilization of reserve follows from the homeostasis assumption. The mobilized reserve is divided according to the κ-rule: a fixed fraction is allocated to the processes of growth and somatic maintenance, the rest to development, maturity maintenance, and reproduction.
Somatic maintenance	F3	$p_S = p_M + p_T$	Energy flux to basic metabolic processes that keeps the organism alive. We differentiate between the structural-volume related metabolic costs (p_M), such as costs of protein turnover, and surface area related metabolic costs (p_T), such as costs of heating for endotherms. For ectotherms such as sea turtles, $p_T = 0$ and $p_S = p_M = [p_M]L^3$.
Growth	F4	$p_G = \kappa p_C - p_S$	Increase of structure (change in size), without the increase in complexity (see Maturation). It includes the costs of converting the energy reserve into structure $[E_G]$, because the chemical composition of two compartements is different.
Maturity maintenance	F5	$p_J = k_J E_H$	Maintenance of complexity of structure (see Maturation).
Maturation	F6	$p_R = (1-\kappa) p_C - p_J$	Increase of complexity of structure, as a preparation for the adult stage. At certain levels of maturity the organism undergoes metabolic switches. See text for details.
Reproduction	F7	$p_R = (1-\kappa) p_C - k_J E_H^p$	Conversion of mother's energy reserve into the energy reserve of an egg. The reproduction flux is a a continuation of the maturation flux (hence the same notation), where E_H in the maturity maintenance flux is now replaced with constant E_H^p.
*Reproduction (eggs/time)	R2	$R = \kappa_R p_R / E_0$	Reproduction output, where E_0 is the cost of (or initial energy in) an egg.

Table 3.2: The list of standard DEB model primary parameters, with symbols, units, processes they control, and summarized descriptions. Notation: symbols marked with square brackets, [ ], indicate that the parameter relates to structural volume (volume specific parameter), and symbols marked with curly brackets, , indicate that the parameter relates to structural surface area (surface area specific parameter). More details are available in Lika et al. [126], and the online DEB notation document `www.bio.vu.nl/thb/deb/deblab/`.

Core parameters	Symbol	Unit	Description	Process
Maximum searching rate	$\{F_m\}$	l/d.cm^2	Controls food intake if food is not abundant and has no effect at abundant food.	Feeding
Digestion efficiency (of food to reserve)	κ_X	-	Specifies the fraction of energy in food that is fixed in reserve.	Digestion
Defaecation efficiency (of food to faeces)	κ_X^P	-	Specifies the fraction of energy in food that ends up as faeces.	Product formation
Maximum specific assimilation rate	$\{p_{Am}\}$	J/d.cm^2	Not directly estimated, but calculated using the parameter z - the zoom factor that controls the maximum length via the specific assimilation: $\{p_{Am}\} = z[p_M]/\kappa$	Assimilation
Energy conductance	v	cm/d	Controls the reserve mobilization.	Mobilisation
Allocation fraction to soma	κ	-	Controls the allocation of mobilised reserve to somatic maintenance and growth as opposed to maturity maintenace and maturation or reproduction.	Allocation
Reproduction efficiency	κ_R	-	The fraction of reserve allocated to reproduction that is fixed in the reserve of offspring.	Reproduction
Volume-specific somatic maintenance	$[p_M]$	J/d.cm^3	Controls the sink of reserve linked to structural volume, mostly due to turnover of structure and behavior.	Maintenance
Surface-specific somatic maintenance	$\{p_T\}$	J/d.cm^2	Controls the sink of reserve linked to structural surface area.	Maintenance
Maturity maintenance rate coefficient	k_J	1/d	Controls the sink of reserve linked to maturity.	Development
Specific cost for structure	$[E_G]$	J/cm^3	The reserve energy that is required to synthetise a unit volume of structure.	Growth
Maturity at birth	E_H^b	J	Controls the timing of and the size at birth, i.e. the moment assimilation is switched on.	Life cycle transitions
Maturity at puberty	E_H^p	J	Controls the timing of and the size at puberty, i.e. the moment at which investment into maturation is redirected to reproduction.	Life cycle transitions
Weibull aging acceleration	h_a	1/d^2	Controls the mean life span in a way that hardly depends on food density.	Ageing
Gompertz stress coefficient	s_G	-	Controls the mean life span, but in ways that depend on food density.	Ageing

Table 3.3: The list of standard DEB model auxiliary parameters, with symbols, units, and summarized descriptions. More details are available in Lika et al. [126].

Auxiliary parameters	Symbol	Unit	Description
Reference temperature	T_{ref}	K	The temperature for which the rates and times are given; $T_{ref} = 293K$
Arrhenius temperature	T_A	K	Controls the effect of temperature on rates.
Shape coefficient	δ_M, δ_{SCL}, δ_{CCL}	-	Convertes physical to volumetric structural length. The general notation (δ_M) has been replaced with a more specific one (δ_{SCL} or δ_{CCL}) that contains information on the type of measurement (straight or curved carapace length)
Specific densities	d_V, d_E, d_X, d_P	g/cm^3	Convert volume to mass for each organic compound (structure V, reserve E, food X, faeces P).
Chemical potentials	μ_V, μ_E, μ_X, μ_P	J/mol	Convert moles to energy for organic compounds V, E, X, and P.
Chemical indices	η_V, η_E, η_X, η_P	#/C	Relate the frequency of chemical elements (C, H, O, and N) to C for organic compounds V, E, X, and P.
Molecular weights	w_V, w_E, w_X, w_P	g/mol	The molecular weight of each compound is obtained by multiplying the chemical indices with the atomic mass of each element (C=12g/mol,H=1 g/mol, O=16 g/mol, N=14 g/mol).

After estimating the parameter values and obtaining the model predictions, the differences between the data and the model predictions were calculated. The relative error, *RE*, was calculated by dividing the absolute value of the difference between the value of the data point, *data*, and the value estimated by the model, *prdData*, by the value of the data point: *RE=|data-prdData|/data*. For data sets with more than one data point (univariate data), the relative error was calculated as the sum of relative errors of all data points in a data set, divided by the number of datapoints in the data set. The mean relative error of all data points and datasets (*MRE*) was then used to compute the FIT value as $10 \times (1 - MRE)$, and compare the goodness of fit to other DEB models in the add_my_pet library [110]. The possible FIT values range from $-\infty$ to 10 [127].

3.2.2 Data used

3.2.2.1 Zero-variate data

Zero-variate data are single data points (numbers) for various physiological observations such as age at birth, weight at puberty, maximum reproduction rate, etc. [126], and are presented in Table 3.5. Data that are connected to rates, such as age at birth or puberty, or reproduction at a certain size, are coupled with the corresponding temperature. The temperature was assumed to be 20° C before puberty, and 21.8° C after puberty based on the average value and suggested temperature range for loggerhead turtles [85]. Model calculations were corrected for temperature to account for the effect of temperature [109]. All data refer to the western North Atlantic population of loggerhead turtles, and are given for the average relative food abundance in the North Atlantic (f_{NA}, see description of **maximum length** below).

Hatching, emergence, and birth (start of feeding) Age at hatching (a_h, leaving the egg), emergence (a_e, leaving the nest), and birth (a_b, starting to feed) are connected, and often defined by the environment rather than internal (maturity) thresholds. For example, hatching takes up to 24 hours [8], and the time from hatching to emergence (reaching the surface of the nest) depends on the temperature, depth of the nest, compactness of the sand above the nest etc., and on average lasts 4.1 days [70], a value used here as well for incubation at T=30ºC. The yolk bag is absorbed and hatchlings start feeding 24-48 hours after emerging and swimming frenzy [115], or within 3 days if held at 27ºC (Stokes, pers. comm.). The age at emergence was calculated as the average of incubation durations from Ref. [223]: $a_e = 55.4$ days, T=30ºC. Age at hatching was then calculated as $a_h = (a_e - 4.1)$ days, T=30ºC and age at birth as $a_b = (a_e + 2)$ days, T =27ºC.

Physical length at hatching (L^h_{SCL}), emergence (L^e_{SCL}), and birth (L^b_{SCL}) were taken as 4.5 cm SCL (straight carapace length), the average value of length at emergence calculated from Refs. [223, 90, 175]. Length does not significantly change from hatching to emergence [8], and was assumed to remain constant until the onset of feeding (birth). The assumption was justified because SCL was considered a proxy for structure (in DEB terms), and no significant changes in size of structure occurred in that period, as indicated by no significant changes in dry mass of the yolk-free hatchling from hatching to 96 hours post emergence [115]. As an upper limit of the range for length at birth, we can tentatively use the mean of the first measurements taken four days after the onset of feeding, which is 5.06 cm SCL (SD=0.3437) [223].

Wet weight at birth (W^b_w) and emergence (W^e_w) were assumed the same and calculated as 19.41 g (mean from values reported in Refs[223, 185]). Wet weight at hatching, W^h_w, was calculated as $W^h_w = 1.1 W^b_w = 21.35$ g, to account for the approximately 10% of wet mass loss between hatching and emergence reported by some authors [8]. The calculated W^h_w is in accordance with the mass of hatchlings and yolk at hatching reported in Ref. [1]. Wet weight at birth (precise moment when the feeding was observed) was usually not reported, but as the upper limit the first measurements taken a few days after the onset of feeding (in total 7 days after emergence) can be used: 25.28 g (SD=4.1895) [223]. The water content of the tissue was assumed to be around 72% [115] and constant.

Puberty. Puberty (the moment when an average female sea turtle becomes physiologically capable of vitallogenesis and consequently oviposition) was indirectly assumed equivalent to the first nesting (first oviposition). As the age of wild nesting females is generally not known, an estimated value (28 years, [219]) for age at puberty (a_p) was used. Length and weight at puberty were calculated as the means of low end values of the size ranges reported for nesting females in Refs. [28, 54, 224, 165]: the value of 80 cm SCL was used as physical length at puberty (L^p_{SCL}), and the value of 79 kg as weight at puberty (W^p_w). The puberty data was assigned small weight because of large uncertainty and variability of reported values, and are discussed in more detail in the section 3.4.

Maximum lifespan and ultimate size. Maximum lifespan (a_m) was assumed to be 65 years based on the information about a nesting female that is at least 60 years old [78], and a record of a wild individual living 38 years after reaching sexual maturity [215]. Since the estimated age to maturity for animals in the wild is 16-35 years (see also the Section 3.4), even wild individuals could live to at least 50 or even 70 years of age.

The ultimate length (L^i_{SCL}) is the length most individuals reach by the end of their life cycle under given conditions. A value of 105.89 cm SCL was used, calculated as the mean of the largest nesting females reported in Refs. [28, 54, 224, 165].

The maximum length (L^m_{SCL}) denotes the biologically determined length that individuals can reach under ideal conditions, i.e. when $f = 1$. It is a species-specific biological trait that does not depend on the environment. A value of 130 cm SCL was used, reported as the largest nesting female in South Carolina [65]. The ratio of the ultimate and the maximum length can serve as a proxy for the relative food abundance in the environment where the ultimate length is reached, so $f_{\text{NA}} = L_m/L_i = 0.814$ was calculated.

The ultimate weight (W^i_w) of 162.62 kg was used, calculated as the mean of largest values reported in Refs. [54, 165]. Reported range for North Atlantic nesting females is 75-150 kg [165].

Reproduction. The maximum reproduction rate (R_i) was expressed as eggs per day (the standard DEB model assumes a continuous reproduction) using the number of eggs per clutch (assumed to be 140 on average, [232, 204]), the number of clutches per nesting season [237, 81], and the number of nesting seasons per year (an inverse of the remigration interval, [81]): two combinations (4 clutches every 2 years, and 5 clutches every 2.5 years) yielded the same value of the maximum reproduction rate. The maximum reproduction rate was then calculated as $R_i = 4 \times 140/(2.5 \times 365) = 0.7671$.

Initial energy content of an egg (E_0) was assumed to be 210 kJ based on the assumption that the volume-specific energy content of the loggerhead eggs is the same as that of the green turtle eggs [88]. The eggs of North Atlantic loggerhead turtles on average have a diameter of 42.53 mm [232], so the energy content should be between 165 kJ calculated for the 38.2 mm diameter loggerhead turtle eggs [88], and 259.7 kJ measured for the 44.4 mm diameter green turtle eggs [10]. The green turtle eggs could have more yolk compared to the loggerhead eggs of the similar size [1], so a conservative value of 210 kJ was used. This data point was given high weight for the covariation method of the parameter estimation because energy was measured directly, and obtaining the correct order of magnitude for E_0 greatly improved the realism of the prediction for the maximum reproduction rate (R_i).

3.2.2.2 Uni-variate data

Observations that consist of a list of one or more pairs of numbers, where one member of each pair represents an independent variable (e.g. time) and the other a dependent variable (such as length or weight) are referred to as uni-variate data [126]. Several types of data-pairs were used, with each type of data contributing a different type of information for the parameter estimation [126]. Each data set is decried, and the number of data pairs are indicated.

- Age at emergence vs incubation temperature (Ta_e): one data set, N=61. Temperature was recorded during the incubation in natural nests,and was reported together with the incubation duration (which is equivalent to the age at emergence) [223] .

- Posthatchling length vs time (tL_{Stok}): three data sets with average values of length up to 10 weeks of age, and three data sets with average values of length up to 8 weeks of age ($N = 3 \times 10 + 3 \times 8$). Hatchlings were captive reared for 8 to 10 weeks at 27±2°C. Food was provided daily as 20% of body mass during the first two weeks, and as 8% of body mass for the remainder of the experiment ; Experiment setup and explanation of data sets were published in Stokes et al. [223], and data sets were obtained directly from L. Stokes. For the purpose of parameter estimation, temperature and food were assumed constant with T=27°C, and scaled functional response $f = 0.99$.

- Posthatchling weight vs time (tW_{Stok}): three data sets with average values of wet weight up to 10 weeks of age, and three data sets with average values of wet weight up to 8 weeks of age ($N = 3 \times 10 + 3 \times 8$). Wet weight data was collected simultaneously with length data, so same temperature and food conditions were assumed for the parameter estimation.

- Posthatchling weight vs length (LW_{Stok}): three data sets with 10 pairs of length-wet weight measurements, and three data sets with 8 pairs of length-wet weight measurements ($N = 3 \times 10 + 3 \times 8$). The fact that the weight and length measurements were taken simultaneously allowed for the construction of weight - length data pairs, using the average weight and the average length from the two previously described data sets. Temperature does not play a role in this data set, and $f = 0.99$ was used as the scaled food availability.

- Juvenile length vs time (tL_{Park1926} and $tL_{\text{HildHats1927}}$): two data sets, one with two length at age measurements (from Ref. [174]) and the other with three length at age measurements (from Ref. [90]), (N=2+3). The sets contain data on captive reared juveniles held in semi-natural conditions. Precise values for temperature and food availability were not reported, but were probably more optimal than in the wild. For the purpose of parameter estimation, temperature and food were assumed constant with T=23°C for one data set [174], and T=21°C for the other [90], while scaled food availability was assumed to be nearly *ad libitum* (f=0.99).

- Juvenile weight vs time (tW_{Park1926}, tW_{Park1929}, and $tW_{\text{HildHats1927}}$): six data sets, each with different number of wet weight at age measurements. Data for four individuals had been reported in [175], with data for one of those individuals previously partially reported in [174], and data for two individuals had been reported in [90] (N=5+6+6+5+2+2). Temperature was assumed as T=23°C for four data sets (from Refs. [174, 175]), and as T=21°C for two datasets (from Ref. [90]), while scaled food availability was again assumed to be nearly *ad libitum* (f=0.99).

- Juvenile mass vs juvenile length ($LW_{\text{WabnPaul2008}}$): one data set, N=369 (from Ref. [244]). The set contains data on the individuals encountered in the wild. The scaled food availability for the individuals in the wild had to be assumed already for the zero-variate data (f_{NA}=0.81), and the same scaled food availability was used for this data set. The temperature does not play a role in the weight to length relationship, so it was irrelevant for this data set. ,

- Eggs per clutch vs female length (LF): one data set, N=48, (from Ref. [232]). The set contains data from one season on females nesting in the wild. Because conditions are assumed identical for all individuals in the wild, temperature and food availability were assumed the same as for the maximum reproduction rate (T_{am}, f_{NA}).

To calculate the growth of posthatchlings and juveniles, ODEs were solved for the change in structure, scaled reserve, and scaled maturity, and then length and weight were calculated using equations 3.1 and 3.2 (see section 3.2.1 for details).

Age at emergence (Eae) was predicted by solving the ODEs for scaled age, scaled maturity and scaled length at emergence (function `dget_aul` integrated in the DEBtool package), using E_H^e as an additional maturity threshold, $E_H^0 < E_H^e < E_H^b$, and correcting for the effect of temperature. Fecundity at length (EF) was calculated using the daily reproduction rate (output of the Matlab function `reprod_rate.m` integrated in the DEBtool package) and then correcting for the length of the remigration interval, number of clutches per season, and average number of eggs per clutch.

3.2.2.3 Pseudo-data

The final result of the covariation method (i.e. the parameter estimates) does not depend on the initial values of the parameters. However, several initial parameter values (that serve as "prior knowledge" in the covariation procedure) do influence the parameter estimations. Therefore, they are conceptually treated as data, and are hence referred to as 'pseudo-data' [126]. Most often, the parameter set for a generalized animal [126, 109] is used, and all pseudo-data are given low weight, so they do not play a significant role in the parameter estimation if sufficient real (zero- or uni-variate) data are available [126, 127]. In addition to data for specific densities, chemical potentials, chemical indices, and molecular weights (values from [109]), values for the generalized animal were used for energy conductance ($v = 0.02\,\mathrm{cm/d}$), allocation fraction to soma ($\kappa = 0.8$), reproduction efficiency ($\kappa_R = 0.95$), volume-specific somatic maintenance ($[p_M] = 18\,\mathrm{J/d.cm^3}$), surface-specific somatic maintenance ($\{p_T\} = 0\,\mathrm{J/cm^2}$), maturity maintenance rate coefficient ($k_J = 0.002\,\mathrm{1/d}$), and growth efficiency ($\kappa_G = 0.8$) [126].

3.3 Results

The parameter set (presented in Table 3.4) was realistic when compared to the parameter values of other sea turtles in the "Add my pet" data library [110] , and the overall fit of the model was extremely good (mean relative error, MRE = 0.1776, producing a FIT value of 8.22). The mean relative error of the zero-variate data was 0.1956, with the best model estimate for energy content of an egg (relative error, $RE(E_0) = 0.0017$), and the worst model estimate for the wet weight at hatching ($RE(W_w^h) = 0.5773$) (Table 3.5). The mean relative error of the uni-variate data was 0.1689, with the best model predictions for the length at age for one dataset obtained from L. Stokes ($RE(tL_{\text{Stokes}}) = 0.0280$), and the worst model predictions for wet weight as the function of age for one of the individuals reported in Ref. [175] ($RE(tW_{\text{Park1929}}) = 0.4944$). The relative errors were similar between uni-variate and zero-variate data sets: two (14.29%) zero-variate data had a relative error larger than 0.3, whereas this was the case for five (17.24%) of uni-variate datasets. Uni-variate data contains many more data points, so a larger relative error was expected. Also, many uni-variate data sets were reported for individuals, which means that the fit could be improved by estimating parameters for each data set independently (DEB models are individual-based), but also that a single parameter set cannot reproduce the interindividual variability.

3.3.1 The model parameters

All parameter values were realistic, and are discussed further in section 3.4.1.

The Arrhenius temperature (T_A) was calculated as the slope of the relationship between the inverse incubation temperature (in Kelvin) and the natural logarithm of incubation duration (in days), using the published data on incubation and temperature for the North Atlantic, the Mediterranean, and the Australian population (Data sources: [223], [187], and [258], respectively). The latter two datasets, even though they were smaller, were reported for a controlled environment with constant incubation temperature, and data from the North Atlantic were obtained from incubation in natural nests with fluctuating temperature. The curve fitted on all datasets described the relationship extremely well, and suggested an Arrhenius temperature value of 7000 K (see Figure 3.3). The value is within the range of values reported for other reptiles in the "Add my pet" data library (6 000-10 000 K, mostly between 7 000 and 8 000 K), and similar to values for the other two sea turtle species (Table 3.4).

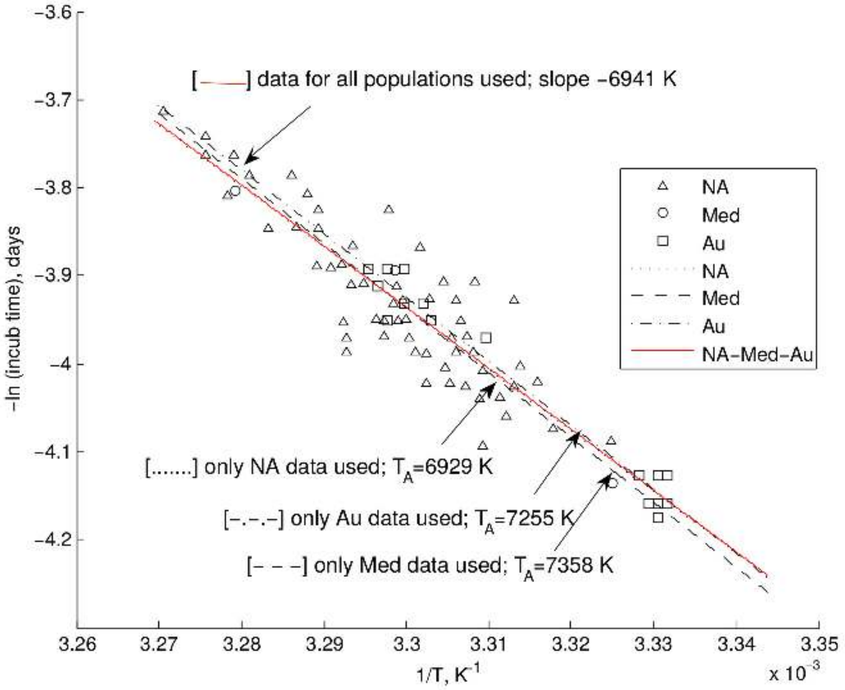

Figure 3.3: Data and corresponding relationships between inverse of the incubation temperature and the logarithm of incubation duration. The model slopes, i.e. the Arrhenius temperatures obtained this way are: North Atlantic (NA): 6929 K; Mediterranean (Med): 7358 K; Australian (Au): 7255 K. Fitting the relationship on all data yields a value of 6941 K (95% confidence intervals: 6298, 7584), $R^2 = 0.8555$, RMSE: 0.0399. When the slope was fixed at -7000, the goodness of fit did not deteriorate ($R^2 = 0.85554$, RMSE: 0.03965). Data sources: NA: [223]; Med: [187]; Au: [258].

The set of parameters that produced the best fit to zero-variate and uni-variate data is presented in Table 3.4. Some of the parameters (κ_R, κ_G, k_J, $\{p_T\}$, and chemical indices and densities) included as pseudo-data were not estimated with the covariation method, mostly because the information available was not sufficient to estimate them reliably, and also because when estimated, they show little variation across different taxa [127].

Table 3.4: List of primary and auxiliary parameters for the North Atlantic loggerhead turtle (*Caretta caretta*). Parameters that where estimated using the covariation method Lika et al. [126] are indicated by ’1’ in the third column. The additional shape parameter δ_{CL} was used for the data where the length measurement type had not been specified (in Refs. [174, 90]). Parameter values for two other sea turtles in the "Add my pet" library are given for comparison: Kemp’s ridley (*Lepidochelys kempii*) [179], and leatherback turtle (*Dermochelys coriacea*) [105]. Typical values for a generalized animal with maximum length $L_m = zL_m^{ref}$ (for a dimensionless zoom factor z and $L_m^{ref} = 1\,\mathrm{cm}$), were taken from Refs. [126, 109], Table 8.1, p300. All rates are given for the reference temperature K. For parameter descriptions see Tables 3.2 and 3.3.

Parameter	Est.	*C. caretta*	*L. kempii*	*D. coriacea*	Typical value	Unit
z	1	44.32	25.02	51.57	L_m/L_m^{ref}	-
$\{F_m\}$	0	6.5	6.5	6.5	6.5	l/d.cm^2
κ_X	0	0.8	0.8	0.206503	0.8	-
κ_X^P	0	0.1	0.1	0.2	0.1	-
v	1	0.07084	0.0424059	0.0865079	0.02	cm/d
κ	1	0.6481	0.692924	0.916651	0.8	-
κ_R	0	0.95	0.95	0.95	0.95	-
$[p_M]$	1	13.25	20.1739	21.178	18	J/d.cm^3
k_J	0	0.002	0.002	0.002	0.002	1/d
$[E_G]$	0	7847	7840.77	7843.18	$2800 d_V$	J/cm^3
E_H^h	1	3.809e+004	-	-	-	J
E_H^b	1	3.809e+004	1.324e+04	7.550e+03	$0.275\ z^3$	J
E_H^p	1	8.73e+007	3.6476e+07	8.2515e+07	$166\ z^3$	J
h_a	1	1.85e-010	1.42057e-09	1.93879e-09	$10^{-6}z$	1/d^2
s_G	0	0.0001	0.0001	0.0001	0.01	-
T_{ref}	0	293.15	293.15	293.15	293.15	K
T_A	0	7000[a]	8000	8000	8000	K
δ_{SCL}	1	0.3744	0.3629	0.3397	>0	-
δ_{CL}	1	0.3085				-
$d_V = d_E$	0	0.28[b]	0.3	0.3	0.3	-
$\{p_{Am}\}$	J/d.cm^2	0	906.1[b]	728.426	1191.41	$22.5\ z$

[a] Estimated independently by data fitting, see Figure 3.3 [b] Value from Kraemer and Bennett [115].
[c] Primary parameter not directly estimated; calculated as $\{p_{Am}\} = z[p_M]/\kappa$

The surface area maintenance ($\{p_T\}$) is mostly connected to heating costs, so for ectotherms like the loggerhead turtle it is assumed to be zero. The value from the generalized animal was used for the assimilation efficiency ($\kappa_X = 0.8$), because the assimilation efficiency of 0.8 has been reported for other reptiles [182], and should be fairly constant in nature [127].

3.3.2 Zero-variate data

The model predictions for data describing life history traits such as age and length at hatching, length at maturity etc, are presented in Table 3.5. The model predictions are realistic, but some differences exist when compared to data used as input (columns two and three of Table 3.5), especially regarding age at puberty. The model suggests that the loggerhead turtles start allocating energy to reproduction approximately a decade sooner than is currently thought, and probably several years prior than nesting is observed. The predictions fall within the range of observed values, and/or the discrepancies can be explained in ways consistent with the model. This will be discussed further in the Section 3.4.2.

The mean relative error of the zero-variate data was 0.1956.

Table 3.5: Comparison between observations and model predictions, at the temperature that had been used for the corresponding zero-variate data (for details see the Section 3.2.2.1), and the assumed scaled functional response $f = 0.81$.

Data	Predicted	Observed	Relative error	Observed, range	Unit	Reference
age at hatch	48.62	51.30	0.0522	45.8-55.8	d	[223, 70]
age at birth	52.51	57.40	0.0853	2-3 d after emergence	d	§
age at puberty	14.17	28.00	0.4939	19-30+	yr	[219, 25, 173]
life span	66.69	67.00	0.0046	65+	yr	[215, 78]
SCL at hatching	5.56	4.50	0.2360	3.9-5.01	cm	§,[185]
SCL at birth	5.56	4.50	0.2357	3.9-5.06	cm	§,[90, 175]
SCL at puberty	76.75	80.00	0.0406	76.8-84	cm	[28, 54, 224, 165, 232]
ultimate SCL	96.35	105.26	0.0846	98-110	cm	[28, 54, 224, 165, 232]
wet weight at hatching	9.02	21.35	0.5773	14-24	g	[1]
wet weight at birth	23.62	19.41	0.2171	14-24	g	[223, 185]
wet weight at puberty	62.08	79.00	0.2142	75-89.7	kg	[54, 165]
ultimate wet weight	122.82	162.62	0.2447	148.9-180.7	kg	[54, 165]
initial energy content of the egg	209.64	210.00	0.0017	165-260	kJ	[88]
maximum reprod rate	0.8556	0.7671	0.1153	0.3452-0.8630	eggs/c	[151, 204, 81]

§ unpubl.data obtained from L. Stokes

3.3.3 Uni-variate data

The model described the uni-variate data well (Figures 3.5 to 3.7), with mean relative error for all uni-variate datasets 0.1689, and mean relative errors of individual datasets from 0.0280 to 0.4944.

The fit of the model to data for the age at emergence with respect to incubation temperature (Figure 3.4) was relatively good, suggesting that the temperature can explain most of the variation in the incubation duration. The underprediction for the age at hatching (and birth) could imply additional metabolic processes or abiotic factors not accounted for by the model.

The fit of the model predictions to data for post-hatchling growth was satisfactory when the predicted length at birth was used as a starting point (Figure 3.5, full line in panels a and b). However, when the observed length at birth was used, the predicted growth curves were consistently lower than the data (gray dashed line, Figure 3.5, panels a and b). For the relationship of posthatchling wet weight to length (Figure 3.5, panel c), the mean relative error was 0.0829 , with the underpredicted weight up to approximately 6.5 cm SCL.

The model fitted very well to the weight-to-length data of juveniles and adults from the wild (Figure 3.6, panel c) (relative error of the dataset 0.2026), and reasonably well to growth data of captive reared juveniles (Figure 3.6, panels a,b) (relative errors of the datasets ranging from 0.0413 to 0.4944).

The reproduction to length relationship was described reasonably well by the model (relative error of 0.2106, Figure 3.7), but the trend of the model slope did not correspond to the trend evident from the data. The reproduction was underpredicted for smaller sizes, and overpredicted for larger sizes, suggesting a clutch size as small as 20 eggs for small lengths, and larger than 150 eggs for large lengths.

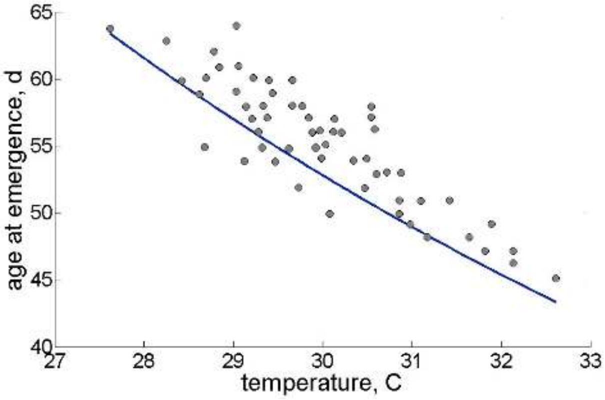

Figure 3.4: Model predictions for incubation duration as a function of incubation temperature. Data source: [223]

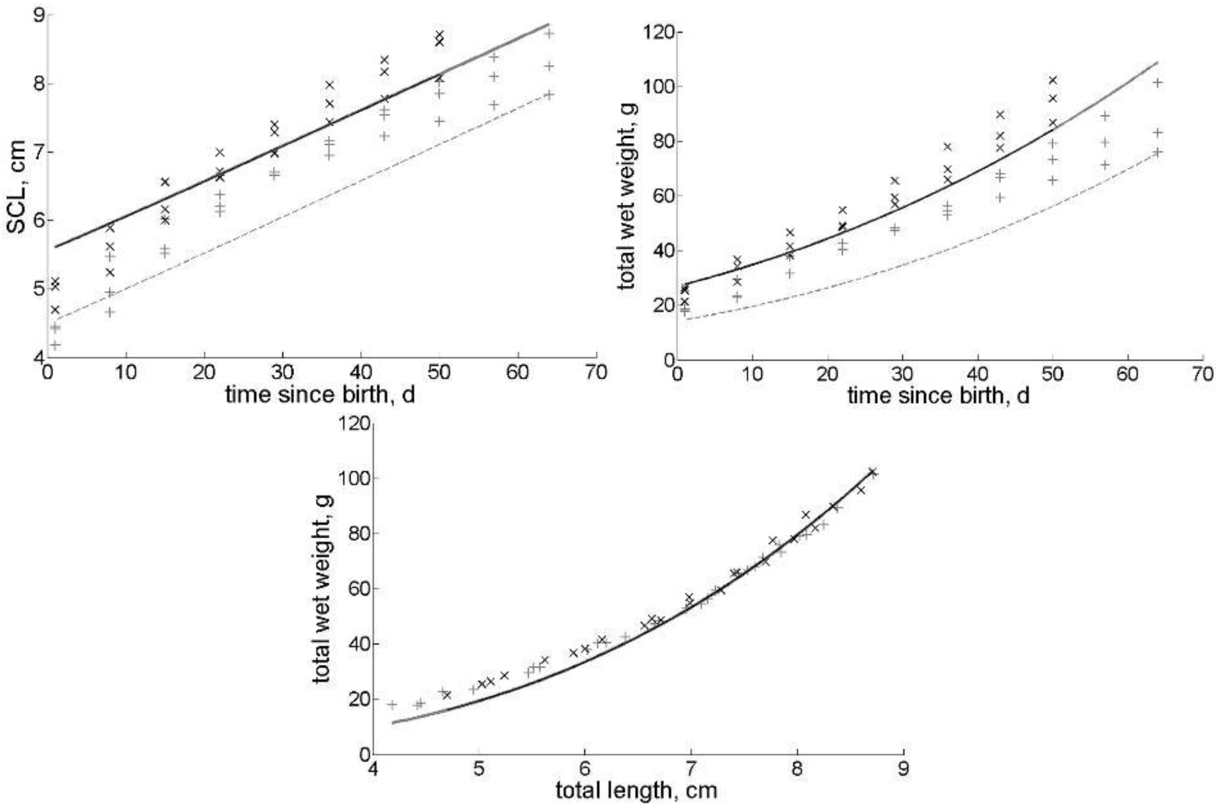

Figure 3.5: Model predictions for posthatchlings up to 10 weeks old. (a) carapace length in relation to age, (b) mass in relation to time, and (c) mass in relation to length. Data source: unpublished data obtained from L. Stokes. The gray dashed line in panels (a) and (b) are the model predictions when 4.5cm SCL instead of predicted L_b is used as the initial length for the model predictions.

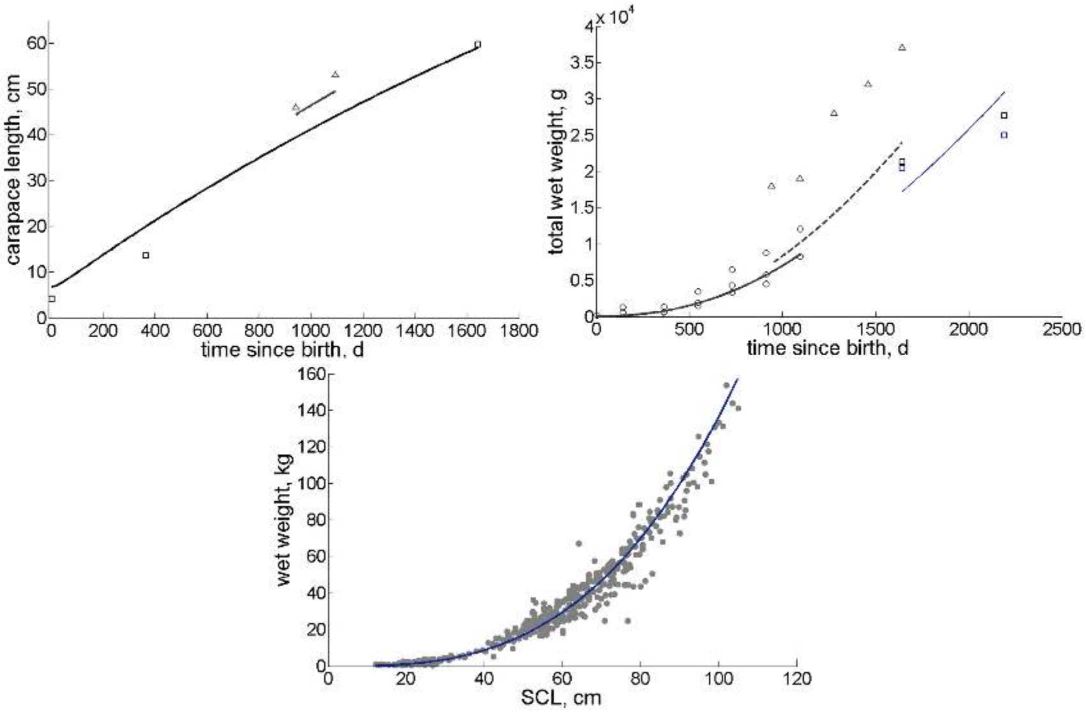

Figure 3.6: Model predictions for uni-variate data concerning juveniles and adults. (a) Carapace length in relation to age. Data from: Parker [174] (triangles), and Hildebrand and Hatsel [90] (squares). (b) Mass in relation to age. Data from: Parker [174] (triangles), Parker [175] (circles), and Hildebrand and Hatsel [90] (squares). (c) Mass in relation to straight carapace length (SCL). Data from Wabnitz and Pauly [244]. Data containing individual growth rates (panels a and b) show large variability within a relatively short time span, while data for the length to weight relationship show small variability over the whole size range (panel c).

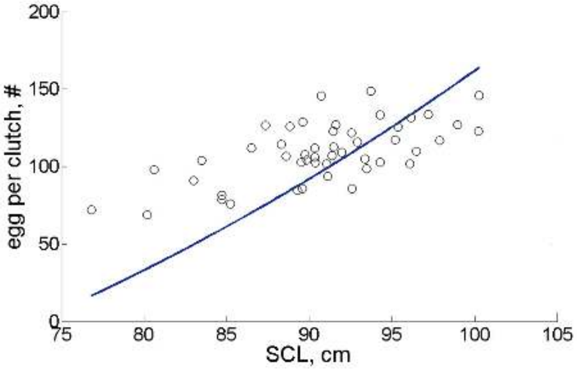

Figure 3.7: Model predictions for number of eggs per clutch as a function of the straight carapace length (SCL). The predictions are smaller than observed for small SCL, and larger than observed for large SCL, suggesting that there is a optimal clutch size. Data from Tiwari and Bjorndal [232].

3.4 Discussion

This study took into consideration the current knowledge about the biology of the loggerhead turtle, *Caretta caretta*, with a particular focus on the life-history traits, and the effects of environmental characteristics on the life cycle of the loggerhead turtle. The needed data were extracted from the available literature, some of it published as early as 1926 [174]. To reduce variance introduced by different measurement techniques, length data expressed as straight carapace length (SCL) were preferred. The measurement exhibits less variability than curved carapace length, and is therefore recommended [212]. Wet weight (W_w) was used for consistency, even though using dry weight would have been more accurate, and would have negated the effects of weight decrease due to dehydration [1] or weight increase due to drinking of sea water [8]. Even though some dry weight data exist for hatchlings (e.g. [115]), mostly wet weight is reported for loggerhead turtles. The completeness of data of 3 (on a scale of 1-10 presented in Table 1 in [126, 127]) is comparable to other entries in the add-my-pet library, where most entries having the completeness of 2.5-3. Goodness of fit is also satisfactory considering the variety of data sources and data types (mean relative error of 0.1776), especially if we take into account the fact that the predicted values for zero-variate data fall within the observed range of values (Table 3.5), and predictions for uni-variate data are biologically plausible (Figures 3.4 to 3.7). The score of the goodness of fit is also influenced by the choice of data, and possibly differently chosen data would yield a higher goodness of fit, but at the price of consistency. FIT value of 8.22 (on a scale from $-\infty$ to 10) is also a somewhat typical value and within the range of 8-8.5 expected for “Add my pet” entries [127]. In the next sections, I will discuss the parameter values, choice of data, and the model predictions in the context of observations.

3.4.1 The model parameters

The parameter values are realistic because they produce a good fit and fall within ranges of DEB parameters for other sea turtles listed in the "Add my pet" data library [110]. When compared to the values of parameters estimated for two other sea turtle species, Kemp’s ridley (*Lepidochelys kempii*, [179]) and the leatherback turtle (*Dermochelys coriacea*, [105]), values for the loggerhead turtle mostly fall in between. This especially makes sense for the parameters that are related to size (z, E_H^b, E_H^p, h_a, and the compound parameter $\{p_{Am}\}$), because the loggerhead turtle is larger than Kemp’s ridley, and smaller than the leatherback turtle [219]. The estimated value $[p_M]$ of around 13 J/d.cm^3 is smaller than that estimated for the other two sea turtle species (around 20 and 21 J/d.cm^3), and that used for the generalized animal (18 J/d.cm^3), but this difference is small compared to the range of values found in the "Add my pet" library.

The maturity parameters (E_H^b, E_H^p) are specific to DEB theory. They allow that age and size at birth and puberty are food-dependent, but the maturation levels are not. However, there are not many comparable parameter values in the literature available for sea turtles. The value for E_H^b can be inferred indirectly, as it in fact represents the amount of energy (estimated as 37 kJ) that has been invested into maturation before birth. The total energy of the hatchling and the yolk sac was calculated to be around 140 kJ at hatching, and 125 kJ at birth (using values in [115]). The total energy available at the beginning of the embryonic development (i.e. energy of an egg) was assumed to be around 210 kJ (between 165 and 260 kJ, [88]), suggesting that somewhere between 70 and 85 kJ are used during the embryonic development for costs of maturation, maintenance, overheads of growth etc, an approximation consistent with the measured respirometry value (62 kJ, [187]). A proportion of around 43% was used for maturation, while the rest was distributed between maintenance and growth overheads seems realistic (see also Figure 3.8). Because both maturity parameters scale with size in the same way, one can assume that maturity at puberty is predicted well, too.

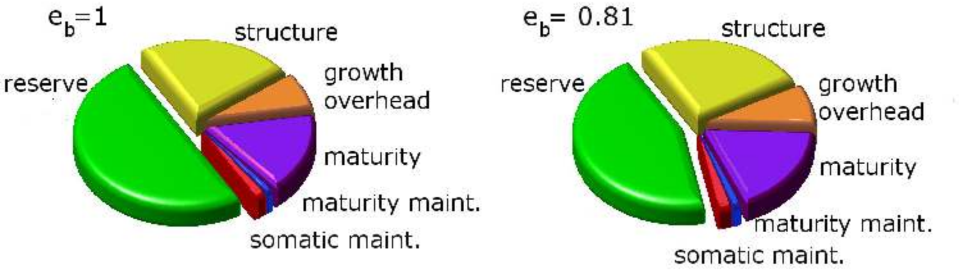

Figure 3.8: Cumulative energy investment observed at the moment of birth, plotted for two food availabilities resulting in different scaled functional responses ($e_b = f = 1$, and $e_b = f = 0.81$), the second one being the food availability assumed for the North Atlantic. In the environment with high food availability ($f = 1$), the hatchling still has approximately half of his reserves available at the moment of birth. When the food availability is assumed lower, more than half of the reserves have been used for the processes of growth, maturation, and other processes. This has important implications for, e.g. predicting the period a hatchling can survive before it reaches the feeding grounds.

Obtaining the parameter values allowed us to predict many features of the energetics quantitatively, and study the implied properties which were never measured directly. As an example, I plotted the cumulative energy investment during the embryonic development, i.e. the cumulative energy invested into growth and maturation, or dissipated as maintenance and overheads of growth, when observed at the moment of birth (Figure 3.8). Another example is a visualization for the energy allocation at birth, puberty, and ultimate size (fully-grown adult), see Figure 3.9. A visualization of the fluxes at three life stages provides insight into the changes in allocation throughout the ontogeny of the loggerhead turtle, and provides a powerful tool for exploring additional properties. For example, most of the energy budget at birth is allocated towards the processes of growth and maturation, whereas the processes of (somatic and maturity) maintenance add up to be over three quarters of the daily energy budget of the fully grown individual. The maturity maintenance, an energy flux allocated towards maintaining (among other things) the immune system [109], is an energy sink for almost one quarter of the mobilized energy of a fully grown adult (Figure 3.9). The process of maintaining maturity is therefore an important part of the whole energy budget, yet it is rarely discussed outside of DEB literature.

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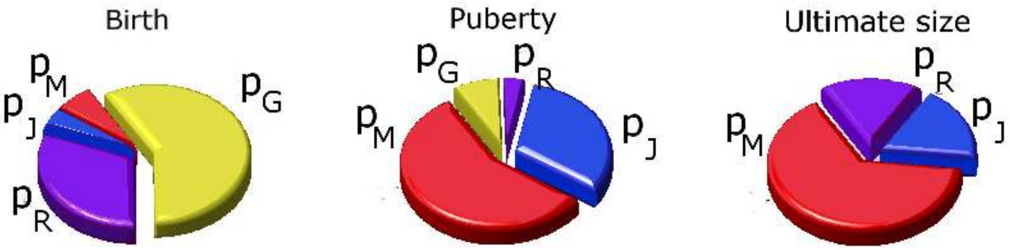

Figure 3.9: A visualization of the energy budget at birth, puberty, and ultimate size: p_G - growth flux, p_R - maturation/reproduction flux, p_M - somatic maintenance, and p_J - maturity maintenance (marked with F4, F6/F7, F3 and F5, respectively, in Table 3.1), as fractions of the mobilization flux (marked with F2 in Table 3.1). Fluxes are calculated using the estimated parameter values for the individuals of the North Atlantic (see Table 3.4) at the scaled functional response of $f = 0.81$.

3.4.2 Zero-variate data

Hatching, emergence, and birth. Age at hatching, emergence, and birth were modeled as a function of temperature and food availability experienced by the mother, and were underpredicted by the model by 3 to 5 days (5-9%). Factors that were found to influence the duration and success of the incubation include the grain size, humidity, and compactness of the sand, salinity of the water around the nest, the number of eggs in the clutch, and gas exchange of the eggs [70, 151, 176, 257, 1]. Values predicted by the model fall within the observed range for all traits except for wet weight at hatching, which is considerably underpredicted (Table 3.5). The standard model was not able to reproduce the observed wet weight decrease between hatching and emergence, and emergence and onset of feeding [8, 1]. The decrease in wet weight might be a consequence of the water loss due to evaporation during emergence, and/or due to higher salinity of the sea water compared to the amniotic fluid. This could result in a slight tissue density reduction, without reduction of structural (dry) mass [115], and could be reversed by direct water intake [8]. Another important fact that needs to be kept in mind is that 50% of the external yolk sac is utilized during the period between the hatching and birth (96 hours post-emergence), with no significant difference in the dry mass of hatchlings (measured without the yolk) during the same period [115]. In the model, the average 72% water content [115], or tissue density of $d_V = 0.28$ was used. However, the relative water content of the yolk is much lower (45%, [115]). The yolk sac is considered reserve, i.e. part of the individual [109], and is therefore included in the prediction of the wet mass at hatching. The external yolk sac can weigh 2-4 g at the moment of hatching [115, 1], and with such high density of around 0.5 its contribution to weight is greater than the model predicted while assuming the average density of 0.28.

Generally, smaller observed length at hatching, emergence, and birth than the model predicts, in combination with larger wet mass at hatching but smaller wet mass at birth than predicted, could be explained by the DEB parameters not being constant throughout the life of the loggerhead turtle, differing between the embryonic and post-embryonic phases. It is possible that the metabolic heating present in the last third of the embryonic development [124, 98, 156, 262] speeds up the processes of growth and maturation ("T-acceleration", see [113]), effectively resulting in earlier hatching/birth, and smaller size than the model predicts, with the previously mentioned environmental factors such as decreased respiratory gas exchange prolonging the incubation [1]. If the embryo development is the focus of a study, an extension of the standard model should be made. The extension should include the additional environmental factors, as well as changes in the tissue density during the embryonic development, possibly by characterizing the yolk as an additional (reserve) state variable.

Puberty. The model predicts that the loggerhead turtles reach puberty at around 14 years of age, with a size of 76 cm SCL and a weight of 62 kg. If length at puberty is assumed around 80 cm SCL, weight should be around 67 kg [244], a value closer to the model prediction. A loggerhead turtle has been recorded to obtain puberty at a similar weight (70 kg,[210]), but taking the average of the lowest reported values from the literature (see the Section 3.2.2) suggested a value of 79 kg which was used as the "observed data" (a value consistent with 78 kg, the weight of the other mature turtle, [210]). On a more technical note, model predictions of physical length were determined by the shape coefficient (δ_{SCL}) that was used to convert the predicted (abstract) structural length to the observable (physical) carapace length. Because structural length is by necessity a volumetric one (the cubic root of structural volume), this parameter combines the effect of shape and the separation of the contribution of structure and the contribution of reserve to the total volume (and weight) of the individual. The loggerhead turtles were assumed to be isomorphic (of constant shape), i.e. the shape coefficient was assumed to be constant [137], but a small (4%) deviation from isomorphy has been detected [137]. The small reduction (-4%) in the shape coefficient yields predictions of physical length at maturity of around 80 cm SCL, which is in accordance with the data used as input. Introducing an additional shape coefficient parameter to improve the prediction for length at maturity, however, does not seem justified considering the previously mentioned uncertainties regarding data that describe maturity, and hence 80 cm SCL and 79 kg being estimates rather than certain information. Furthermore, loggerhead turtles have been reported to mature at 75 cm SCL [210], and even nest at that size [232]. Prediction for the weight at puberty would probably be improved by taking the weight of the reproduction buffer into account. The reproduction buffer can have a substantial contribution to weight [94], and its dynamics could be included in the DEB model (e.g. see R1 in the Table 3.1).

While the model predictions are lower compared to the data used as "observations" (a_p=28 yr, L_p=80 cm SCL, Ww_h= 79 kg), the predictions are reasonable. Two points need to be kept in mind. First, as noted in the Section 3.2.2, age and size (length and weight) at maturity show large variability. Second, this variability is further enhanced by the discrepancies between age at nesting and age at puberty, and the somewhat arbitrary choice of length that best represents length at puberty. These points can be summarized with the following four relationships: (i) a_p vs a_{nest}, (ii) a_p vs L_p, (iii) L_p vs L_{nest}, and (iv) SCL vs CCL.

(i) a_p vs a_{nest}: As mentioned in the Section 3.1, the assumption that the **age at sexual maturity** (a_p) is equal to the **age at first nesting** (a_{nest}) is a simplification. One of the main hurdles in elucidating a_p and a_{nest} or differentiating the two is that puberty is hard to observe, and that the age of wild loggerhead turtles is very hard to accurately obtain. In general, a_p is taken as the age when the individual has finished the morphological and physiological changes and the reproductive system is fully developed. Maturation is a long process starting from age zero (egg fertilization) and culminating in what is observed as "puberty". In an energetic sense, energy that was thus far being used for maturation ('building up' and preparing the reproductive system), can from this moment be used for reproduction (mating and offspring production). In the theoretical (DEB) world, puberty is a moment which occurs when the maturity level E_H^p is reached. In the real world, puberty in female loggerhead turtles is a period rather than a moment, lasting for 4 years during which morphological changes in oviduct and ovary occur [128]. Next, investment into reproduction, observed as vitallogenesis, starts, followed by mating and ovulation. Vitallogenesis requires up to 12 months for completion, and is triggered by the right combination of endogenous (fat levels, hormones) and exogenous (photo period etc) factors [18, 151]. The first vitallogenesis is observed 2-4 years after maturation has finished (end of puberty) [128], and is not necessarily followed by ovulation and nesting [128, 134]. So, for females that didn't mate, ovulate and oviposit during their first vitallogenesis, the first nesting (the event that many studies take equivalent to obtaining sexual maturity) can occur after the second vitallogenesis cycle is finished, which is on the 2nd or 3rd year after the first cycle, or even later [128]. Consequently, these two values (a_p and a_{nest}) could be as much as a decade apart [128], and the age at puberty predicted by the model (15 years) could translate into 25 years as the age at first nesting.

The prediction of age at puberty at 14 years is in accordance with age at sexual maturity reported by several authors ([208, 264, 210, 48]) who characterized sexual maturity as a change in behavior or morphology, rather than the event of first nesting. Frazer [63] gives an overview of several studies estimating the age at sexual maturity, i.e. the age at which sea turtles grow to the size of sea turtles observed to reproduce, to be around 6 to 7 years based on the growth rates of captive reared loggerhead turtles.

(ii) a_p vs L_p: The estimation of **age at sexual maturity** (a_p) is sometimes obtained as age at which loggerhead turtles grow to/reach a certain length, with the choice of **length at sexual maturity** (L_p) being in fact arbitrary [264]. The prediction for age at maturity in this case largely depends on the growth rates, and the growth model used in the calculations [264]. Even though the estimates of some authors [148, 264] are in accordance with the estimation obtained by the DEB model (around 14 years), Zug et al. [264] in their discussion, point to the reported interindividual variation in growth rates that should be taken into the account. The variation in growth rates is present in all size classes [264, 17, 181, 223], and was reported also for other species of sea turtles [16]. Consequently, the estimated age of individual loggerhead turtles encountered at sizes corresponding to L_p can range from 6 to 25 years [264]. Other studies combining the estimates for the average duration of specific life stages (posthatchling, oceanic juvenile, neritic juvenile, and adult), estimate that loggerhead turtles mature at a mean age of 30.8 (±3.2) years [215], or that they need on average as much as 41-45 years to reach sexual maturity [209].The variation in growth rates cannot be captured by a general growth model, or by a single (individual based) DEB model, but could be reproduced by allowing certain DEB parameters to be dataset-specific.

(iii) L_p vs L_{nest}: Even though Bjorndal et al. [16] found no significant correlation between a_p and length or mass, the authors still suggest the length to be the best indicator of sexual maturity . Since a sample of nesting turtles has a range of lengths rather than a single "**length at nesting**" (L_{nest}), the question as to which length should be used as the "**length at maturity**" (L_p) still remains. In the previously mentioned studies the authors either used the length reported as the lower range for nesting loggerhead turtles [264], or the average length of nesting females in the US [215, 209]. The latter choice could be justified by the advice given by Parham and Zug [173] saying that "Selection of the minimum size of nesting females for determining age at sexual maturity would significantly lower the age estimates derived from either growth models or average growth rates. Minimum size, however, is biologically unjustified. Evidence continues to mount that the turtles maturing at the smaller sizes are not the youngest maturing individuals but some of the oldest ones." Additionally, the assumption that is silently made is that the length at nesting is equal to length at maturity, because most studies suggest that the growth after maturity is negligible. However, looking at the size range of nesting turtles, e.g. 80.5 - 107.0 cm SCL in Cumberland Island [224], or 70-109 cm reported in Ref. [151], it is evident that sea turtles do grow after becoming sexually mature, some of them substantially [16]. In other words, using the average size of nesting females might produce significantly *higher* estimates of a_p.

(iv) *SCL* vs *CCL*: As mentioned already, the measurements of **straight carapace length** (*SCL*) are recommended because they have shown less variability than that of **curved carapace length** (*CCL*) [212]. However, in some cases the measurements of CCL are more appropriate (e.g. for carcases, [264]), are preferred by the authors (e.g. authors studying the Mediterranean loggerhead turtles), or describe individuals that had been measured years or decades ago and therefore cannot be re-measured. This is why conversion formulae for SCL-CCL relationships are useful, but need to be used appropriately [137].

Ultimate size. The ultimate size is slightly underpredicted by the model (92.42 cm SCL and 105.38 kg, compared to 105.26 cm SCL and 162.62 kg used as zero-variate data), but very close to the observed average length of nesting females (92.4 cm SCL, calculated from values in Refs. [224, 54, 27]) and the average weight of adults (116.4 kg, [54]). The predictions of weight do not include the mass of the reproduction buffer, because in the basic model the reproduction was assumed to be continuous. Weight of nesting females can vary with respect to the nesting season [89] as they do not eat while nesting [49]. The cumulative (annual) wet mass of clutches produced by a 100 kg heavy sea turtle can be as much as 10 kg [94], possibly accounting for a large portion of variability of wet weight. Assuming that both the life span and the scaled food availability were realistic, the underpredicted maximum size could be a consequence of a more complex life cycle than the standard model was capable of reproducing. It is possible that the ontogenetic shift to neritic habitats is not just connected to a different type and quality of food (which could be included as a change in the value of f), but also to metabolic changes of the individual. One of the consequences of such metabolic changes could be the change in growth pattern, resulting in the hypothesized polyphasic growth [40, 38]. Extending the standard DEB model, for example by incorporating an additional metabolic switch (and a maturity threshold) connected to the recruitment to neritic habitats, could result in a different growth rate and a different ultimate length.

Three main factors that affect the model predictions for the ultimate length are (i) the shape coefficient, (ii) the maximum age, and (iii) the scaled food availability.

(i) The value of the shape coefficient could be corrected by 4% to account for slight deviations from isomorphy 137, however this does not substantially change the predicted value, corroborating the assumption of a constant shape coefficient.

(ii) The age of large loggerhead turtles could be substantially larger than the assumed maximum life span of 65 years, but this is not likely. Considering the threats and pressures all sea turtles are facing, the number of turtles older than 65 years is likely to be low, and the contribution of the age underestimate to the size underestimate is likely to be limited.

(iii) The scaled food availability of the adults might be higher than the average of $f = 0.8$ assumed in the model, which would allow the individuals to grow to a larger size. Because of maternal effect it would imply that the hatchlings have a larger scaled energy density at birth, resulting in weight larger than 22 g for the predicted $L^b_{\text{SCL}} > 5\,\text{cm}$. However, assuming a higher scaled functional response of adults would have consequences on other life history traits: for example at the *ad libitum* food availability ($f = 1$) the reproduction would be twice that observed. To obtain a realistic reproduction output without changing the values of other parameters, the allocation to reproduction would need to decrease. Allocation to reproduction is directly connected to the allocation to maturation (via the $(1 - \kappa)$ part of the mobilization flux, see Figure 3.1), implying that the metabolic switches (birth and puberty) would happen at a later age and an even larger size, which is not consistent with the observations for birth, and hard to unequivocally determine for puberty.

Assuming that both the life span and the average scaled food availability were realistic, the underpredicted maximum size could have been a consequence of a more complex life cycle than the current standard model was capable of reproducing. The ontogenetic shift to neritic habitat connected to different food type and quality [177] could be included as a change in the value of certain parameters such as f or $\{p_{Am}\}$). It is also possible that the individuals experience different temperatures [177], and undergo certain metabolic changes. One of the consequences of such metabolic and/or environmental changes could be the change in growth pattern, resulting in the hypothesized polyphasic growth [40, 62].

Reproduction. The model slightly overpredicted the maximum reproduction rate, but the predicted value was biologically realistic, suggesting that a fully grown adult nesting every two years allocates enough energy to reproduction to produce 625 eggs, or between 4 and 5 clutches of approximately 140 eggs each. The maximum reproduction rate (R_i) was expressed as eggs per day to be consistent with the standard DEB model which assumes continuous reproduction. To calculate what would the continuous maximum reproduction rate be, three components needed to be taken into account: the number of eggs per clutch, the number of clutches per nesting season, and the number of nesting seasons per year. *The number of eggs per clutch* was assumed to be 140 used in the model, but lower (e.g. 126 eggs per clutch, [232, 204]) and higher (e.g. 198 eggs per clutch, [151]) values would also be realistic. We assumed that extremely large clutch counts reported were mostly likely outliers, and/or the yolk-less eggs had not been removed during egg counting [151]. *The number of clutches per season* was assumed to be 4 [81], but a value of 5 clutches per season had also been suggested [15, 237]. Most studies report an average clutch size per beach or population, without distinguishing first-time nesters from remigrants. For the calculation of the *maximum* reproduction rate, only the remigrants were taken into account, and hence only the upper parts of the reported ranges were used. *The number of nesting seasons per year* is an inverse of the remigration interval, reported to be 2–3 years for the North Atlantic loggerhead turtle [81]. It was not clear whether larger (and probably older) turtles exhibit a longer [103] or a shorter [80] remigration interval. Two combinations (4 clutches every 2 years, and 5 clutches every 2.5 years) yielded the same value of the maximum reproduction rate (0.7671 eggs/day) so this value was used as input.

The reproduction output, i.e. the number of eggs produced from the energy allocated to reproduction, is correlated to the energy content of an egg. The initial energy content of an egg needs to be sufficient for both embryonic development ($>$ 60 kJ, [187]), and for the embryo itself ($>$ 120 kJ, [115]), suggesting that the predicted value of 210 kJ is realistic. Females of different sizes within a population lay eggs of similar sizes [248, 232], and presumably the egg energy content does not vary even when some intrapopulation variability in egg size is present. The intrapopulation variability in egg size has been explained by varying amounts of albumin [248], which accounts for most of the egg volume ([52] in Ref. [125]), and has not been significantly correlated to the hatchling size but rather to the amount of water the egg can osmotically absorb [248].

3.4.3 Uni-variate data

Data which measured both the turtles, and their environmental conditions (water temperature and food availability) was scarce. As a consequence, detailed information about loggerhead turtle growth is limited to the first 10 weeks of the sea turtle's long life cycle (captive reared loggerheads). Even this short period was sufficient, in combination with the data of life-history traits, to see whether the standard DEB model can capture the patterns in the post-embryonic development and growth. Due to the large number of available measurements for the same age, the data also provides a glimpse of the inter-individual variability of growth rates present even under controlled conditions (Figure 3.5, panels a and b). The data for juveniles (from Refs. [90, 174, 175]) was also included, because the age of the individuals was known, while the food availability and water temperature, even though unknown, were probably adapted to fit the needs of the animals and can be considered optimal.

Age at emergence as a function of temperature. The model described the relationship of the age at emergence and the incubation temperature qualitatively well (the slope of the predicted linear relationship was correct). Quantitatively, age at emergence was slightly underpredicted. Age at emergence in the DEB model is a result of several factors: the maturity thresholds for hatching and birth, the mobilization and the maturation rates (see Table 3.1), and various constraints set by the model that ensure the parameter values are consistent and do not violate the model assumptions. As already mentioned, the duration of incubation (and emergence) is also influenced by factors such as humidity and grain size of the substrate, which were not accounted for in the model, and might explain some of the variation. The average time from hatching to emergence (4.1 days,

[70]) was initially considered a constant, assuming it was not a function of temperature, but rather other intrinsic or extrinsic factors such as sand humidity and grain size, nest depth, duration of the day etc. However, including the temperature correction for that phase of the emergence period improved the trend line and described the data better (in terms of the slope) compared to the non-corrected prediction. The authors Godfrey and Mrosovsky [70] calculated the average hatching to emergence time by calculating the difference between the incubation duration in the laboratory (oviposition to hatching), and the incubation duration in the naturally incubated nests (oviposition to emergence). This was done for each incubation temperature (approximated via the produced sex-ratio of the clutch), and the values were then averaged, however it is unclear whether the hatching to emergence time was tested for correlation to temperature. The better fit of the temperature-corrected model suggests that the duration of the period from hatching to emergence is also determined by the physiological processes that need to take place before emergence, the rates of which are affected by temperature. The significant utilization of the yolk sac during this period [115] is probably connected to the required processes of preparations for emergence.

Growth of posthatchlings. The model predicted the growth of posthatchlings reasonably well, but when the (lower than predicted) observed length at birth was used as a curve starting point, the plotted curves were consistently lower than data. The food was modeled as constant and *ad libitum* throughout the simulation, assuming the decrease in food availability from 20% to 8% body weight per day [223] did not have a substantial effect at such a high food level. Initial optimal conditions (head starting) were correlated to the higher growth rates in later life-stages for other reptiles [132], suggesting that any change in food availability experienced early in life could have an effect on growth rates. It is not certain whether the reported change in food availability would result in a significant change of the scaled functional response (f), because the relationship is hyperbolic and at high food availability ($f > 0.9$) a relatively large change in absolute food abundance shows up as only a minor change in the functional response [109]. When the food availability (value of the parameter f_{Lab}) was estimated by the covariation method with the other parameters, the estimated value was only slightly higher (1.043) than the initially assumed value of 1 (a value higher than 1 is possible here, as the parameter f includes food abundance *and quality*). Changing just the value of f did not reproduce the growth pattern, suggesting that the observed high growth rate was a result of factors other than food and temperature. Calculating the growth rate involved parameters related to physiology and the energy budget, which made it possible to explore other factors.The specific growth rate (r) of structural length (D2 in Table 3.1, eq. 3.1), and consequently wet weight (eq. 3.2), was calculated using the equation 3.3. Two parameters of interest in the equation 3.3 are the energy conductance (v), which is a core (primary) parameter, and the energy investment ratio (g), which can be written as:

$$g = \frac{[E_G]}{\kappa[E_m]} = \frac{[E_G]v}{\kappa\{pA_m\}} = \frac{[E_G]v}{z[p_M]}. \tag{3.4}$$

A change in the values of the parameters v and/or g would change the observed growth rate, because they both affect the specific growth rate (r), and directly or indirectly affect the processes of reserve mobilization, and growth (fluxes F2 and F4 in Table 3.1). The parameter g is a compound parameter, so a change in its value has at a first glance more possible sources. The volume-specific cost of structure, $[E_G]$, is generally assumed constant throughout the life cycle because the strong homeostasis is assumed to always hold [109]. The parameter kappa (κ), in order to yield values consistent with other model predictions and assumptions, would need to drastically change just after birth from a value of 0.648 to a value above 0.8 in order to maximize growth at cost of development and maturation, and then gradually return to the 'standard' adult value within next couple of weeks. The parameter κ was shown to be constant throughout life for most species [109], so drastic changes do not seem plausible. Changes in the volume-specific somatic maintenance ($[p_M]$) were, however, observed in other species [113, 106].

Changes in parameters $[p_M]$ and v would be intuitive: the sea currents that posthatchlings need to swim against would show up as an increase in $[p_M]$, and an increase in v would be required to mobilize the reserves and provide enough energy for all processes. Since the data for the posthatchlings were obtained from the laboratory setting, the possible increase in $[p_M]$ might have been caused by the flow-through system in the holding tanks, or it is possible that the shift in parameter values is intrinsically triggered, and occurs regardless of the environmental conditions. The increase in the value of parameter $[p_M]$, resulting in faster growth at a higher energetic cost, was recorded in various other species and named the "waste to hurry" strategy [106]. It is very likely that the objective of small posthatchling loggerhead turtles is to maximize growth in order to lower the risk of being eaten by predators [202], and that faster growth is achieved via the waste to hurry mechanism. This is corroborated by the metabolic rates of posthatchling loggerhead and other sea turtles being higher than metabolic rates of larger juveniles (see [247] for an overview of metabolic rates of sea turtles). When parameters $[p_M]$ and v change proportionally, then the maximum reserve density ($[E_M] = \{p_{Am}\}/v$) is maintained, however it is possible that the change in parameter values is such that the $[E_m]$ of post-hatchlings is different compared to that of the adults. This would probably improve the fit of the weight-to-length relationship for the post-hatchlings as it is determined by the ratio of the shape coefficient (δ_{SCL}, assumed constant, [137]) and the maximum reserve density. Nesting females are capable of prolonged periods of fasting combined with nesting - a very demanding behavior in terms of energy expenditure [89, 49], and it would be interesting to see the relation of $[E_m]$ in adults and post-hatchlings.

The hypothesized change in parameter values is measured in days, and probably can be considered negligible when the general life cycle of loggerhead turtles is studied.

When a species needs more than a decade to obtain sexual maturity, and lives almost as long as a human, 10-15 days seem insignificant. However, this could have significant consequences on a life cycle of the loggerhead turtle [106], and the implications should be studied further. The increased growth rate and a different fasting ability compared to adults should be taken into account for captive rearing, reproduction and head-starting programs.

Growth of juveniles. Growth of juveniles (Figure 3.6, panels a and b) was modeled with the same set of ODEs as the posthatchling growth was, and the model predicted the growth curves well. There was some uncertainty relating to the holding conditions (Hildebrand and Hatsel [90] stated a concern about the suitability of holding tanks), and whether the data was representative of turtle growth (Parker [175] hypothesized that the individual showing extremely rapid increase in weight and length might be more of an exception than a rule). The data, though far from perfect, did provide valuable information related to growth and metabolism, which was taken into account for the parameter estimation. Additionally, the evident interindividual variation under controlled conditions suggests an even larger interindividual variation in the wild, placing the data and predictions of life-history traits into a more realistic context.

Weight as a function of length. Weight to length relationship for the whole size span (Figure 3.6, panel c) was described very well by the model, indicating that the dependence of morphometric scaling on size identified previously (see Chapter 2 and [137]) does not result in drastic changes of the weight-to-length relationship, i.e., that the straight carapace length is a valid approximation of structural length.

Reproduction as a function of length. The reproduction to length relationship was described reasonably well by the model (Figure 3.7), but the trend of the model slope did not correspond to the trend evident from the data, with the reproduction underpredicted for smaller sizes, suggesting a clutch size as small as 20 eggs, and overpredicted for larger sizes, suggesting clutches larger than 150 eggs. Calculating the reproduction output as was done in this study had the advantage of simplicity, but it also had limitations. The two simplifications that most influenced the predicted clutch size were: (i) the number of nests per season was the same (four) for sea turtles of all sizes (and age); and (ii) there were no constraints on the clutch size in terms of the minimum and maximum clutch size. Clutches with more than 140 eggs are rarely observed in nature [204, 151], possibly because of mass and resource limitations. Another limiting factor can be the metabolic heating, which is positively correlated to the number of embryos. The metabolic heating can be significant in the last third of incubation ($+1.6^\circ$ C, [262]), and the excess heat can be lethal to embryos [142]. Generally a trade-off between clutch mass and clutch frequency was observed for most turtles, including sea turtles [94, 249, 26]. Furthermore, loggerhead turtles nesting for the first time produce on average half the number of clutches than those that had nested previously [81]. Each clutch includes the energetic cost of crawling to the beach, digging the nest, and returning to the sea [246], with the associated risk of predation, but more clutches per season increase the survival with respect to the risk of predation, inundation, seasonal environmental pressure etc. Constraining the model predictions by a minimum and maximum clutch size, and allowing the number of clutches per season to vary between turtles of different sizes, would result in more realistic predictions. The modified model could provide insight into the energy allocation during the nesting events, including optimization of the number and size of clutches produced in a nesting season. While this is not necessary for studying the general life cycle of the loggerhead turtle, or estimating the seasonal reproductive output, it would be interesting to see whether the modified model can reproduce the trend observed in the wild.

3.5 Conclusion

With a relatively small set of data on life-history traits and growth curves, we characterized the energy utilization patterns in the loggerhead turtle by estimating values for the core parameters of a standard DEB model. The Dynamic Energy Budget (DEB) model of the North Atlantic loggerhead turtle describes the life history traits and growth curves well (mean relative error = 0.1776, and FIT=8.22 of 10).

The standard DEB model predicts the growth, maturation, and reproduction as a function of temperature and food (or energy reserves provided by the mother, in case of the embryo), and therefore can be useful for studying the environmental effects on these processes. However, sometimes other factors not included in the model play an important role, and including them might increase the predictive capabilities and accuracy of the model. For example, for the embryonic development the humidity of the sand affects the incubation duration, the compactness of the sand influences time needed from hatching to emergence, and the temperature inside the nest can increase by 1.5° C due to metabolic heating; including the additional environmental parameters might be required if embryonic development is the focus of the study. For the reproduction output, the optimal clutch theory should be taken into account; including constraints on the size of the clutch and possibly an additional parameter to account for the variable number of clutches per season might improve the trend of the model predictions.

Observed deviations of model predictions from the measured and published data might have been caused by errors in some of the assumptions used. However, the deviations suggest interesting hypotheses that should be explored further:

(i) During the first 10-15 days of development, post-hatchlings might use the "waste to hurry" [106] strategy resulting in faster growth at a larger metabolic cost when compared to older juveniles and adults.

(ii) Seemingly contradictory estimates of age at maturity ranging from 15-39 years 173, 209, 215, 25, might be a consequence of sea turtles maturing at smaller sizes (75-80cm SCL) and at younger ages (15 years), but beginning to nest at larger sizes (85-90cm SCL) and older ages (20-35 years), but also a result of large interindividual variability in growth and maturation rates [5].

(iii) Polyphasic growth proposed or indirectly assumed by some authors 40, 38, 173 might explain the variability in reported growth rates. Polyphasic growth might also explain variability in estimates of life stage durations and age at maturity when calculated on the basis of size.

In addition, the set of DEB parameters for North Atlantic loggerhead turtles, model predictions, and possible model extensions provide insight into the physiology, biology, and ecology of the loggerhead turtle, and make a powerful tool for conservation biology and management of sea turtles. The parameter values now allow us to predict many features of the energetics quantitatively, which were never measured directly. Examples are the plotted energy budget at birth, puberty, and fully-grown adult, see Figures 3.8 and 3.9. We here see some remarkable implications, namely the drastic change in proportion of energy fluxes during ontogeny, and the energy invested into the (rarely discussed) maturity maintenance comprising almost 25% of the daily energy budget.

Obtaining a set of DEB parameters for a different loggerhead turtle population, for example the Mediterranean population, might provide further insight into the ecology of the species, and the previously observed [232, 181] differences in growth, maturation, and reproduction between these two populations. Information on relevant processes and life history traits (duration of life cycle phases, reproduction output etc.) can be further studied for a range of temperatures and/or food abundances to gain additional insight into the biology and ecology, and construct better conservation strategies for this endangered and protected species.

Chapter 4

Comparison of Mediterranean and North Atlantic populations using DEB models

Abstract

Loggerhead turtles that nest in the Mediterranean sea are geographically and genetically distinct from the North Atlantic (NA) other populations of loggerhead turtles. The individuals belonging to the Mediterranean population, even though belonging to the same species, are consistently (at birth, puberty, and ultimate size) smaller than their conspecifics of the North Atlantic population. Because of their geographical and genetic isolation, the smaller size could be a result of environmental and/or physiological characteristics. The aim of this study was to study the morphological (size) and physiological (metabolism, growth, reproduction) traits of the Mediterranean loggerhead turtles, and to compare the studied traits with those of the North Atlantic loggerhead turtles.

The research and comparison were performed in two steps: First, an analysis of size (length and weight) data of the individuals from the two populations was performed. Information gained by this approach was limited to empirical observations, and gave only limited insight into the possible drivers and mechanisms for observed size differences. Second, a mechanistic modeling approach was used to study the physiology of the Mediterranean loggerhead turtles, and thus obtain insights into the possible metabolic responses of the Mediterranean loggerhead turtles to their environment. An energy model based on the Dynamic Energy Budget (DEB) theory was developed for the Mediterranean loggerhead turtle population, and was then compared to the previously developed DEB model for the North Atlantic loggerhead turtle population.

Results suggest that the Mediterranean loggerhead turtles have physiologically adapted to the higher salinity and lower food availability of the Mediterranean sea. The physiological condition indices (expected to be smaller for the Mediterranean loggerhead turtles due to lower food availability) are similar between the populations, but markedly different between life events (hatching and nesting) within a population. Parameter values and model predictions specific to the Mediterranean population suggest faster growth and earlier maturation at smaller sizes compared to their North Atlantic relatives. This is consistent with the pattern that has been observed previously, but in this study is linked to physiological adaptations, some of which are empirically very hard to detect, such as

a relatively small increase in the energy required for somatic maintenance, and a relatively large decrease in the energy investment required to reach puberty and maintain that level of maturity.

In addition to gaining an insight into the Mediterranean population, we detect interesting patterns related generally to growth of earlier life stages, and to growth and maturation in a variable environment. Namely, the faster growth of posthatchlings compared to older life stages is predicted extremely well by the model while allowing the somatic maintenance rate ($[p_M]$) and energy conductance (v) to be estimated specifically for the posthatchling data. The somatic maintenance rate is generally related to maximum assimilation rate ($\{p_{Am}\}$), and all three parameters seem to have a higher value in the posthatchling stage. Parameters $[p_M]$ and $\{p_{Am}\}$ have been related to the "waste to hurry" strategy, i.e. maximizing growth at a higher energetic cost during the period of food availability, a pattern which is in accordance with the ecology of the posthatchlings. Parameter v regulates reserve mobilization and maximum reserve density, influencing on how much energy can an individual store, and how long it can survive starvation. Additionally, using the same mechanistic model and simulating a drastic change in the food availability, we obtain a pattern of biphasic growth consistent with polyphasic growth patterns suggested by other authors.

4.1 Introduction

Compared to the North Atlantic, the Mediterranean Sea is a relatively small basin (2.5 million km^2 [150], compared to 106.5 million km^2, NOAA-facts), and its only communication with other sea basins is with the Atlantic Ocean via the narrow Strait of Gibraltar. The main characteristics of the Mediterranean Sea are (adapted from Refs. [136] and [263, 150, 133]):

- From an oceanographic point of view, it is an evaporation basin. The resulting difference in salinity and water deficit sustain permanent currents across the Strait of Gibraltar: a strong incoming surface current, and a weaker subsurface countercurrent.
- Can be roughly divided into two basins, the western and the eastern, connected by a shallow Sicily Channel and the narrow Messina Strait.
- The two basins have different hydrological conditions, the eastern being more saline and warmer.
- Due to a lack of nutrients, the Mediterranean is an oligotrophic sea and one of the less productive seas in the world.

- The large number of species inhabiting different types of environments make it a biodiversity hotspot.

The loggerhead turtles present in the Mediterranean sea have probably evolved from the North Atlantic loggerhead turtles more than 10,000 years ago [136]. Currently they are the most abundant sea turtle species in the Mediterranean, having evolved into local populations, and are one of two sea turtle species that nest in the Mediterranean (the green turtle is the other one, [236]). All sea turtles in the Mediterranean are listed as endangered and are protected [238, 236].

Defining the protection measures, apart from identifying the most important pressures, requires an in depth knowledge of the ecology (habitat types, distribution, feeding, nesting, and migration areas), and the biology (physiology, maturation, growth, and reproduction) of the species. Integrating and combining the information published on the subject of the loggerhead turtles, and using the knowledge from the laboratory and field studies focusing on incubation or physiology, satellite tracking, tagging and monitoring programs, rescue and rehabilitation centers, and rearing and reproduction programs, provides an overwhelming pool of information. In addition to research specific to the Mediterranean region, the physiological and biological characteristics of the Mediterranean loggerheads can be elucidated from the information about other loggerhead turtle populations, and even other species of sea turtles or reptiles.

Generally the life cycle of the Mediterranean loggerhead turtles is very similar to that of their North Atlantic conspecifics.

The Mediterranean loggerhead turtles need between 14 to 28 years to reach sizes between 66.5-84.7 cm curved carapace length (CCL), signifying sexual maturity [39, 181, 136, 232]. Mating and nesting occurs primarily in the east Mediterranean, with major nesting sites and rookeries in Greece, Cyprus and Turkey [136]. In general, sea turtles nesting in Greece are larger (mean size 83.55 cm CCL or 78.52 cm straight carapace length, SCL) and have on average larger clutches (>105 eggs/clutch), than those nesting in Cyprus, Libya, Tunisia, and Turkey: mean size 76.44 cm CCL (72.36 cm SCL), and clutches of <100 eggs/clutch (all values from Refs. [136, 77]). With a remigration interval (a period between two nesting seasons) of approximately 2 years [26], the females lay on average 1.8 to 2.2 clutches [26] of on average 100 to 200 eggs each [136, 77]. The incubation lasts lasts 50-60 days (duration of incubation being inversely proportional to the incubation temperature [61, 142, 223, 187]), and the sex of the embryos is determined by temperature in the last third of the embryonic development [156, 265]. Generally hatchlings weigh around 16 g with a carapace length of 4.2 cm, but the size of hatchlings can vary between nesting areas [136].

The loggerheads from the Mediterranean and the North Atlantic belong to the same species, but are genetically different [181, 31], and do not interbreed even when sharing the same feeding grounds [32]. The individuals from the two populations are morphologically similar, but the Mediterranean loggerheads are smaller than their North Atlantic relatives [232, 136]. Nesting Mediterranean females are possibly also younger than the North Atlantic ones [39, 181], and compared to that of the North Atlantic loggerheads, the reproduction output of the Mediterranean loggerhead includes shorter remigration intervals [26, 88], fewer clutches per season [26, 88, 237], but more eggs per clutch [136, 77, 232].

Size dimorphism was also noticed within other species from both sea basins [45, 93, 69]. The observed variability in size and reproductive output is probably a result of multiple factors. In general, possible explanations include:

- environmental effects, where more favorable conditions (higher food abundance and temperature) in the North Atlantic result in faster growth rates and larger sizes (e.g. in Ref. [17, 45]), while energy limited environments such as the Mediterranean reward earlier maturation at smaller sizes [232, 106];
- environmental conditions, where more favorable (constant) conditions (such as those in the Mediterranean with smaller environmental oscillations) support a longer reproduction season and more individuals (denser populations), resulting in smaller individuals due to less resources per individual ([109], p297);
- the genetic differences cause different growth and maturation potentials [9];
- the ecological pressures such as long (trans-oceanic) migrations favor larger sizes, or higher adult predation favor earlier reproduction at smaller size (references in [232]);
- the adaptations in feeding behavior result in different ecological niches [45].

The smaller size of Mediterranean hatchlings can probably be attributed to the smaller size of the eggs [69, 96, 232, 1], even though incubating conditions such as humidity, salinity, and temperature of the sand, have also been correlated to the size of hatchlings within a population [223, 69, 184, 79, 169, 24], but see [187, 96, 184, 176].

The complex interactions between different environmental and physiological factors require a systemic approach, and are particularly hard to study because of the large number of possible combinations. Furthermore, with large variability within and between different loggerhead turtle populations, the consistent pattern of the smaller sizes reported for the Mediterranean populations of the loggerhead turtles ([136, 64], and Appendix I in [232]), in combination with the observed faster growth compared to that of the North Atlantic loggerhead turtles in the Mediterranean [181], make a very interesting puzzle. An additional part of the puzzle is whether the difference in size and the reproduction output is also a difference in the general condition of the animal, with smaller sizes at specific life stages being correlated to the poorer condition of the individuals. The answer to the puzzle might be found by exploring some or all of the mentioned possible explanations, and testing various combinations of the environmental and physiological factors. Even when taking only two populations into account, the Mediterranean and the North Atlantic, an experimental setup for a study of such a large scope includes a number of logistic constraints (time, equipment, permits, statistically valid number of replicates, finances, etc.). Valuable insights can also be obtained by simulating specific scenarios and analyzing the individual responses using mechanistic models that combine existing data and knowledge, which was done in this study.

The aim of this research was to study the Mediterranean loggerhead turtle population, and to gain additional insights by comparing the Mediterranean and the North Atlantic populations. The comparison was done using two approaches: (1) a static approach, by taking a "snapshot” of the morphology and the physiological condition of the individuals within the two populations, and (2) a dynamic approach, by developing and then using an energy-budget based mechanistic model specific to the Mediterranean population. Results of the model specific to the Mediterranean population were compared to the results of the previously developed model for the North Atlantic population (Chapter 3). The environmental characteristics of the two sea basins were taken into the account for the comparison. By explicitly modeling the environmental factors, it was possible to elucidate the environmental effects and possible causes of the observed differences between the populations.

4.2 Methods

Data necessary for the analysis was obtained by a comprehensive literature search for data specific for the Mediterranean population, and by contacting personnel in various aquaria and research programs to obtain data from controlled rearing conditions. All code was written and executed in Matlab R2011b.

4.2.1 The static approach - Analyzing the “snapshots” of size and physiological condition of individuals from the two populations

Size and physiological condition at two life-history events (hatching and nesting) were studied for individuals from the Mediterranean and North Atlantic basins. Size is mostly described by weight and length, so data on weight and carapace length were of primary interest. For the analysis of the size (length and weight) at hatching, data reported at the moment of hatching (leaving the egg) was pooled together with the data reported for the moment of emergence (leaving the nest). This was justified as the length does not significantly change between hatching and emergence, and the decrease in wet weight can be explained by dehydration [8]. Pooling the data for analysis increased the sample size, as length and/or weight were mostly reported at emergence for the field incubated nests (e.g. [223]), and at hatching for the laboratory incubated nests (e.g. [187]).

The ratio of the weight and length, generally referred to as the "condition index" of the individual, is often used as an indication of the physiological condition of the animal: a larger condition index means that the animal is better fed, i.e. has more energy available for various processes. The Fulton's condition index ($K = W/L^3$, g/cm^3) was used to test whether the difference in size correlates with the difference in the physiological condition. The Fulton's condition index was originally developed for fish, but has already been applied to many species of vertebrates, including sea turtles (see [222] for an overview), and has the advantage of not assuming a "standard" or "healthy" value that e.g. relative mass indices assume [222]. The condition index was calculated on the basis of the mean size and weight at hatching and at nesting for each population. The chosen condition index is dependent on the choice of the length measurement [222], but using only one measure of length (SCL) prevented potential measurement-induced bias from affecting the analysis. The average egg sizes were also compared, because it may explain a large part of the hatchling size variation [69, 96, 232, 1]. The "condition index" of the egg, akin to the Fulton's condition index, i.e. the ratio of the egg mass (in grams) and egg diameter (in centimeters) was used for the comparison.

All data used for the comparison is listed in Tables 4.1 and 4.2. The data was either available as a dataset, or reported as a mean with a range and/or standard deviation (SD). For the North Atlantic population available datasets consisted of 94 weight and length data points for hatchlings at emergence (each data point was a mean of measurements of 10 hatchlings from one nest, unpublished data obtained from L. Stokes), and 214 length data for nesting females, reconstructed from the plotted length-to-width relationship in Refs. [54, 28]. Weights of the North Atlantic nesting females were calculated using the allometric equation $W = 0.000282 \cdot SCL^{2.823}$ [244], which yielded values consistent with the reported weight range [204]. For the Mediterranean population, substantially fewer datasets were available. The data for wild populations was mostly reported as a mean with a range and/or standard deviation. In addition, a previously unpublished dataset was obtained from a rearing and reproduction program in Antibes (France) (courtesy of S. Cateau). The dataset contained measurements of weight at hatching and weekly weight increase up to the age of 2 years for 21 juveniles, and measurements of size and weight of two females reproducing in captivity. This data cannot be analyzed as a part of the wild Mediterranean population even though the adults were obtained and reproduced in the Mediterranean. The captive reared individuals experience considerably more favorable conditions than the turtles in the wild, and are therefore expected to have a better condition index and produce heavier hatchlings [109]. Consequently, the data from the reproduction program was analyzed separately.

Different types and amounts of data hindered an advanced statistical comparison. However, it was possible to gain some insight into the size distribution by visually analyzing the distribution of values. The datasets available from the North Atlantic population were first analyzed by a Lilliefors (normality) test at 5% significance level, and then plotted as normalized histogram and normality plots. Data from the Mediterranean population was included in the histograms as individual data points. In addition, the dataset for captive-reared Mediterranean hatchlings was analyzed by a Lilliefors test at 5% significance level as well, and then statistically compared to the analogous dataset for North Atlantic hatchlings. All data was used to calculate average condition indices for each life stage within a population.

4.2.2 The dynamic approach - Development and applications of the Dynamic Energy Budget (DEB) model

4.2.2.1 Constructing a DEB model for the Mediterranean loggerhead turtle population

Zero-variate data consisted of data points containing the life history traits (age and size at hatching and puberty, maximum reproduction etc) of the wild Mediterranean loggerhead turtles. For length, straight carapace length (SCL) was preferred because of better accuracy of the measurements [212] and also consistency with Chapter 3, but length at puberty and ultimate length are reported also as curved carapace length (CCL) because it is the measurement of choice in most published literature on the Mediterranean loggerhead turtles. Some data is explained in more detail, and all data is presented in Table 4.3.

- *Hatching, emergence, birth.*
  Hatching (leaving the egg), emergence (leaving the nest), and birth (starting to feed) occur several days apart. Age at hatching (49.08 d for 30ºC incubation, [187]) was used to calculate the age at birth (55.18 d) by adding the average time required for emergence (4.1 d, [70]), and two additional days until the onset of feeding [115], assuming that the time from hatching until birth is relatively constant within a species. From an energetic perspective, birth is the most important event, as it denotes the transition between the embryo (does not feed or reproduce) and juvenile (does feed but does not reproduce). Birth was considered to be determined by a single maturity threshold, because separate maturity thresholds could not be differentiated using the available data (see Table 3.4 in Chapter 3). No substantial difference in length has been detected between hatching and birth [115]. Length ($L^b_{\text{SCL}} = 4.1\,\text{cm}$) and wet weight ($W^b_w = 16.1\,\text{g}$) at birth were calculated as mean values from Refs. [136, 187].

- *Puberty*
  Puberty (start of allocation to reproduction) was, as for the North Atlantic population, considered equivalent to the event of first nesting. Only estimates were available for age at puberty because puberty is generally not observed directly. Estimates mostly depend on the length defined as the "length at puberty" and on the method used for estimation, and are reported as a range from 14.9 to 28.5 yr (CCL of 66.5 to 84.7 cm, [39]), 24 yr (69 cm CCL, [181]), and 23.5 to 29.3 yr (80 cm CCL, [34]). A value of 20 years was used as the "observed value", but was given low weight in the parameter estimation procedure [126] due to a large variability of estimated values. The length at puberty ($L^p_{\text{SCL}} = 64.2\,\text{cm}$) was calculated as the average of the smallest females nesting in the Mediterranean region (Greece, Turkey, Cyprus, Tunisia) [136, 77, 232]. Using sources that report only curved carapace length (CCL), the length at puberty was $L^i_{\text{CCL}} = 69\,\text{cm}$ [136, 77]. For wet weight data only one report [77] was found for the nesting Mediterranean loggerhead turtles. The report is for the population nesting in Greece, the average size of nesting females was reported to be 80 cm CCL, and the range of weight values to be 52.5-87 kg. A lower value of this range was used as wet weight at puberty.

- *Maximum life span and ultimate size*
  The maximum life span was assumed to be relatively consistent within a species, so the same value as for the North Atlantic population was used (65 yr, [78, 214]). The ultimate length ($L^i_{\text{SCL}} = 87\,\text{cm}$) was calculated as the average of the largest females nesting in the Mediterranean region (Greece, Turkey, Cyprus, Tunisia) [136, 77]. The ultimate length calculated from the sources that report length in CCL was $L^i_{\text{CCL}} = 91\,\text{cm}$ [136, 77]. The length of the largest nesting female has been reported for Greece as 95 cm SCL [136], but the maximum length (the length that individuals can reach under *ad libitum* food) was assumed to be consistent within a species, so a value of 130 cm SCL [65] was used. Data for the maximum weight was equally scarce as data for the weight at puberty. The value indicated as the higher end of the range for loggerhead turtles nesting in Greece (87 kg, [77]) was used.

- *Reproduction*
  The maximum reproduction was expressed as the number of eggs per day by taking into account the 3 nests (clutches) per nesting season [26], 160 eggs per clutch [136, 77, 232], and remigration interval of 2 years [26]: $R_i = 3 \times 160/(2 \times 365) = 0.6575\,\#/\text{d}$. The energy content of an egg was 170 kJ [88].

The conditions in the Mediterranean were simulated using an average sea surface temperature of the eastern Mediterranean basin ($T_{\text{Med}} = 21^\circ\text{C}$, [133]), because most of the activities of the Mediterranean loggerhead turtles are concentrated in the eastern part of the Mediterranean sea [136].

Uni-variate data are data sets that include different types of data-pairs of dependent and independent variables. Temperature vs. incubation duration (data from [187]) and length vs. clutch size (data from [232]) data sets relate to the individuals from the wild. Age vs.length, age vs.weight, and length vs.weight data sets were obtained from the Marineland (Antibes) rearing program (courtesy of S. Cateau), and represent captive-reared individuals. The data includes measurements of two loggerhead turtles that hatched in 2010 and were measured until they were 4 years of age, and of twelve or six (depending on the data set) loggerhead turtles that hatched in 2011 and were measured until they were 3 years of age. The food was assumed *ad libitum*, and the reported temperature (22 - 26°C) was explicitly included by modifying all rates to the specific temperature (see section Model formulation).

In addition to the listed zero-variate and uni-variate data, the same **pseudo-data** as for the North Atlantic population was also used (see the Section 2.2 of Chapter 3).

Model formulation The set of assumptions made during model formulation for the North Atlantic population of loggerhead turtles (see the Section 2.1 of Chapter 3) were assumed to hold for the Mediterranean population of loggerhead turtles as well. The main assumption was that loggerhead turtles of the Mediterranean population follow the standard DEB theory and that their life history traits and important processes can be described well by the standard DEB model.

The mechanistic modeling was performed in two steps (Figure 4.1). In the first step, the parameter set specific to the North Atlantic (NA) population of the loggerhead turtles ($pars_{\mathrm{NA}}$), obtained in the Chapter 3, was used in the combination with the environmental conditions (f and T) assumed for the zero-variate Mediterranean data. In this step, no parameter estimation was performed, i.e. the parameter values were fixed. The first step effectively simulated the responses of the North Atlantic individuals to the Mediterranean environment, predicting their life history traits (i.e. zero-variate data), and growth and reproduction (uni-variate data).

The second step was analogous to the model formulation for the North Atlantic loggerhead turtles (see Figure 3.2 in Chapter 3). In the second step, new parameter values ($pars_{\mathrm{Med}}$, specific to the Mediterranean population) were estimated using the covariation method, and the $pars_{\mathrm{NA}}$ parameters as initial parameter values.

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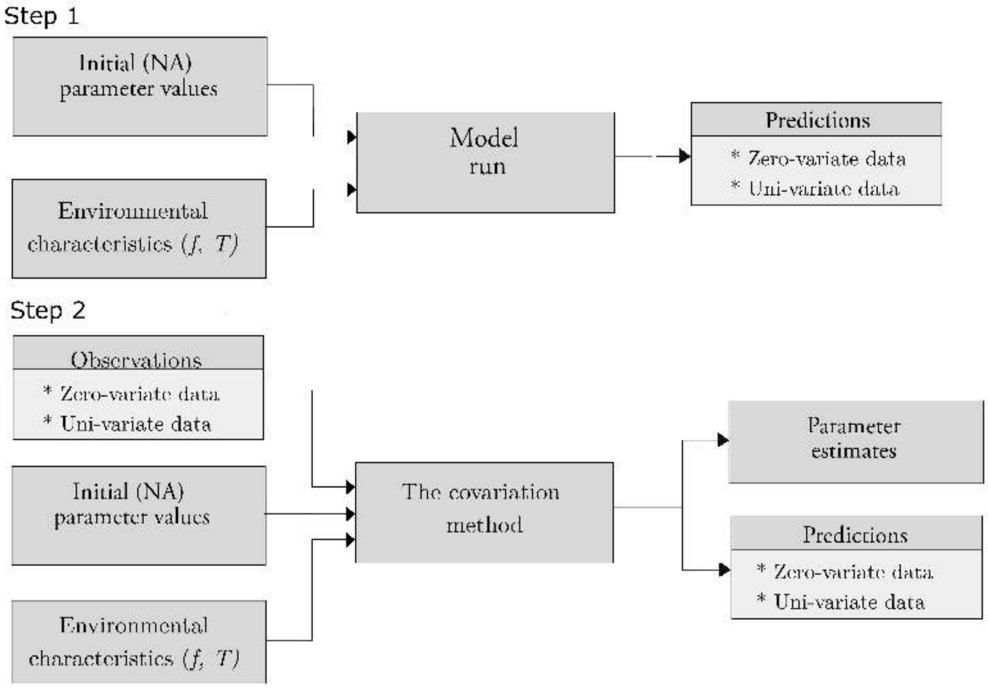

Figure 4.1: A scheme of the modeling process. In the first step, the model was used to obtain the set of predictions while keeping the parameter values specific to the North Atlantic (NA) population ($pars_{\mathrm{NA}}$) and simulating the environmental conditions specific to the Mediterranean sea (f_{Med}, T_{Med}). In the second step, the covariation method was used to estimate the parameter values specific to the Mediterranean population ($pars_{\mathrm{Med}}$), and obtain the predictions for zero-variate (life history traits) and uni-variate (dependencies) type of data.

Environmental conditions that most influence the energy budget (and consequently the parameter estimates and model predictions) are temperature and food availability, that were either known (for captive-reared individuals) or assumed (for individuals in the wild). The rates predicted by the model (k_{ref}) are all predicted for a reference temperature ($T_{\mathrm{ref}} = 273\,\mathrm{K}$), and then corrected to the temperature of the data set (T) in Kelvin using the Arrhenius temperature (T_A) (equation 1.2 in [109]):

$$k(T) = k_{\mathrm{ref}} \exp\left(\frac{T_A}{T_{\mathrm{ref}}} - \frac{T_A}{T}\right). \tag{4.1}$$

Food availability was included as the scaled functional food response (f). The scaled functional response is a saturating function denoting the feeding rate as a fraction of the maximum for an individual of the same size [109]. As an estimate for the scaled functional response, the ratio of the ultimate length and the maximum length can be used, so $f_{\mathrm{Med}} = L_m / L_i = 0.706$.

As with the North Atlantic population (see the Section 2.1 in Chapter 3), an individual turtle was characterized in the model by three state variables: structure (i.e structural length, L), reserve, E, and maturity, E_H, and ordinary differential equations (ODEs) were solved for changes in the state variables to obtain model predictions such as growth curves and reproduction output. The DEB model specifies that the size at specific life events (birth, puberty) can be different at different food levels if the maturity maintenance rate coefficient (k_J) and somatic maintenance rate coefficient (k_M) differ. It was assumed that the $k_J \neq k_M$ condition might reproduce the observed size dimorphism between the two (North Atlantic and Mediterranean) populations.

Conversion of the abstract DEB variables into measured (observed) data such as length and weight, requires conversion parameters (see Table 3 in Chapter 3). Converting different measures of length required special attention because data included two types of carapace measurements, straight (SCL) and curved (CCL). One could convert one measurement into the other using a conversion formula, and over the course of time, many different conversion formulae were developed (cf. [64, 215, 4, 13, 178, 234]). However, by using a conversion formula, a set of implied assumptions must also be made [64, 137], and the step of converting the structural length (estimated by the model) to CCL via SCL, and vice versa, would introduce an unnecessary source of error. Instead, in addition to the shape coefficient parameter used to convert the structural length into SCL (henceforth marked as δ_{SCL}), a shape coefficient parameter δ_{CCL} was introduced to convert the predicted structural length into CCL:

$$L_{\text{SCL}} = L/\delta_{\text{SCL}},\ L_{\text{CCL}} = L/\delta_{\text{CCL}}, \tag{4.2}$$

The shape coefficients not only depend on shape, but also on the contribution of reserve to length. The compound parameter ω was used to account for the contribution of reserve to weight, i.e. to convert the model output to total weight:

$$W = L^3(1 + f\omega), \tag{4.3}$$

where f is the scaled food availability. The weight of adult (female) loggerhead turtles will also have a contribution from the reproduction buffer [94], but the contribution of the reproduction buffer to weight was here not included because the reproduction, i.e. egg production, was assumed continuous (calculated by the function `reprod_rate.m` of the DEBtool package,[112]). The clutch size (for the relationship of the clutch size to the carapace length of the nesting female) was calculated by calculating the reproduction rate for a certain length, and then transforming the value by using the average length of the remigration interval, number of clutches per season, and number of eggs per clutch.

After the predictions were obtained using the parameter set specific to the North Atlantic population ($pars_{\text{NA}}$) (step 1 in the Figure 4.1), the parameters were estimated for the Mediterranean population ($pars_{\text{Med}}$)(step 2 in the Figure 4.1). Out of the 19 parameters of the standard DEB model for the North Atlantic population, five parameter values were estimated specifically for the Mediterranean population: $[p_M]$, v, E_H^b, E_H^p, and h_a, and the additional parameter δ_{CCL}. Parameters z, κ, T_A, and δ_{SCL} were considered species-specific and the values estimated for the NA population were used. Consequently, maximum specific assimilation ($\{p_{Am}\} = z[p_M]/\kappa$) also differed between the populations. The parameters defining the maturity thresholds (E_H^b, and E_H^p) were initially considered species-specific and their values were not estimated, but the observed size difference at birth and puberty could not be reproduced.The values of these parameters were therefore estimated as well.

The differences between the data and the model predictions obtained using the estimated parameters were expressed as the relative error. The relative error, *RE*, was calculated in the same way as in Chapter 3: by dividing the absolute value of the difference between the value of the data point, *data*, and the value estimated by the model, *prdData*, by the value of the data point: *RE=|data-prdData|/data*. For data sets with more than one data point (uni-variate data), the relative error was calculated as the sum of relative errors for each data point, divided by the number of datapoints. The mean relative error of all data points and datasets (*MRE*) was then used to compute the FIT value as $10 \times (1 - MRE)$, and compare the goodness of fit to other DEB models in the "Add my pet" library [110]. The possible FIT values range from $-\infty$ to 10 [127].

4.2.2.2 Simulating the biphasic growth with the change in food availability

Chaloupka [40] had suggested that the growth of pelagic North Atlantic loggerhead turtles is polyphasic, with a few growth "spurts" during the life cycle. Casale et al. [38] concluded that the growth of the Mediterranean loggerhead turtles encountered in the Adriatic cannot be represented using a single von Bertalanffy growth curve, suggesting a polyphasic growth [38]. By describing the growth of pelagic loggerhead turtles (CCL$<$ 30 cm) by one von Bertalanffy growth curve [37], and that of larger (CCL$>$ 30 cm) loggerhead turtles by another von Bertalanffy growth curve [38, 39], the authors had indirectly assumed a biphasic growth.

In this study, a biphasic growth was assumed based on a drastic change in the environmental factors (temperature, and food type and availability) that loggerheads turtles experience during the ontogenetic habitat shift, i.e. recruitment to neritic habitats [23]. In this simplified scenario, the first phase would represent the oceanic juvenile life stage, with the loggerheads feeding on nutrient-poor pelagic prey, and the second phase the neritic juvenile (and adult) phase(s), with the loggerheads feeding on nutrient richer food. The phases were characterized by temperature and food availability. The temperature was assumed to be constant and (because the data available for validation [37, 39] describes only individuals in the Adriatic) equal to that experienced by the wild Mediterranean loggerhead turtles in the Adriatic sea ($T = 20$°C, [133]). The food was assumed to differ between phases: the scaled functional response (f) in the second phase was assumed to be equal to that of the wild Mediterranean loggerheads ($f_2 = 0.7$), while the scaled functional response in the first phase was assumed to be half of that: $f_1 = f_2/2$. Growth was assumed to be of the von Bertalanffy type during the periods of constant food, with the difference in the food availability resulting in different length at birth, asymptotic length, and the von Bertalanffy growth rate.

The length at birth has in Chapter 3 been overpredicted by 22%, explanations for which were discussed in Section 4.2. To reduce the error introduced by the model prediction, the observed physical length at hatching (practically equal to the length at birth, [8]) was used to calculate the structural length at birth using the shape coefficient ($L_b = \delta_{\text{SCL}}4.1$).

The switch between phases was assumed to be triggered by reaching a certain size (length) at which the loggerhead turtles can move to the habitat with the food of better quality. Length of 30 cm CCL, used previously as the upper [37] or lower [38, 34] length for a certain growth phase, was used to calculate L', the length triggering the phase switch ($L' = \delta_{\text{CCL}}30$). The asymptotic length was marked as $L_{1\infty}$ and the von Bertalanffy growth rate as r_{1B} for the initial (lower) food availability that resulted in the scaled functional response f_1, and as $L_{2\infty}$ and r_{2B} for the later (higher) food availability that resulted in the scaled functional response f_2. The length at time t during the first phase was then calculated as:

$$L(t) = L_{1\infty} - (L_{1\infty} - L_b)e^{-r_{1B}t}, L_b = L(0). \tag{4.4}$$

The time t', i.e. the age when the switch occurs was calculated as:

$$t' = \frac{1}{r_{1B}} ln \frac{L_{1\infty} - L_b}{L_{1\infty} - L'},$$

which made it possible to calculate the length at time t in the second phase as:

$$L(t) = L_{2\infty} - (L_{2\infty} - L')e^{-r_{2B}(t-t')}, L' = L(t'). \tag{4.5}$$

The model predictions were calculated using two parameter sets: $pars_{\text{NA}}$ and $pars_{\text{Med}}$ (see the Section 4.2.2.1 for details). The von Bertalanffy growth rate was calculated using the somatic maintenance rate coefficient, k_M, and the energy investment ratio, g, as:

$$r_{*B} = \frac{k_M/3}{1 + f_*/g}, \tag{4.6}$$

and then corrected for the effect of temperature using the equation 4.1.

Two data sets were used to reconstruct the growth curve, i.e. validate the model predictions for the growth of loggerhead turtles: (i) growth rates calculated by length-frequency analysis (5 datapoints in [37]), and (ii) age-at-length estimated by skeletochronology (33 datapoints in [39]). The relative error and the value of FIT were calculated in the same way as described in the section 4.2.2.1.

4.2.2.3 Modeling posthatchling growth

Results of the data analysis for captive reared posthatchlings of the North Atlantic population (Chapter 3) suggested that posthatchlings grow faster than older life stages, possibly due to intrinsic reasons (see Discussion of Chapter 3). Posthatchling growth was explored further in this chapter because the obtained data (unpublished, courtesy of S. Cateau) included data for posthatchling growth under known and controlled conditions. Because higher observed growth rates might be specific to the posthatchling stage, only data for this life stage was used for parameter estimation. The previously analyzed data (Chapter 3) for the 'NA' population (unpublished, obtained from L. Stokes) consisted of two data sets: one of individual weekly measurements until the turtles were 10 weeks (64 days) of age, the other of individual weekly measurements taken until the turtles were 13 weeks (85 days) of age. The data for the 'Med' population consisted also of two data sets: one data set of 3 measurements per individual taken until the turtles were approximately 8 weeks (55 days) of age, and the other of 12 measurements per individual taken until the turtles were 13 weeks (65 days) of age. All data that had been collected simultaneously within a population (i.e. when the posthatchlings were of the same age), were pooled together and reported as mean values to reduce the scatter introduced by inter-individual variability. For the 'NA' population, this yielded thirteen tL, tW, and LW data pairs for one dataset (length and weight values calculated as a mean of 40 samples), and ten tL, tW, and LW data pairs for the other data set (length and weight values calculated as a mean of 435 samples). For the 'Med' population, one data set yielded three tW data pairs, and the other nine tW data pairs. Because only wet weight-at-age data was available for the 'Med' population, size-at-age estimates obtained by length-frequency analysis for loggerhead turtles encountered in the Adriatic sea [37] were used to validate the model estimations.

The experimental conditions, i.e. the temperature and food availability, were reported for all data sets. The posthatchlings from the Mediterranean ('Med') population experienced temperature of 23.5ºC, and food was assumed *ad libitum* ($f = 1$) because the turtles were fed to maximize growth (S. Cateau, pers.comm.). The temperature experienced by the North Atlantic ('NA') posthatchlings was modeled to be a constant 27°C, but the food availability was modeled in more detail, to include the change in the feeding regime: food had been provided daily as 20% of the posthatchling's mass for the initial 15 days, and 8% of the posthatchling's mass for the remainder of the experiment [223]. It was modeled as $f = 1$, and $f = 0.9$, respectively.

Growth was assumed to be of the von Bertalanffy type during the periods of constant food, with the difference in the food availability resulting in different length at birth, asymptotic length, and the von Bertalanffy growth rate. The observed physical length at hatching (practically equal to the length at birth, [8]) was used to calculate the structural length at birth to reduce the error introduced by the model prediction (see Section 4.2.2.2). The physical length at hatching had not been reported for the captive-reared posthatchlings, but due to the similarity in weight at hatching with the North Atlantic hatchlings, a similar length as that reported for the North Atlantic hatchlings was assumed. Consequently, structural length at birth for both populations was calculated using the shape coefficient and the average length at hatching of the North Atlantic hatchlings (4.5 cm SCL, see Table 4.1) as $L_b = \delta_{\text{SCL}}4.5$. The asymptotic length and the von Bertalanffy growth rate, marked as $L_{1\infty}$ and r_{1B} (respectively) for the higher food availability, and as $L_{2\infty}$ and r_{2B} for the lower food availability were calculated independently for each scaled functional response (f_1 and f_2, respectively). The length at time t was calculated using the equations 4.4 and 4.5, with the time at change in food availability known ($t' = 15\,\text{d}$).

Most parameters were assumed species-specific, with the exception of those most directly related to the metabolism. Metabolic rates of loggerhead hatchlings had been observed to be several times higher than those of loggerhead juveniles (see Wallace and Jones [247] for an overview of metabolic rates of sea turtles). The energy conductance (v) and the maximum surface-area specific assimilation rate ($\{p_{Am}\}$) control the reserve dynamics, which fuels metabolism: v controls the mobilization of the reserve, whereas $\{p_{Am}\}$ controls its buildup. The surface-area specific assimilation rate is a primary parameter, which is fixed by the compound parameter, z, known as zoom factor: $\{p_{Am}\} = z[p_M]/\kappa$. Assuming κ and z to be species-specific, we directly coupled $\{p_{Am}\}$ and $[p_M]$. Parameters which were estimated specifically for the datasets were therefore $[p_M]$ and v, but $\{p_{Am}\}$ was affected as well. Parameters $[p_M]$ and v were used to calculate the compound parameters k_M and g, and therefore, together with food availability (f) and temperature, determined the growth rate (see equation 4.6).

The primary parameters estimated specifically for each population were marked as $'v_{\text{NA}}'$ and $'v_{\text{Med}}'$, and $'[p_M]_{\text{NA}}'$ and $'[p_M]_{\text{Med}}'$, and estimated simultaneously from all population-specific data using the weighted sum of squared deviation between data and predictions as estimation criterion. These estimates were obtained from guessed initial estimates with DEBtool routine `nmregr.m`, which uses the Nelder-Mead simplex method to find the parameter estimates. The relative error and the value of FIT were calculated in the same way as described in the section 4.2.2.1.

4.3 Results

4.3.1 Analyzing the "snapshots" of the size and physiology of individuals from the Mediterranean and North Atlantic populations

Results of the data analysis for eggs, hatchlings, and nesting females implied that the size difference is present in all three compared instances (Tables 4.1 and 4.2). Data availability hindered a more in-depth statistical analysis, but a visual analysis of the Mediterranean data in the context of North Atlantic data distributions was consistent with the reported [232] size dimorphism between the individuals from the Mediterranean ('Med') and the North Atlantic ('NA') populations, with the difference being more pronounced at the later life stage event (nesting).

For the size at hatching, all three datasets that were tested for normality (length and weight at hatching for the 'NA' population, and weight at hatching for the captive-reared loggerheads reared in the Mediterranean), rejected the null hypothesis of the samples coming from a normal distribution ($p > 0.05$). Histograms and normality plots (Figure 4.2, panels a and b) suggested a few outliers with larger sizes, skewing the distribution that otherwise resembled the normal one. The (non-parametric) Wilcoxon rank sum test at a 5% significance level ($p < 0.05$) rejected the null hypothesis that the weight samples of 'NA' hatchlings and captive-reared hatchlings are independent samples from the same distribution ($p = 0.1148$). Captive-reared hatchlings were heavier than the wild ('NA' and 'Med') hatchlings (Figure 4.2, panel b), which is consistent with the maternal effect, as implemented in the standard DEB model. The skewed distribution towards larger hatchlings, i.e. the lack of outliers on the low end of the distribution range, implied that a critical minimum, but not a maximum exists for size at hatching.

For the size at nesting, the distribution of length and weight of 'NA' loggerhead turtles was statistically not different (Lilliefors test, $p < 0.05$) from a normal distribution (Figure 4.3, panels a and b). The reported mean values for length and weight of the 'Med' nesting loggerhead turtles were smaller than the mean of the 'NA' loggerhead turtles (Table 4.2). The reported minimum and maximum values suggested generally smaller sizes at nesting, but we need to assume a normal distribution of nesting 'Med' loggerhead turtle sizes as well. On one hand, the *lengths* of two females from the reproduction program were within the observed range for 'Med' loggerhead turtles, and were at the lower end (80 cm SCL for one turtle) or below (70 cm SCL for the other turtle) of the range reported for 'NA' nesting females (Figure 4.2, panel a), suggesting that the females grew up in the Mediterranean sea. The *weights* of the two females, on the other hand, were above the Mediterranean range, and were close to the median values of the 'NA' weight distribution (Figure 4.3, panel b). The length of the females had not markedly changed in the four years that the length had been measured, and the weight during that period had been oscillating (unpublished data from S. Cateau). It can therefore be assumed that both females had reached the asymptotic (ultimate) length. The observed weight, but not length oscillations are consistent with a DEB-based interpretation. Length is directly related to the size of structure in DEB terms (via equation 4.2), and is expected not to decrease unless under the conditions of prolonged starvation [109]. Weight has contribution from both structure and reserve ([109], also see eq. 4.3), and can oscillate depending on the food availability, reproduction cycle, etc [109, 94]. The relatively small size of females in the reproduction program has two possible explanations: (i) The females experienced low food availability before they reached ultimate length, resulting in the small asymptotic (i.e. ultimate) length ($L_i = fL_\infty$). (ii) Their ultimate length is the result of the smaller genetically determined growth potential, implying that the females are of the Mediterranean origin.

The calculated condition indices suggested that the differences between life stages were markedly larger than the differences between the populations (Tables 4.1 and 4.2). The condition indices were higher at hatching than at nesting: the condition index of the hatchlings was 0.2140 for the North Atlantic individuals, and 0.2385 for the Mediterranean individuals, whereas the condition index of the nesting adults was 0.1413 for the North Atlantic individuals, and 0.1544 for the Mediterranean individuals. We refrained from directly comparing the condition indices at certain life stages (e.g. the condition index of the North Atlantic hatchlings to that of the Mediterranean hatchlings) because the condition index calculated using just the mean size and mean weight of the each population could not account for the interindividual variability. A more advanced analysis would require raw data for which individual condition indices could be calculated. The egg "condition index" was also similar between the two populations (0.5340 for the North Atlantic eggs, and 0.5578 for the Mediterranean eggs), suggesting that the ratio of the weight and cubed diameter of the egg has an evolutionary constraint.

The condition indices of the two females in the reproduction program suggested a large inter-individual variation (0.1959 and 0.2525), and was relatively low considering the high food availability of the reproduction program. As noted, weight has been oscillating during the four years that the measurements have been taken, and a larger weight than the one used in the analysis has been recorded at occasions. A possible explanation of the weight reduction is the event of reproduction, however this explanation is not very likely because the weight oscillations have been recorded in the same year for both females whereas the reproduction events were a year apart (unpublished data obtained from S. Cateau). Additionally, the measurements have been taken in December, while the reproduction events usually take place from April until June (S. Cateau, pers.comm.). Other possible explanations include water retention (K. Gobic Medica, pers.comm.), a decrease in the food availability, illness (causing a weight reduction), etc. The difference in the condition indices of two females kept in the same rearing facility (i.e. under controlled conditions), even if unexplained, does highlight the need for a statistically more comprehensive analysis of the hatchlings and nesting females of each of the two studied populations that would include the standard deviations of the calculated condition indices.

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Table 4.1: Overview of the data used in the analysis, and the calculated condition indices at *hatching* calculated for the mean values on a population level. To avoid biases introduced by the choice of length measurement, only straight carapace length (SCL) measurements were used. Length is expressed in centimeters, and weight as wet mass in grams. The range (minimum and maximum) and/or the standard deviation (SD) of the sample is given in the brackets where the information was available. The mean (in bold font) was calculated as the average value of all available values (or means) reported for a population. Data from the reproduction program is included as a separate group, with SCL at hatching unknown. Data sources are indicated next to each data set, and the number of data points (N) is provided in the table footer where the information was available.

Population		Length (SCL) (cm)	Weight (g)	Condition index
North Atlantic	Egg size	egg diameter (cm) 4.25 (SD 0.14) [232]	39.4 (SD 3.8) [232] 42.58 (SD 1.78) [1]	(0.5340)
	mean	**4.25**	**40.99**	
	Hatching	4.53 (SD 0.20) (4.17-5.23) [§] 4.6 (SD 0.11) (4.3-4.6) [185]	19.42 (SD 2.31) (14.9-29.47) [§] 19.8 (SD 1.33) (15.3-22.4) [185] 22.08 (SD 1.49) [1]	
	mean	**4.57**	**20.43**	0.2140
Mediterranean	Egg size	egg diameter (cm) 3.76 (SD 0.142)) [232]	27.6 (SD 3.1) [232] 30.48(SD 1.62) [187] 30.21 (SD 1.65) [187] 30.31 (SD 1.79) [187]	(0.5578)
	mean	**3.76**	**29.65**	
	Hatching	4.29 (SD 0.09) [187]$^{(a)}$ 4.24 (SD 0.10) [187]$^{(a)}$ 4.22 (SD 0.10) [187]$^{(a)}$ 4.04 (SD 0.7) [136]$^{(a)}$ 4.0 (2.49, 4.93) [136]$^{(b)}$ 4.1 (3.6, 4.5) [136]$^{(b)}$ 3.98 (2.8, 4.5) [136]$^{(c)}$ 3.91 (3.6, 4.2) [136]$^{(c)}$	16.74 (SD 0.82) [187]$^{(a)}$ 16.72 (SD 1.02) [187]$^{(a)}$ 16.59 (SD 0.90) [187]$^{(a)}$ 15.30 (9.4, 21.4) [136]$^{(b)}$ 16.30 (12, 21.5) [136]$^{(b)}$	
	mean	**4.1** (4.14)$^{(\text{a-b})}$	**16.33**	0.2385 (0.2301)$^{(\text{a-b})}$
Mediterranean reprod. program [§§]	Hatching		21.02 (SD 5.80) (16.7, 37.5)	

Data sources for North Atlantic: *Tiwari and Bjorndal [232]*, egg size: $N = 48$; *Ackerman [1]*, egg size: $N = 45$, hatchling size: $N = 41$; [§] *Stokes (unpublished.data)*, $N = 94$ for length, and $N = 94$ wet weight data; *Reich et al. [185]*, $N = 120$ for length and $N = 120$ for weight,
Data sources for Mediterranean: Tiwari and Bjorndal [232],egg size: $N = 23$; *Reid et al. [187]*, $N = 10$ for each SCL data group, and for each wet weight data group; *Margaritoulis et al. [136]*, overview of published data on the Mediterranean population of loggerhead turtles; [§§] unpublished data obtained from S. Cateu, reproduction program in Marineland (Antibes)
Data for loggerhead turtles in: $^{(a)}$Greece; $^{(b)}$Cyprus; $^{(c)}$Turkey

Table 4.2: Overview of the data used in the analysis, and the calculated condition indices at *nesting* calculated for the mean values on a population level. To avoid biases introduced by the choice of length measurement, only straight carapace length (SCL) measurements were used. Length is expressed in centimeters, and weight as wet mass in kilograms. The range (minimum and maximum) and/or the standard deviation (SD) of the sample is given in the brackets where the information was available. The mean (in bold font) was calculated as the average value of all available values (or means) reported for a population. Data from the reproduction program is included as a separate group, with SCL at hatching unknown. Data sources are indicated next to each data set, and the number of data points (N) is provided in the table footer where the information was available.

Population		Length (SCL) (cm)	Weight (kg)	Condition index
North Atlantic	Nesting	90.9 (SD 5.0) (76.801-100.276) [232]	103.95 (SD 17.21) (63.9-152.44) [†]	
		92.01 (SD 5.34) (78.89-104.47) [54]*	118.2 (SD 17.5) (89.70-170.90) [54]	
		90.9 (SD 4.9) (82-103) [54]	116.3 (SD 17.1) (71.70-148.90) [54]	
		92.3 (SD 5.6) (81-110) [54]	114.7 (SD 20.3) (79.60-180.70) [54]	
		94.73 (SD 5.29) (80.72-107.34) [28]*		
		94.3 (SD 5.5) (83.8-106.7) [28]		
		95.1 (SD 4.8) (80.7-107.4) [28]		
	mean	**92.89**	**113.29**	0.1413
Mediterranean	Nesting	79.43(SD 4.4) (74.308, 84.37) [232]$^{(a)}$	67.26 (52.5, 87.00)[77]$^{(a)}$	
		78.45 (63.5, 87.0) [136]$^{(a)}$		
		78.85 (66, 95.00) [136]$^{(a)}$		
		78.75 (68.5, 90.00) [136]$^{(a)}$		
		73.1 (60.2, 83.90) [136]$^{(c)}$		
		73.2 (66, 87.50) [136]$^{(c)}$		
		72 (58, 87.00) [136]$^{(c)}$		
		78.7 (62.3, 83.20) [136]$^{(d)}$		
	mean	**75.81** (78.68)$^{(a)}$	**67.26**	0.1544 (0.1381)$^{(a)}$
Mediterranean reprod. program [++]	Nesting	80	100.3	0.1959
		70	86.6	0.2525

Data sources for North Atlantic: *Tiwari and Bjorndal [232]*, nesting females: $N = 51$; *Ehrhart and Yoder [54]*: *values for SCL digitalized from Figure3, $N = 102$; other values from Table1: for SCL $N = 84$, and $N = 110$, and for weight $N = 47$, $N = 93$, and $N = 121$. textitByrd et al. [28]: *values for SCL digitalized from Figure3, $N = 112$; other values from Table1 for SCL $N = 41$, and $N = 84$; [†] The weight was calculated from data in Ehrhart and Yoder [54] and Byrd et al. [28] using the allometric equation from Wabnitz and Pauly [244], $N = 214$;

Data sources for Mediterranean: Tiwari and Bjorndal [232], nesting females: $N = 14$ (Greece); *Groombridge [77]*; *Margaritoulis et al. [136]*, overview of published data on the Mediterranean population of loggerhead turtles; [++] unpublished data obtained from S. Cateu, reproduction program in Marineland (Antibes)

$^{(a)}$ Data for loggerhead turtles nesting in Greece; $^{(b)}$ Data for loggerhead turtles nesting in Cyprus; $^{(c)}$ Data for loggerhead turtles nesting in Turkey; $^{(d)}$ Data for loggerhead turtles nesting in Libya;

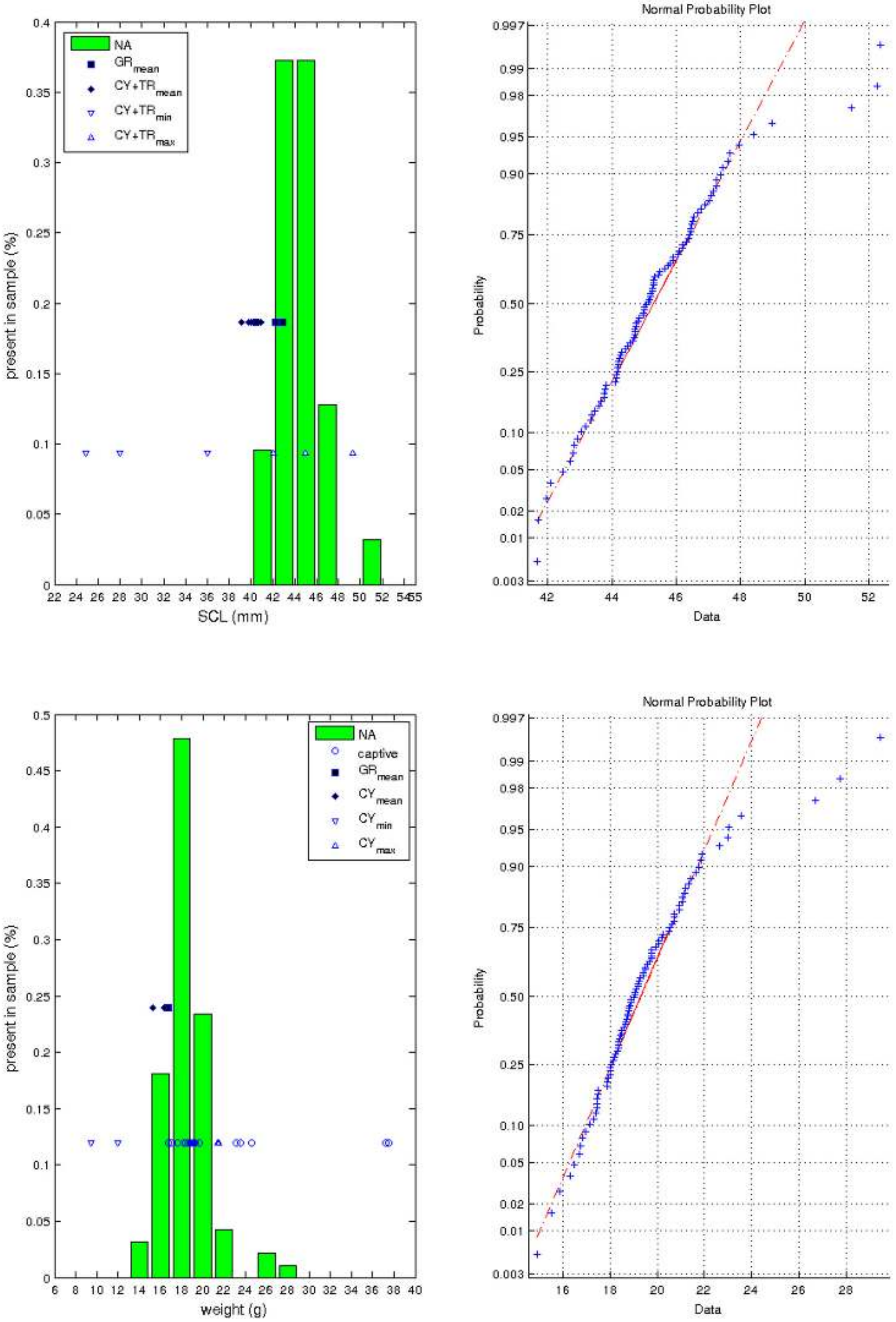

Figure 4.2: Results of the morphological size comparison: the distribution and measurements of length (panel a) and weight (panel b) at hatching. The data for the North Atlantic population is presented as histogram and normality plots. The data for the Mediterranean population is included in the histogram plots as individual data points, with the type of data (mean from a sample, or an individual measurement) taken into account by adjusting the "visual weight" of data: the height of the plotted data point corresponds to 1/2 (if point represents the sample mean) or 1/4 (if the point is an individual data point) of the height of the highest histogram bar in that plot. To account for the difference between the Mediterranean subpopulations and also to identify the individuals from rearing facilities, data for hatchlings are plotted with different symbols.

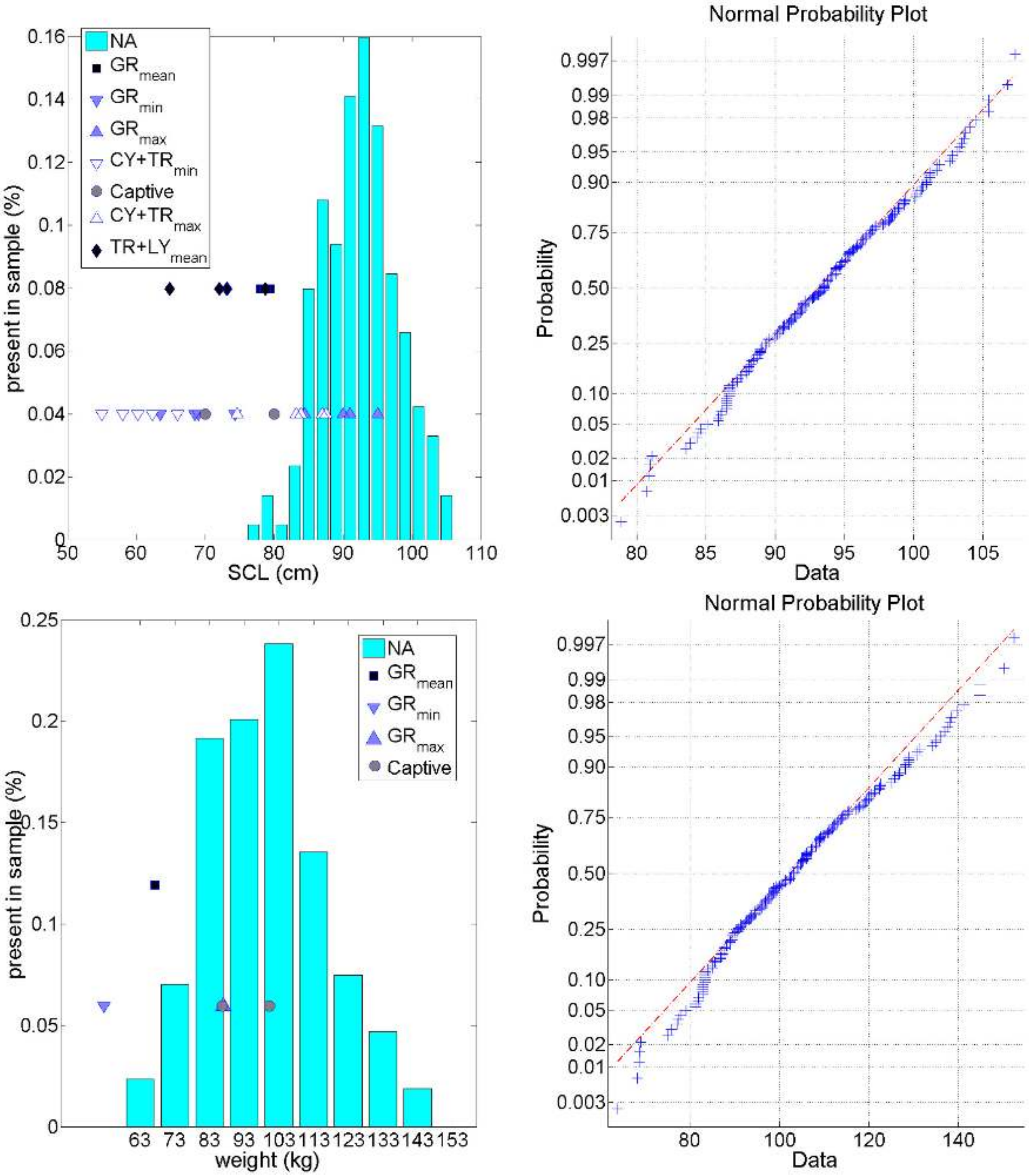

Figure 4.3: Results of the morphological size comparison: the distribution and measurements of length (panel a) and weight (panel b) at nesting. The data for the North Atlantic population is presented as histogram and normality plots. The data for the Mediterranean population is included in the histogram plots as individual data points, with the type of data (mean from a sample, or an individual measurement) taken into account by adjusting the "visual weight" of data: the height of the plotted data point corresponds to 1/2 (if point represents the sample mean) or 1/4 (if the point is an individual data point) of the height of the highest histogram bar in that plot. To account for the difference between the subpopulations nesting in Greece and those nesting in Turkey, Libya and Cyprus, and also to identify the individuals from rearing facilities, the points are plotted with different symbols.

4.3.2 DEB model

Model predictions were first obtained using the parameter set specific to the North Atlantic population, 'pars_{NA}', and then using the parameter set specific to the Mediterranean population, 'pars_{Med}'. Initially, individuals were assumed to experience a lower food availability in the Mediterranean sea than in the North Atlantic [130, 171, 263], resulting in a lower scaled functional response: $f_{\text{Med}} < f_{\text{NA}}$. The analysis of the physiological indices suggested however a similar condition index of the individuals belonging to the two populations (see Section 4.3.1). To account for the possibility of the individuals having the same scaled functional response, both scaled functional responses were simulated in each step. In total, four scenarios were tested:

- 1.1 '$pars_{\text{NA}} + f_{\text{Med}}$': parameter set estimated for the North Atlantic population and the assumed scaled functional response of 0.71
- 1.2 '$pars_{\text{NA}} + f_{\text{NA}}$':parameter set estimated for the North Atlantic population and the assumed scaled functional response of 0.81
- 2.1 '$pars_{\text{Med}} + f_{\text{Med}}$': parameter set estimated for the Mediterranean population and the assumed scaled functional response of 0.71
- 2.2 '$pars_{\text{Med}} + f_{\text{NA}}$': parameter set estimated for the Mediterranean population and the assumed scaled functional response of 0.81

Model predictions with the parameter set specific to the North Atlantic population: Scenarios 1.1 and 1.2 The calculated mean relative error of all predictions was similar in both scenarios: 0.2190 (FIT=7.8164) in scenario 1.1 ('$pars_{\text{NA}} + f_{\text{Med}}$'), and 0.2308 (FIT=7.69) in scenario 1.2 ('$pars_{\text{NA}} + f_{\text{NA}}$').

Model predictions for zero-variate data differed between scenarios 1.1 ('$pars_{\text{NA}} + f_{\text{Med}}$') and 1.2 ('$pars_{\text{NA}} + f_{\text{NA}}$') because the predictions for life history traits, such as size and reproduction, strongly depend on the assumed food availability [109]. Zero-variate data predictions for scenario 1.1 are presented in Table 4.3. Scenario 1.2 yielded the same zero-variate data predictions that were obtained as the model estimations for the North Atlantic population and can be found in the Table 4 of Chapter 3. Mean relative error of the zero-variate data was 0.1695 for scenario 1.1, and 0.2077 for scenario 1.2. Compared to the observations, predictions for the lower food level (scenario 1.1) suggested approximately 35% larger size (length and weight), 20% larger length at puberty, 7% larger weight at puberty, 4% smaller ultimate length, 15% smaller ultimate weight, and 64% smaller maximum reproduction rate. The age at puberty was predicted to be close (19.8 years) to the value taken as the observed age at puberty (20 years). Predictions for the higher food level (scenario 1.2) were also 36% larger for the length at birth, and 20%

larger for the length at puberty, but the weight at birth was now overpredicted by 46% and the weight at puberty by 19%. The ultimate size was overpredicted by 10% (length) and 38% (weight), and the maximum reproduction rate by 30%. The prediction for the age at puberty (13.20 years) was lower than the value used as "observed" data.

Model predictions for uni-variate data were mostly lower than the observed data (Figures 4.5, 4.6, and 4.7, with predictions of the 1.1 scenario plotted as dashed gray lines). When comparing the two scenarios (1.1 and 1.2), predictions did not differ for the captive-reared individuals, because the food availability for that data was in both cases assumed *ad libitum*. The mean relative errors of uni-variate data were similar (0.2467 for scenario 1.1 and 0.2437 for scenario 1.2).

Table 4.3: Comparison between observations and model predictions, for the scenarios marked as 1.1 (parameter set estimated for the North Atlantic population and the assumed scaled functional response of 0.71), and 2.1 (parameter set estimated for the Mediterranean population and the assumed scaled functional response of 0.71). The relative errors (column 5) were calculated for the predictions in the scenario 2.1. Temperature was assumed constant with $T = 21^\circ$ C [133].

Data	Predicted (scenario 1.1)	Predicted (scenario 2.1)	Observed	Relative error	Observed, range	Unit	Reference
age at hatching	49.55	**48.45**	**49.08**	0.0128	45.8-55.8	d	[187]
age at birth	57.79	**56.53**	**55.18**	0.0245	2-3 d after emergence	d	[70][§]
age at puberty	19.79	**11.67**	**20.00**	0.4167	14-28	yrs	[39, 181]
life span	66.40	**61.51**	**67.00**	0.0090	65+	yrs	[215, 78]
SCL at birth	5.563	**5.56**	**4.10**	0.3560	2.5-4.9	cm	[187, 136]
SCL at puberty	77.11	**66.03**	**64.20**	0.0285	55-69	cm	[136, 77, 232]
CCL at puberty	83.21	**71.25**	**69.00**	0.0326	60-78	cm	[136]
ultimate SCL	83.57	**83.57**	**87.00**	0.0394	77-91	cm	[136, 77, 232]
ultimate CCL	90.19	**90.19**	**91.00**	0.0089	85-99	cm	[136]
wet weight at birth	21.7	**21.77**	**16.10**	0.3523	9.4-21.5	g	[136]
wet weight at puberty	36.47	**57.78**	**52.00**	0.2986	52.5	kg	[77]
ultimate wet weight	73.96	**73.57**	**87.00**	0.1499	87	kg	[77]
initial energy content of the egg	197.75	**197.93**	**170.00**	0.1643	165-260	kJ	[88]
maximum reproduction rate	0.2389	**0.5867**	**0.6575**	0.1077	0.3452-0.8630	eggs/day	[136, 77, 232]

[§] unpubl. data obtained from L. Stokes

Estimating the parameters for the Mediterranean population: Scenarios 2.1 and 2.2. The parameters $[p_M]$, v, E_H^b, E_H^p, and h_a were first estimated assuming the lower food availability (scenario 2.1, 'pars$_{\text{Med}} + f_{\text{Med}}$'), and the estimated parameters are presented in Table 4.4. The main differences in the values were for the following three primary parameters: volume-specific somatic maintenance ($[p_M]$), energy conductance (v), and maturity at puberty (E_H^p). The estimated value of the somatic maintenance rate (13.65 J/d.cm^3) was higher than the value for NA (13.25 J/d.cm^3), the estimated value of the energy conductance (v) was slightly higher (0.0723 cm/d compared to 0.0708 cm/d), and the maturity at puberty (E_H^p) was lower ($5.713e + 07$ J compared to $8.73e + 07$ J) (Table 4.4). The estimated value for the maturity at birth parameter (E_H^b), which was expected to differ in the same way between populations, was the same as the value estimated for the North Atlantic population, suggesting it was not defined well by the data as the maturity level at puberty was. The model predictions obtained in this scenario ('$pars_{\text{Med}} + f_{\text{Med}}$') had the best fit to the observed data (mean relative error 0.1909; FIT=8.1) and are presented in Table 4.3 (columns 2 and 4) and Figures 4.4, 4.5, 4.6, and 4.7. These model predictions will be discussed later in more detail.

Next, the scenario 2.2 ('pars$_{\text{Med}} + f_{\text{NA}}$') was tested. The parameters were again estimated using the Mediterranean data, and the obtained parameter values were close to those of the North Atlantic population ($v = 0.07225$ cm/d, $[p_M] = 13.89$ J/d.cm^3, $E_H^b = 3.81e + 04$ J, $E_H^p = 1.241e + 08$ J; see column 3 of Table 4.4 for comparison). Most zero-variate predictions were larger than observed, the reproduction rate by as much as a factor of two (1.054 egg/day). The age at hatching and age at birth were close to the values observed in nature, and the age at puberty was underpredicted. The mean relative error of all predictions was 0.2233 (FIT=7.77), with the mean relative error of zero-variate predictions being 0.2347, and the mean relative error of uni-variate predictions being 0.2170. Some uni-variate predictions (for datasets tL and tW) had a relative error close to 1.

Finally, the parameter f was allowed to be estimated together with other parameters that were estimated for the Mediterranean population. The value of around 0.72 was obtained ($f = 0.7228$), with values of parameters $[p_M]$, E_H^p, and v being very similar to that estimated in scenario 2.1 ($[p_M] = 13.72$ J/d.cm^3, $E_H^p = 5.953e + 07$ J, $v = 0.07907$ cm/d; see column 2 of Table 4.4 for comparison).

Model predictions with the parameter set specific to the Mediterranean population: Scenario 2.1. All predictions of the model for zero-variate data were realistic (Table 4.3, columns 2 and 6). Predictions for length and weight at birth, and for the initial energy of an egg would probably be improved by adjusting the maturity at birth (parameter E_H^b) which was poorly defined by data. The predicted length at puberty was very close (4% larger) to the average of minimum nesting sizes reported for Mediterranean loggerhead turtles [77, 136]. The average of minimum nesting sizes (used as the “observed data”) was influenced by the proportion of values reported for different subpopulations of the

Table 4.4: List of primary and auxiliary parameters estimated for the loggerhead turtle (*Caretta caretta*) of the Mediterranean population. The shape coefficient δ_{SCL} was used to convert the predicted structural length into straight carapace length (SCL), as was done for the North Atlantic population. The additional shape coefficient δ_{CCL} was used to convert the predicted structural length into curved carapace length (CCL) for the Mediterranean population, and into an unspecified length measurement for the North Atlantic population, therefore the parameter values cannot be directly compared between populations. Parameters estimated in the previous chapter for the North Atlantic population are listed in column two as *C. caretta* $'pars_{\mathrm{NA}}'$. Parameters for two other sea turtles in the "Add my pet" library are given for comparison: Kemp’s ridley (*Lepidochelys kempii*, [179]), and leatherback turtle (*Dermochelys coriacea*, [105]). Typical values for a generalized animal with maximum length $L_m = zL_m^{ref}$ (for a dimensionless zoom factor z and $L_m^{ref} = 1\,\mathrm{cm}$), were taken from Lika et al. [126] and Kooijman [109], Table 8.1, p300. All rates are given for the reference temperature of 20° C. Parameters for the Mediterranean population ($'pars_{\mathrm{Med}}'$) were estimated while assuming $f_{\mathrm{Med}} = 0.71$. Not all parameters were estimated for the Mediterranean population - the estimated parameters are indicated with a number ’1’ in the estimated (Est) column and presented in bold font.

Parameter	Est.	*C. caretta*, $'pars_{\mathrm{Med}}'$	*C. caretta*, $'pars_{\mathrm{NA}}'$	*L. kempii*	*D. coriacea*	Typical value (gen. animal)	Unit
z	0	44.32	44.32	25.02	51.57	L_m/L_m^{ref}	-
$\{F_m\}$	0	6.5	6.5	6.5	6.5	6.5	l/d.cm^2
κ_X	0	0.8	0.8	0.8	0.206503	0.8	-
κ_X^P	0	0.1	0.1	0.1	0.2	0.1	-
v	1	**0.072288**	**0.07084**	0.0424059	0.0865079	0.02	cm/d
κ	0	0.6481	0.6481	0.692924	0.916651	0.8	-
κ_R	0	0.95	0.95	0.95	0.95	0.95	-
$[p_M]$	1	**13.65**	**13.25**	20.1739	21.178	18	J/d.cm^3
k_J	0	0.002	0.002	0.002	0.002	0.002	1/d
$[E_G]$	0	7847	7847	7840.77	7843.18	$2800d_V$	J/cm^3
E_H^b	1	**3.809e+04**	**3.809e+004**	1.324e+04	7.550e+03	$0.275\ z^3$	J
E_H^p	1	**5.713e+07**	**8.73e+007**	3.6476e+07	8.2515e+07	$166\ z^3$	J
h_a	1	**1.44e-10**	**1.85e-010**	1.42057e-09	1.93879e-09	$10^{-6}z$	1/d^2
s_G	0	0.0001	0.0001	0.0001	0.01	-	
T_{ref}	0	293.15	293.15	293.15	293.15	293.15	K
T_A	0	7000	7000	8000	8000	8000	K
δ_{SCL}	0	0.3744	0.3744	0.3629	0.3397	>0	-
δ_{CL}	1	0.3470$^{\mathrm{a}}$	0.347$^{\mathrm{a}}$	-	-	-	-
$d_V = d_E$	0	0.28$^{\mathrm{b}}$	0.28 $^{\mathrm{b}}$	0.3	0.3	0.3	-
$\{p_{Am}\}$	0	**933.1**$^{\mathrm{c}}$	**906.1**$^{\mathrm{c}}$	728.426	1191.41	$22.5\ z$	J/d.cm^2

[a] Shape coefficients cannot be directly compared because they do not convert the same carapace length: for the Mediterranean population the curved carapace length has been reported, whereas for the North Atlantic population the type of length measurement has not been reported.

[b] Value from Kraemer and Bennett [115].

[c] Primary parameter calculated from the primary parameters κ and $[p_M]$ and the compound parameter z as $\{pA_m\} = z[p_M]/\kappa$.

Mediterranean loggerheads. The Greek subpopulation comprises the largest proportion of the Mediterranean population [136], but almost half of the available data related to other subpopulations (see Table 4.2). Females start nesting in Libya and Cyprus at smaller sizes than in Greece [77, 136], lowering the average minimum nesting size. The predictions for age at puberty being lower than data used as "observed values" suggests that, if the loggerhead turtles experience relatively constant conditions throughout their life, they start allocating to reproduction several years prior to the age estimated as age at puberty. This is consistent with the results for the North Atlantic population (Chapter 3).

The model reproduced growth in length, and length to weight relationship of captive-reared juveniles well (Figure 4.6), and growth in weight was reproduced reasonably well (Figure 4.5, and panel a in Figure 4.7). Individuals kept at very similar conditions had exhibited markedly different growth patterns (for example, see Figure 4.5 panel c, and Figure 4.6 panel b), and the relative error for those datasets contributed substantially to the overall mean relative error. The clutch size as a function of carapace length (Figure 4.7 panel b) was predicted satisfactory in terms of the relative error 0.0842), and the trend (slope) of the prediction could be adjusted by considering ecological implications such as the optimal clutch size. Incubation duration as a function of temperature (Figure 4.7 panel c) was predicted reasonably well. The prediction for the incubation duration would probably be improved by adjusting the maturity at birth and initial energy of an egg.

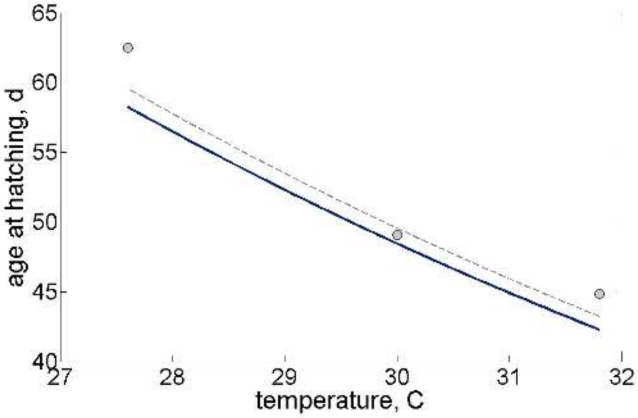

Figure 4.4: Incubation duration as a function of incubation temperature - data and model predictions. The predictions obtained using the parameters specific to the Mediterranean population ($pars_{\mathrm{Med}}$, column 2 of Table 4.4) are shown as full lines, and the predictions obtained using the parameters specific to the North Atlantic population ($pars_{\mathrm{NA}}$, column 3 of Table 4.4) are shown as dashed gray lines. The scaled functional response was assumed to be 0.71. Data from Reid et al. [187].

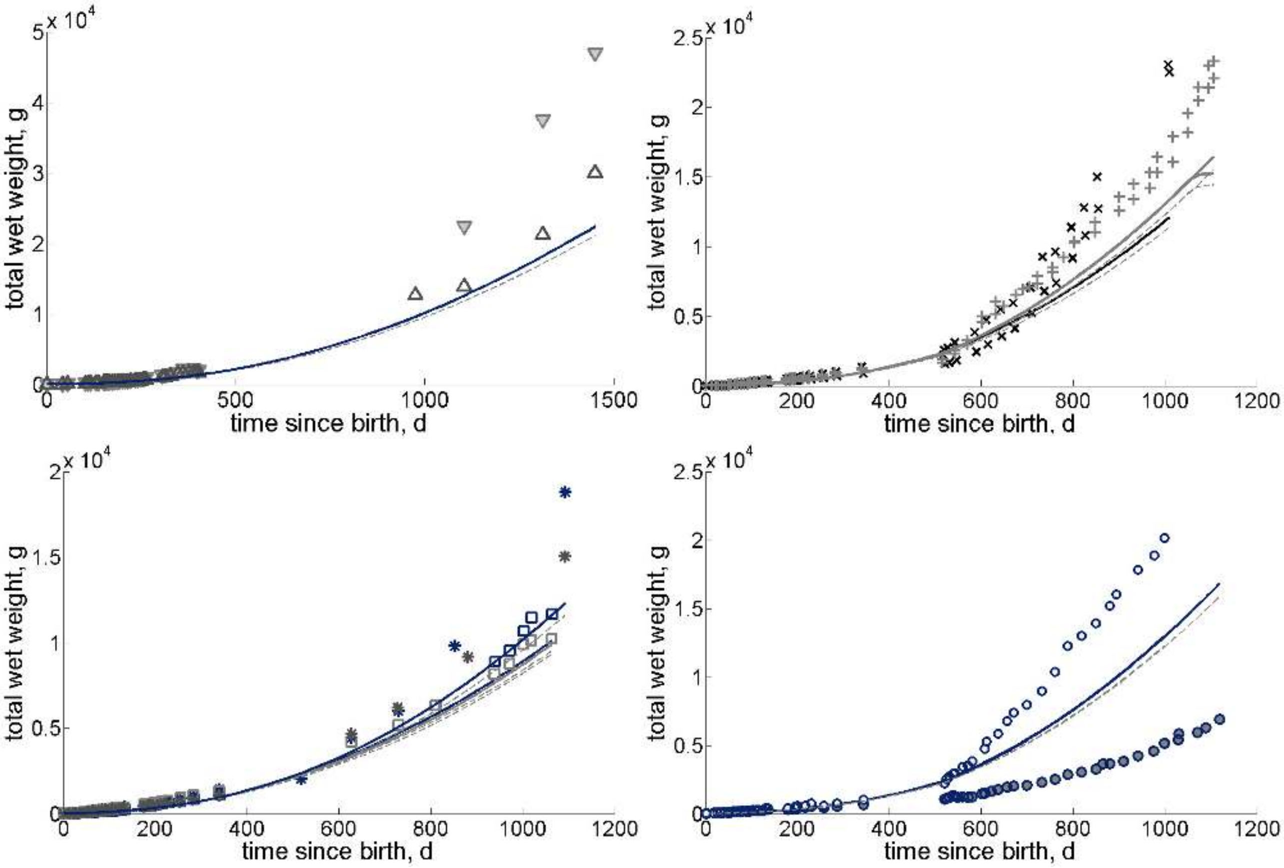

Figure 4.5: Comparison between observations and model predictions for the age to weight relationship of the captive reared posthatchlings and juveniles. Food was assumed *ad libitum* and the temperature differed between data sets (see Appendix A for more details). The predictions obtained using the parameters specific to the Mediterranean population ($pars_{\mathrm{Med}}$, column 2 of Table 4.4) are shown as full lines, and the predictions obtained using the parameters specific to the North Atlantic population ($pars_{\mathrm{NA}}$, column 3 of Table 4.4) are shown as dashed gray lines. Datasets are plotted separately for better readability.

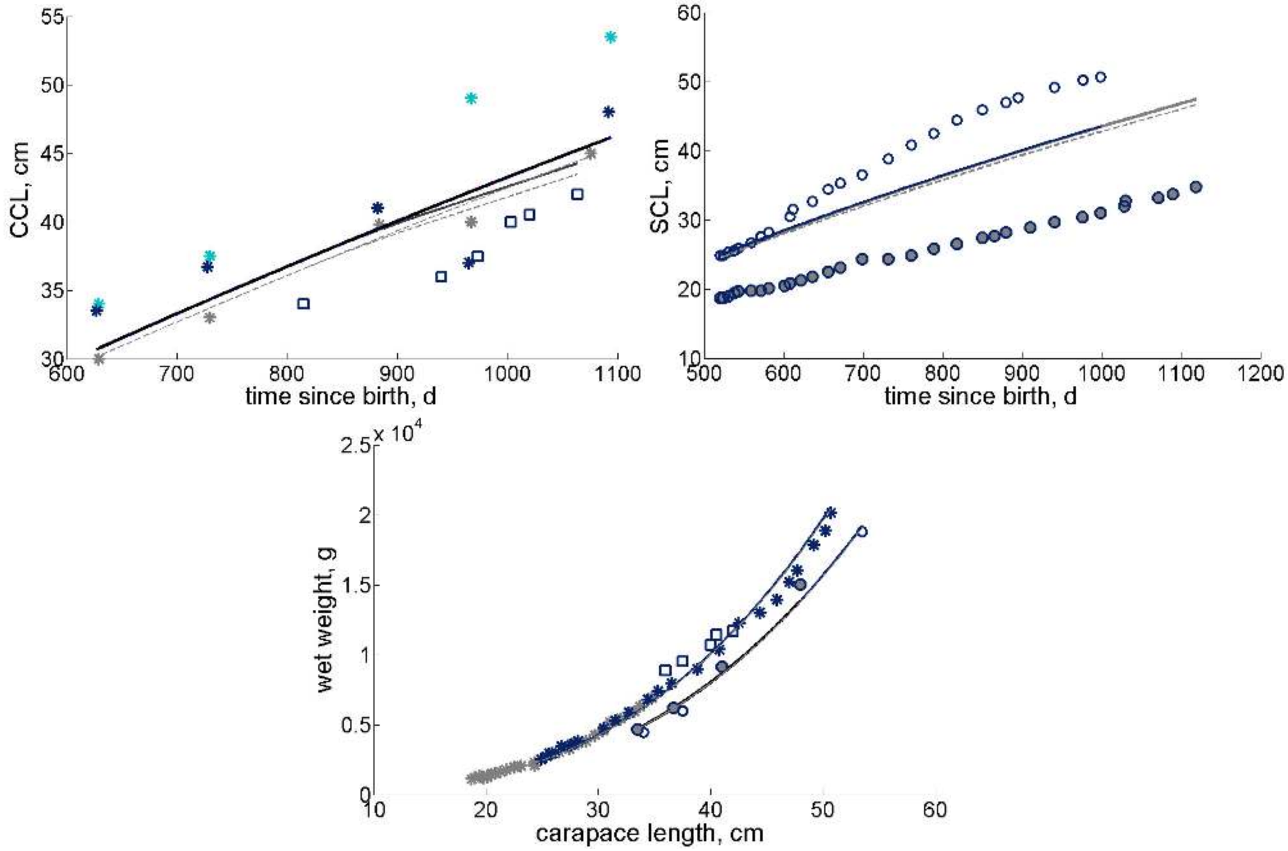

Figure 4.6: Comparison between observations and model predictions for the age to length (panels a and b), and length to weight (panel c) relationships of the captive reared posthatchlings and juveniles. Food was assumed *ad libitum* and the temperature differed between data sets (see Appendix A for more details). The predictions obtained using the parameters specific to the Mediterranean population ($pars_{\mathrm{Med}}$, column 2 of Table 4.4) are shown as full lines, and the predictions obtained using the parameters specific to the North Atlantic population ($pars_{\mathrm{NA}}$, column 3 of Table 4.4) are shown as dashed gray lines. The symbols for data correspond to those in Figure 4.5.

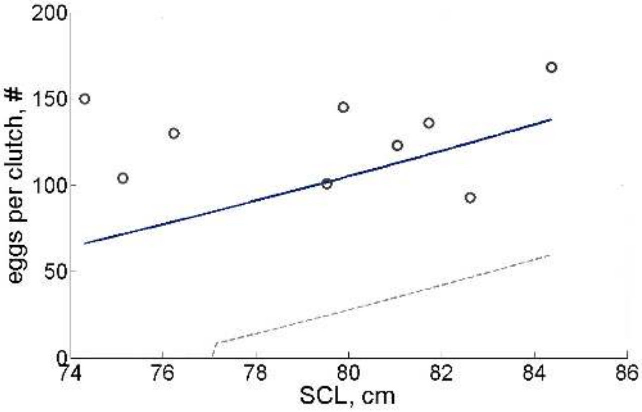

Figure 4.7: Clutch size as a function of carapace length. The scaled functional response was assumed to be 0.71, and temperature to be $T_{\text{Med}} = 21°\,\text{C}$, [133]. The predictions obtained using the parameters specific to the Mediterranean population ($pars_{\text{Med}}$, column 2 of Table 4.4) are shown as full lines, and the predictions obtained using the parameters specific to the North Atlantic population ($pars_{\text{NA}}$, column 3 of Table 4.4) are shown as dashed gray lines.

4.3.3 Simulating biphasic growth with a change in food availability

The change in food availability was modeled as $f_1 = f_2/2$, and $f_2 = 0.71$, while keeping the values of all other parameters constant, reproduced the pattern of biphasic growth and well described the length-at-age data for the Mediterranean loggerhead turtles in the Adriatic (Figure 4.8). Predictions calculated with the parameter values specific to the Mediterranean population ($pars_{\text{Med}}$) had a marginally smaller mean relative error (MRE = 0.1630) and hence a higher value of FIT (8.37) than the predictions calculated with the parameter values specific to the North Atlantic population ($pars_{\text{NA}}$) (MRE = 0.1671, FIT= 8.33). The von Bertalanffy growth rate (at reference temperature, $T_{\text{ref}} = 273\,\text{K}$ and calculated using the $pars_{\text{Med}}$ parameters) in the first nutrient poor ("oceanic") phase (equation 4.4) was $6.36\text{e}^{-4}\,\text{d}^{-1}$, and in the second, nutrient richer ("neritic") phase (equation 4.5), it was lower: $4.73\text{e}^{-4}\,\text{d}^{-1}$. Very similar growth patterns were observed and successfully reproduced by a DEB model for other organisms experiencing periods of two different (constant) food densities (cf. Figures 4.2 and 6.3 in Ref. [109]), with slightly smoother transitions between two parts of the growth curves due to reserve dynamics that smooth out the changes in environmental food availability. The "smoothing out" would probably not be visible for the growth curves of loggerhead turtles because of a very large time scale and therefore low resolution of data and plotted predictions.

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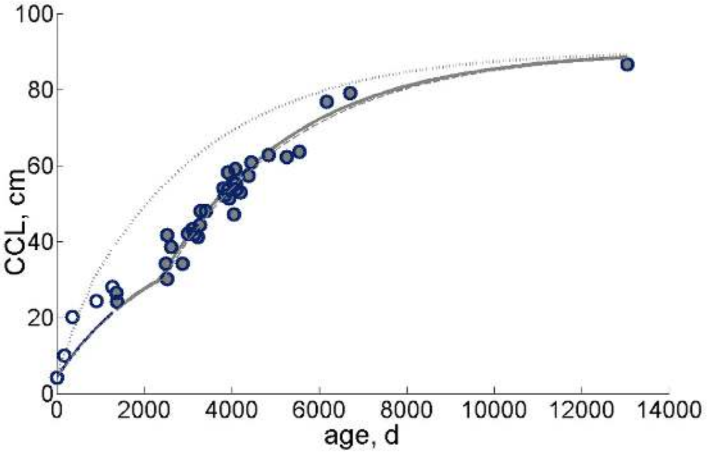

Figure 4.8: The model predictions for the growth of the Mediterranean loggerhead turtles experiencing a change in the food availabilities during their life cycle. Food availability in the first part (oceanic phase) was assumed to be lower than that of the second part (neritic phase), with the relationship between two scaled functional responses being $f_1 = f_2/2$. The predictions calculated using the parameter values specific to the Mediterranean population (pars_{Med}) are shown with full lines, and the predictions calculated using the parameter values specific to the North Atlantic population (pars_{NA}) are shown with dashed lines (for parameter values see Table 4.4). The dotted line represents the classic von Bertalanffy growth curve obtained assuming the constant food availability and using the pars_{Med} parameters. Data taken from Refs. [39] and [37].

4.3.4 Posthatchling growth

The model predictions described the first 10 to 13 weeks of growth for the North Atlantic and Mediterranean captive reared posthatchlings well (Figure 4.9). The overall goodness of fit was extremely high, with the value of FIT=9.1. Somatic maintenance rate ($[p_M]$), energy conductance (v), and maximum assimilation rate($\{p_{Am}\}$) had a higher value than when the parameters were estimated using data for all life stages (cf. Table 4.4), and all three were larger for the Mediterranean posthatchlings than for the North Atlantic posthatchlings (Table 4.5). When calculated for the same (reference) temperature T_{ref} and $f = 1$, the von Bertalanffy growth rate of the posthatchlings reared in the Mediterranean was higher than that of the posthatchlings from North Atlantic: $r_{B_{\text{Med}}} = 3.88e^{-4}\,\text{d}^{-1}$, and $r_{B_{\text{NA}}} = 3.09e^{-4}\,\text{d}^{-1}$. After correcting all rates for the temperature present in the rearing facilities, the von Bertalanffy growth rates, as well as observed and calculated absolute growth rates, were similar (Table 4.5). The contribution of reserve to weight (ω), a compound parameter used to calculate the wet mass (equation 4.3), had a similar value for both populations (Table 4.5) which is an interesting result in the context of similar condition indices of hatchlings obtained in the Section 4.3.1 (Table 4.1).

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Table 4.5: Estimated primary parameters v and $[p_M]$ (with standard deviations in brackets) for the North Atlantic and the Mediterranean captive reared posthatchlings. The values of compound parameters k_M, g, and ω are listed as well. All parameters are listed at the reference temperature, $T_{ref} = 273\,\mathrm{K}$. The von Bertalanffy growth rates (r_B) are presented at the reference temperature and at the actual temperature of the dataset (temperature-corrected von Bertalanffy growth rates, rT_B). Absolute growth rates were calculated as the change in length between the first and the last measurement, divided by the time in days, and then transformed into a yearly growth rate.

Parameter	North Atlantic	Mediter-ranean	Unit	Comment
energy conductance, v	0.07141 (0.002111)	0.08582 (0.00980)	cm/d	standard deviation of the parameters is given in brackets
volume specific somatic maintenance rate, $[p_M]$	17.1 (0.2803)	22.86 (0.7194)	J/d.cm^3	standard deviation of the parameters is given in brackets
surf.area specific maximimu assimilation rate, $\{p_{Am}\}$	1169.37	1563.27	J/d.cm^3	directly linked to $[p_M]$ when κ and z are assumed constant: $\{p_{Am}\} = z[p_M]\,\kappa$
somatic maintenance rate coefficient, k_M	0.0022	0.0029	1/d	$k_M = [p_M]/[E_G]$
energy investment ratio, g	0.7394	0.6647	-	$g = [E_G]/(\kappa * \{p_{Am}\}/v)$
contribution of reserve to weight, ω	2.3690	2.6353	-	
von Bertalanffy growth rate, r_B	3.09e-004	3.884e-004	1/d	at $f = 1$ and T_{ref}
von Bertalanffy growth rate, r_B	1/d	5.45e-004	5.20e-004	at $f = 1$ and $T_{\mathrm{NA}} = 27°\mathrm{C}$, and $T_{\mathrm{Med}} = 23.5°\mathrm{C}$
absolute growth rates	22.04 (23.22)*, 23.06 (23.10)*	(23.49), (23.44)	cm/yr	The values in brackets were calculated for the predicted, rather than measured length.

* For the North Atlantic population, datasets for 10 and 13 weeks yielded different growth rates (see Section 4.2.2.3 for details).

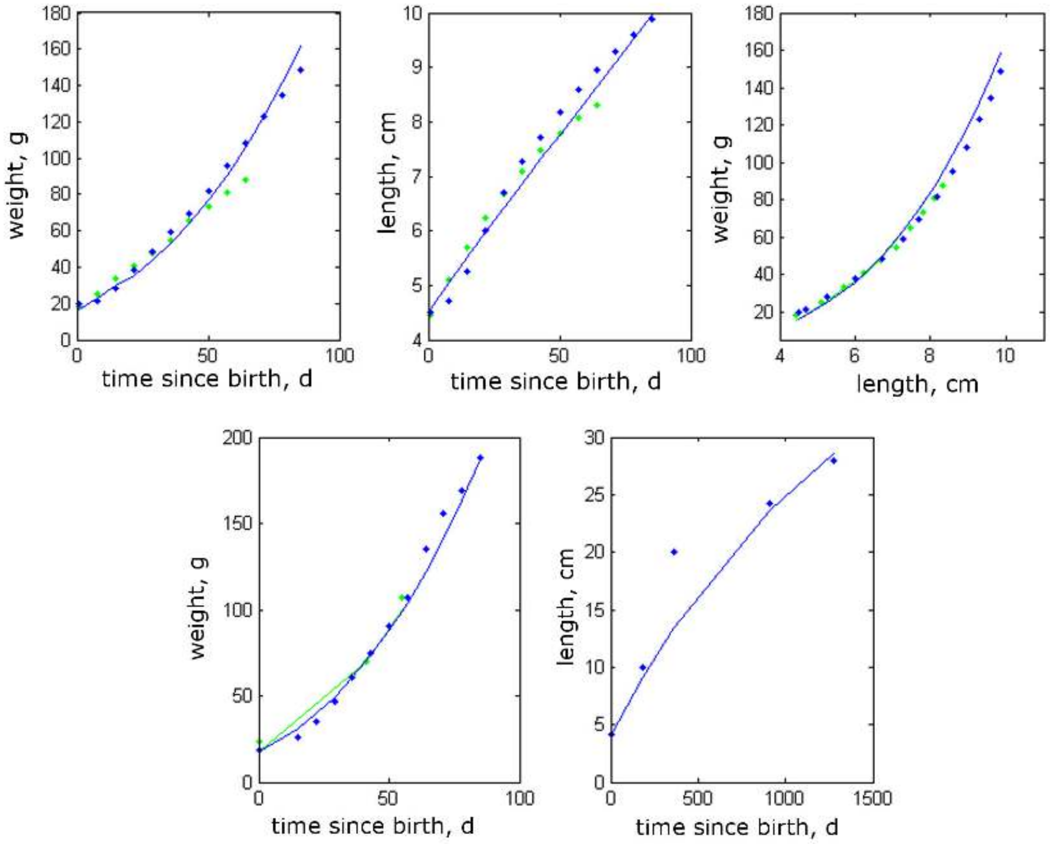

Figure 4.9: Results of the model estimations for hatchling growth. Panels a to c in Row 1: The North Atlantic population - weight increase with time (panel a), length increase with time (panel b), and the relationship of weight to length (panel c); $v = 0.07141\,\mathrm{cm/d}$, $[p_M] = 17.1\,\mathrm{J/d.cm^3}$. Panels d to e in Row 2: The Mediterranean population - weight increase with time (panel d), length increase in time (panel e), not used in parameter estimation (also please note a different scale); $v = 0.08582\,\mathrm{cm/d}$, $[p_M] = 22.86\,\mathrm{J/d.cm^3}$. The parameters $[p_M]$ and v were estimated separately for each population, while the values of other parameters were fixed at species-specific values (see Table 4.4)

4.4 Discussion

In this study the loggerhead turtles from the Mediterranean population were analyzed, and compared to the loggerhead turtles from the North Atlantic population. The result of smaller individuals of the same species residing in the Mediterranean was not surprising [45, 93, 69, 232], and the data analysis suggested that the size dimorphism is present during the whole life cycle. Our results are consistent with that of a previous study [232], reporting that the Mediterranean eggs and nesting adults are smaller compared to the North Atlantic and South Atlantic eggs and adults. In this study, analyzing the morphology, physiological (condition) index, and the physiological processes of loggerhead turtles inhabiting sea basins with different environmental conditions, made it possible to explore how environmental conditions, physiological characteristics, or the interaction of the two might result in size dimorphism.

The results of the first ("static") part of the comparison between the North Atlantic and the Mediterranean population (section 4.3.1), can be discussed in the context of environmental (abiotic) drivers, as the metabolic drivers (processes such as growth, maturation, and reproduction) that influence the morphology have not been addressed at that point. Results of the model simulation which reproduced the biphasic growth (section 4.3.3), provided a glimpse into the effects of food availability on growth.

The second ("dynamic") part of the comparison included the physiological characteristics. Defining and developing an energy-based model of the loggerhead turtle (Chapter 3) allowed us to study and test a variety of properties that would have otherwise been hidden (see the "Discussion" section of Chapter 3). Applying the same theoretical and practical framework to another (Mediterranean) population of the loggerhead turtle made it possible to compare the parameter values and the implied properties between the two (North Atlantic and the Mediterranean) populations. The results are to a large extent discussed in the context of Piovano et al. [181], the only paper to our knowledge explicitly taking into account the origin (North Atlantic or Mediterranean) of loggerhead turtles, while observing their growth rates in the Mediterranean sea. The results are also discussed in the context of the results and insights into loggerhead turtle physiology gained in Chapter 3.

4.4.1 Influence of the environment on the loggerhead turtles

Food availability in the environment. The size of the loggerhead turtles, when analyzed at stages encompassing the whole life cycle (from hatching to reproduction) followed a general pattern that can be interpreted as: smaller (Mediterranean) females produced smaller eggs, smaller eggs resulted in smaller hatchlings, and smaller hatchlings grew into smaller adults, that later produced smaller eggs. In other words, the smaller size of the hatchlings and adults from the Mediterranean population could be a consequence of solely smaller and lighter eggs. Smaller eggs could be a result of the lower food availability in the Mediterranean sea [130, 171, 263] that also induced the smaller size of the adult females. The food availability in the environment generally has a direct link to the ultimate size of individuals [109], which was confirmed in this study for loggerhead turtles while simulating the bi-phasic growth: loggerhead turtles experiencing lower food availability ("oceanic phase") had an asymptotic length which was only half of the ultimate (asymptotic) length of the same loggerhead turtles in the nutrient richer environment ("neritic phase") (Section 4.3.3).

The results are realistic because within the same population the individuals that stay in the oceanic environment indeed experience lower food availability compared to those in the neritic environment [80, 242], which has been correlated to the size and reproduction of the females [80, 242], and the size of the hatchlings [242]. The effect on reproduction has been reported mostly for the length of the remigration interval [80], while the egg size has generally been uniform within a population [232] with most of the egg size variability (within a population) attributed [125] to the amount of albumin (effectively water content), rather than yolk content. Yolk content provides energy for the development and might influence the size of the hatchlings, therefore the egg size variability within a population is probably not correlated with the hatchling size variability within that population, but eggs from different populations, having a different yolk content (e.g. [1]), could be correlated to the size of hatchlings. Egg size could differ between populations exposed to markedly different environmental conditions given enough time for adaptations: loggerhead turtles "follow" the optimal egg hypothesis [232] which describes balancing the energy available for reproduction between the fitness gained by producing a larger egg and the fitness lost by producing fewer eggs (Brockelman, 1975 and Smith and Fretwell, 1974 cited in Ref. [232]). The eggs of the Mediterranean loggerhead turtles are on average smaller than the eggs of the North Atlantic loggerheads [232, 88], which could be a result of a prolonged lower resource (food) availability and could account for most of the observed size differences.

Comparison of condition indices of individuals from the two populations at hatching and nesting produced a somewhat surprising result. Based on the different food availability experienced by individuals [130, 171, 263], the condition indices of individuals belonging to a same life stage but to a different population, were expected to markedly differ. However, the individuals from different populations (represented by an average value for that population) differed more between life events, than they did between populations. It is possible that the Mediterranean loggerhead turtles have physiologically adapted to the nutrient poorer environment of the Mediterranean sea, resulting in their physiological condition being similar to that of the North Atlantic loggerhead turtles despite less favorable conditions. However, a more comprehensive analysis of the condition indices would be required for such conclusions. As noted in the Section 4.2, the condition indices were calculated on the basis of mean population values due to the lack of appropriate data. Consequently, the calculated indices could not account for the interindividual variability.

The incubating environment can also have an effect on the size of the hatchling, and later life stages. Generally, when two populations have been analyzed simultaneously (e.g. [69]), most of the observed size variability between populations was explained by the initial egg size, and the reported correlation between hatchlings sizes and the abiotic factors (temperature, humidity, and salinity of the incubating environment) was mostly reported for individuals belonging to a same population.

Incubating environment in the nests, however, can be very different between the North Atlantic and the Mediterranean nesting beaches, and may be responsible for some of the difference in sizes of hatchlings. Incubation at colder (and moister) sites generally yields heavier and longer hatchlings ([223, 69, 184, 79, 169], but see [187, 96, 184, 176]), and high salinity in the incubating environment (mirroring the physiological effects of a dry incubating environment) yields smaller hatchlings [24]. Nesting locations for each of the populations show great variability in the average incubation duration, hatchling size, and hatchling sex ratio [256], suggesting markedly different incubation conditions within a single population. Making comparisons on the basis of population-specific abiotic factors is therefore extremely susceptible to source-based bias in the available data. Some general patterns can however be explored. The average incubation temperatures of nests on Mediterranean beaches (Cyprus and Turkey, [98, 72]) have been reported to be higher than those on North Atlantic beaches (Georgia, [124]), the higher temperature in natural nests being often combined with a drier substrate [184]. This suggests that the smaller size of the Mediterranean hatchlings is the result of warmer and drier nests. The correlation temperature and humidity with size at hatching has, however, exhibited some inconsistencies. The *temperature* has been observed to negatively correlate with wet (but not dry) mass or length of lizards [96], with weight and flipper area of green turtles [69], and with length, but not wet mass of loggerhead turtles [184]. Weight and length at hatching generally seem to be correlated for loggerhead turtles [223], so the reported [184] correlation of the temperature with length but not weight suggests that the temperature influences the two size traits in a different way. The nest *humidity* has been reported to positively correlate with the length and wet mass of loggerhead turtles [184], but the driest nests produced the longest hatchlings of leatherback turtles [176]. In addition to temperature and moisture (humidity) affecting the hatchling morphology independently [79], a combination of the effects can be present, with higher moisture inducing a lower incubation temperature [79]. Nest temperature and moisture also indirectly affect the size because they affect the incubation duration [262, 79] which has been reported to correlate with the size at hatching [79, 96], and with the utilization of yolk during embryonic development [79].

Salinity of the eastern Mediterranean sea (38-39 ppt, [133]) is higher compared to that of the North Atlantic (35-36 ppt, [226, 172]) which could induce higher salinity of the sand and amniotic fluid, contributing to the abiotic drivers of the size difference. The shell of the sea turtle egg allows the (osmotic) absorption of water by the embryos during embryonic development, resulting in heavier eggs in moister environments [176]. Even though there were no available published studies on the effect of salinity on the embryonic development of sea turtles in general, or of loggerhead turtles specifically, a negative correlation has been reported [24] between salinity and the hatchling mass and hatching success of a (freshwater) snapping turtle. Higher salinity could be lowering the water potential of the nest sand and reducing the osmotic water absorption, or even causing dehydration of the egg. The resulting higher osmolarity of the amniotic fluid would cause less favorable conditions for the embryonic development. Even though the effect of salinity has been significant in the aforementioned study, Bower et al. [24] have investigated a much wider salinity range (0-70 ppt) and lower water potentials (< -10 kPa to < -4900 kPa) than those generally experienced by sea turtle eggs and nests. The salinity of the sand in the parts of the beach where the loggerhead turtles nest is probably more influenced by rainfall than nest inundation by sea water (K. Lohmann, pers.comm.), and the drier environments have been found to produce larger hatchlings and to have a higher hatching success than the wet ones [176], suggesting that the salinity or dryness of the sand does not have a negative effect on the size of the embryos. Furthermore, the water present in the amniotic fluid of leatherback turtles can support the whole embryonic development [176]. All sea turtles are extremely capable osmoregulators [188, 189, 161, 8], and can probably regulate the salinity of the amniotic fluid to match that of favorable developmental conditions. Whether they do this in the Mediterranean at a higher energetic cost (which might have an impact on size) could not be deduced, as there is a lack of reports comparing the salinity and osmolarity of amniotic fluid in the Mediterranean and the North Atlantic turtle eggs.

Other pressures present in the environment. In addition to the abiotic environmental characteristics (food availability, temperature, salinity, etc) that might exert pressures on the loggerhead turtle, factors such as predation and anthropogenic pressures can also result in evolutionary size selection. The lack of small outliers when size at hatching was investigated (Figure 4.2 in Section 4.3.1) suggests that there is a (perhaps ecologically induced) minimum size at hatching. Because of their size, hatchlings are the most vulnerable of the loggerhead turtle post-embryonic life stages [202]. Furthermore, being smaller implies feeding on smaller food particles, and requiring more food particles to be ingested (possibly over a longer period) for a certain energy gain. Hatching at a smaller size would thus prolong the period of sensitivity connected to the size, both in terms of predators, and the available food sources.

Smaller size and younger age at nesting, as identified in this study (see also [181]), might be a result of the selection driven by anthropogenic pressures. The main source of loggerhead turtle mortality in the Mediterranean is bycatch [160], with other significant anthropogenic pressures such as shipping, (over)fishing, and mass tourism [239] also having a high probability of interaction and a negative impact on the loggerhead turtles. Such pressures exert a strong evolutionary incentive for the Mediterranean loggerhead turtles to breed at a younger age, which needs to happen at a smaller size in an energy limited environment. Strong anthropogenic pressure (commercial harvesting) has been reported to drive down the age and size at first breeding of also fish and predators in the marine ecosystems [235, 211].

4.4.2 Influence of physiology on the loggerhead turtles

What happens when a North Atlantic turtle lives in the Mediterranean sea? The Mediterranean sea was characterized by lower food availability than the North Atlantic, resulting in a scaled functional response $f_{\text{Med}} = 0.71$, and the average temperature of the east Mediterranean sea, $T_{\text{Med}} = 21\,\text{C}$ [133]. Using the DEB-parameter set estimated on the data for the North Atlantic population ('pars$_{\text{NA}}$') to obtain the biological response of individuals (as was done in Section 4.2.2.1), we have simulated what the response of the North Atlantic individuals would be to the Mediterranean environment.

Predictions calculated using the 'pars$_{\text{NA}}$' parameter set suggested that the North Atlantic loggerhead turtles would reach the asymptotic size that is now observed for the Mediterranean turtles. They would start reproducing at the similar size as they do in the North Atlantic (model predicts the length at puberty, L_p, to be around 76 cm SCL (83 cm CCL) for the North Atlantic loggerhead turtles in both North Atlantic and the Mediterranean), but at the L_p they would be around 50% older than in the North Atlantic. If a lower sea temperature would be assumed based on the information that the North Atlantic loggerhead turtles mostly forage in the western (cooler, [133]) Mediterranean basin [31, 181], the predicted age difference would be even larger. Additionally, the reproduction rate at the maximum size predicted by the model was only 25% of that predicted (and observed) in the North Atlantic. The predicted size at maturity, and the reproductive output of the North Atlantic loggerhead turtles could not be compared with observed (reported) values, because individuals from the North Atlantic generally do not reproduce in the Mediterranean [32]. However, the physiological (bone structure) changes typical for maturation have been observed in the samples of North Atlantic loggerhead turtles encountered in the Mediterranean [181]. In their discussion, Piovano et al. [181] hypothesize that the North Atlantic individuals grow slower, but mature at a smaller length in the Mediterranean than they do in the North Atlantic. The length at puberty for the North Atlantic environment used in Ref. [181] (87 cm CCL) could have been an overestimate (see Chapter 3), in which case the length at puberty of North Atlantic loggerhead turtles could be similar in both sea basins. The slower growth and consequently larger age, but similar length at puberty in both sea basins, would be consistent with predictions obtained by our model. Furthermore, the extremely low prediction for the reproduction rate would explain why the North Atlantic loggerhead turtles do not reproduce in the Mediterranean.

When the predicted growth of the North Atlantic loggerhead turtles is compared to the data for growth of the Mediterranean loggerhead turtles experiencing the same conditions (dashed and full lines in Figures 4.5 and 4.6), the model predicted that the growth of the North Atlantic loggerhead turtles would be slower than that of the Mediterranean loggerhead turtles, which is in accordance with the results in Piovano et al. [181]. Even though a difference in growth rates was present, it was not drastic (see also Figure 4.8).

Therefore, other factors such as different temperature and food availability, differing between the areas frequented by individuals of the two populations, probably had a significant role in the observed difference in growth rates [181] as well.

What can the DEB parameters specific to the Mediterranean population infer about the population. Testing several scenarios of parameter combinations and environmental characteristics (see Sections 4.2.2.1 and 4.3.2), we were able to identify that the best fit of model predictions to observed data was obtained when parameter values differed between populations while the food level in the Mediterranean sea was assumed to be lower than in the North Atlantic. The estimated parameters that differed the most and have the largest biological and ecological implications were: volume-specific somatic maintenance ($[p_M]$), energy conductance (v), and maturity at puberty (E_H^p).

A slightly higher value of the volume-specific somatic maintenance parameter ($[p_M]$) for the Mediterranean loggerhead turtles might be linked to the energetic costs of osmoregulating via the lachrymal salt gland. The lachrymal salt gland in the orbital cavity allows the sea turtles to regulate their osmolarity within 4 days after drastic change in salinity (between salt and fresh water, or experiencing the double salt load), making them excellent osmoregulators [188, 189, 168]. The higher salinity of the Mediterranean sea might, however, cause higher energetic costs of osmoregulating. On one hand, the loggerhead turtles have often been found in brackish estuarine waters [102], and the "Standard permit conditions for care and maintenance of captive sea turtles" [60] instructs that the water salinity should be maintained between 20 and 35 ppt, suggesting that the lower salinity level has few, if any, negative effects on the sea turtles. On the other hand, it has been hypothesized that areas of higher salinity might act as a barrier for the loggerhead turtles generally experiencing a lower salinity, i.e. that the North Atlantic loggerhead turtles avoid the north part of west Mediterranean, and the east Mediterranean basin because of higher salinity [31, 192]. A physiological (salinity) barrier would explain why the North Atlantic loggerhead turtles mostly feed around the north African coast, while the ones hatched on the Mediterranean beaches feed around the European costs [31]. The "salinity barrier" hypothesis (sensu Carreras et al. [31]) is based on correlations and therefore, as noted by the authors [31] themselves, could be wrong: the North Atlantic loggerhead turtles could be staying in the areas with lower salinity simply because they are following sea currents [31], magnetic cues, or due to other food or habitat preferences (K. Lohmann, pers.comm., R. Reina, pers.comm.). Nonetheless, the higher salinity would influence sea turtle's metabolism through direct drinking of sea water [8], and through ingesting food, as invertebrates have been found to contain three times the concentration of salt compared to sea turtles [161]. With more salt in the bloodstream/organism, the salt glands have to secrete more, also causing dehydration [161]. It would be interesting to see whether the Mediterranean loggerhead turtles drink more sea water to account for possible dehydration, or possibly have a higher tissue density, and whether their salt glands continuously secrete more to balance the higher salinity of the Mediterranean, especially in the eastern part where majority of the Mediterranean loggerhead turtle activities occur.

The costs of somatic maintenance are via $[p_M]$ directly linked to the surface area specific maximum assimilation rate ($\{p_{Am}\}$), under certain assumptions (the values of the zoom factor, z, and allocation to somatic maintenance and growth, κ, not differing between populations) as was done in this study (see Section 4.2.2.1). The parameter $\{p_{Am}\}$ is a primary parameter that controls the assimilation flux, thus resulting in more energy being assimilated (and therefore available for growth and reproduction). The energy allocation strategy which includes a higher value of $[p_M]$ and $\{p_{Am}\}$ has been named "waste-to-hurry" [106].

The energy conductance (v) controls the reserve mobilization. A higher value of v causes faster reserve mobilization, which translates into faster growth and maturation, and lower reserve density, which affects survival during starvation. The combination of higher values of all three parameters ($[p_M]$, $\{p_{Am}\}$, and v) results in individuals growing and maturing faster and at smaller sizes, which captures the pattern observed in the posthatchling stage, but also in the Mediterranean in general [181]. The survival under conditions of low food availability is directly linked to the maximum reserve density, $[E_m]$, a characteristic which is also affected by parameters $\{p_{Am}\}$ and v. $[E_m]$ will largely depend on the proportion of increase in the parameters: relatively larger increase of $\{p_{Am}\}$ will result in a higher maximum reserve density, and *vice versa*.

Faster growth and earlier maturation at a smaller size have three main energetic benefits: (i) cumulative energy invested into growth is smaller in comparison to growing to a larger size, (ii) the total metabolic maintenance, which is paid per unit of (structural) volume is smaller, and (iii) puberty can be reached at lower food levels. The strategy can therefore be beneficial in resource depleted environments with short periods of food availability because faster growth uses the (temporarily) available resources to rapidly increase in size, and smaller size at sexual maturity typically requires less energy to complete a reproduction cycle [106, 103]. The post-hatching period would be one of the examples where such a strategy would be beneficial to rapidly increase in size, and indeed the observed faster growth of posthatchlings was predicted extraordinarily by allowing $[p_M]$ (and consequently $\{p_{Am}\}$) and v to have higher values than estimated for older life stages (see the Section 4.3.4). However, when the whole life-cycle of loggerhead turtles is considered, "waste to hurry" (costly increase in size and shorter period of coping with starvation) does not seem to be a likely evolutionary strategy for a long-lived species in a resource-depleted environment. Furthermore, a relatively large difference in the energy conductance would result in a large difference in condition indices, which was not the case (Tables 4.1 and 4.2). Indeed, when relative differences between the parameters (estimated on data which includes adults) are calculated, the energy conductance is larger by 0.2%, and the volume-specific somatic maintenance by 5.8% (a difference of less than 1 J/d.cm^3 of structure). The smaller size and age at puberty (and birth) could be more directly connected to other parameters, e.g. levels of maturity.

Levels of maturity (maturity at birth, E_H^b, and maturity at puberty, E_H^p) are linked to the transitions between life stages (birth and puberty, respectively), allowing for the transitions to happen once enough energy has been invested into maturation, regardless of size or age [109]. Consequently, different sizes can be observed at the same life stage transitions. The size at birth and puberty are influenced by the maintenance ratio ($k = k_J/k_M$, where $k_M = [p_M]/[E_G]$). A decrease in k (for example due to an increase in $[p_M]$) results in a decrease in size (length) at birth and puberty, and *vice versa* ([109], p306). The estimated maturity at birth (E_H^b) did not differ much between the two populations, probably due to scatter in data resulting in the parameter being less clearly determined by data than maturity at puberty was. When the level of maturity at puberty (E_H^p) was kept fixed (constant) during the parameter estimation, the length at puberty (L_p) predicted for the Mediterranean population was smaller than L_p predicted for the North Atlantic population, but the observed difference in length between the two populations could not be reproduced. Only when the maturity puberty threshold was estimated in conjunction with other parameters, could the observed length difference at puberty be reproduced. The difference between the puberty maturity levels was striking: the Mediterranean loggerhead turtles need to invest 20 MJ less to reach puberty, and allocate 40 kJ/d less to maintain the maximum level of maturity (Figure 4.10, panel b), yet the end result in terms of proportions of allocated energy in a daily energy budget was practically identical (Figure 4.10, pie charts in the first row).

When the estimated maturity levels were analyzed in the context of the observed L_p data, a pattern emerged: 24.6% smaller Mediterranean loggerhead turtles had a 21.33% lower value of maturity at puberty. This suggested that maturity switches happen at a certain ratio of maturity level and structural volume, *maturity density*, rather than the absolute level of maturity. Switches at maturity density could be included in the model with the Matlab functions `get_lb_md` and `get_lp_md` in the DEBtool package [112] (for more details see the online comments file [114], pages 6-9).

DEB model predictions for the Mediterranean loggerhead turtle population. The model fitted most of the observations well, with the largest mismatch between predictions and observations present for age at puberty; a situation consistent with the model predictions for the North Atlantic population (Chapter 3). The predicted length at puberty was very close to the "observed" length at puberty, but it is interesting that the model *over*predicted the length at puberty for the Mediterranean population, and *under*predicted it for the North Atlantic population. As discussed in Chapter 2, "length at maturity" has been found to greatly vary among individuals of the same population, and might not be the best indicator of the loggerhead turtle's sexual maturity [16]. However, considering that the deviations between the model predictions and the data were relatively small (2 cm SCL, i.e. 3%, for the Mediterranean population, and 4 cm SCL, i.e. 5%, for the North Atlantic population), and a more reliable alternative is lacking [16], the length of an individual can be used for a first quick deduction about the developmental stage of the encountered individual.

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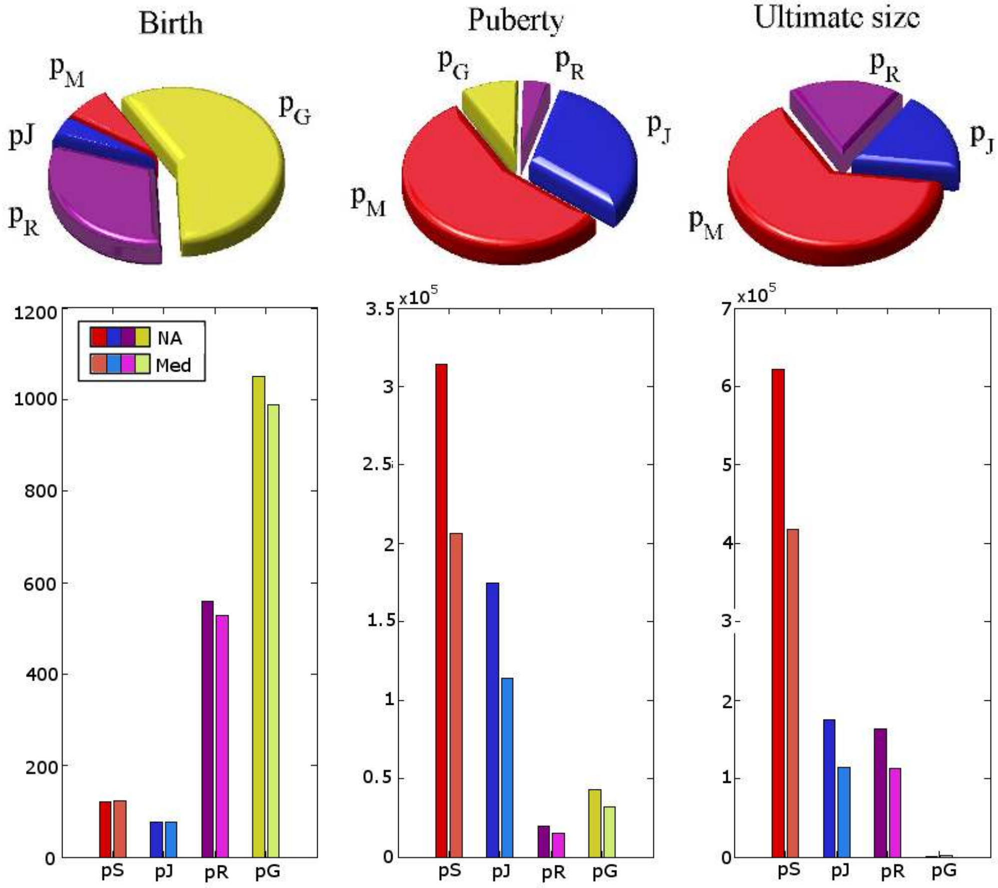

Figure 4.10: A visualization of the energy budget at birth, puberty, and ultimate size: p_G - growth flux, p_R - maturation/reproduction flux, p_M - somatic maintenance, and p_J - maturity maintenance, presented as fractions of the mobilization flux (pie charts) and as absolute values (in Joules) in parallel with the same fluxes calculated for the North Atlantic individuals (histograms). Fluxes for the Mediterranean individuals ('Med') are calculated using the parameters $pars_{\text{Med}}$ (see Table 4.4) experiencing the scaled food availability of $f = 0.71$, and fluxes for values for the North Atlantic ('NA') individuals using the parameters $pars_{\text{NA}}$ (see Table 4.4) experiencing the scaled food availability of $f = 0.81$.

The prediction that the Mediterranean loggerhead turtles reach maturity earlier than their North Atlantic conspecifics (13 years compared to 14 years), is consistent with the earlier maturation of the Mediterranean loggerhead turtles suggested by Casale et al. [38] and Piovano et al. [181], but the predictions for age at puberty are either at the low end of the estimated range [34, 38] or they are lower than the age at puberty [181]. Our results suggest that the Mediterranean loggerhead turtles start allocating to reproduction several years prior to what has been estimated as age at puberty by other authors, which is consistent with the conclusions for the North Atlantic loggerheads in Chapter 3.

One possible explanation is the difference between physiological maturation (and change in energy allocation tracked by the model) and the act of reproduction and/or nesting (often taken as a reference event for other methods of estimating age at puberty) as has been discussed Chapter 3.

Another explanation would be a change in some of the characteristics which were assumed to be constant in the model setup: the environmental parameters (food availability and temperature), and/or the population-specific parameters. When all of the parameters (environmental and individual) are assumed constant during the entire life cycle, the result of a standard DEB model for post-embryonic growth is a classic von Bertalanffy growth curve ([109], see also Figure 4.8, dotted lines). Considering the very good fit of the biphasic growth curve to the observed data (Figure 4.8, full lines), and faster growth of posthatchlings (Section 4.3.4), some of the parameters could change during the life cycle.

The large observed difference in the growth rates between the individuals from the same rearing conditions (Figures 4.5 and 4.6) could not be reproduced well with a single parameter set. DEB theory assumes that parameter values are individual-specific and would have no problem in capturing this pattern if the parameters were estimated for each data-set, but this was not done as one of the aims of this study was to obtain a set of parameters that can describe reasonably well the whole Mediterranean population. Exploring the observed differences, especially between two individuals exhibiting markedly different growth while reared in the same facility (Figure 4.5, panel d, and Figure 4.6, panel b), does however suggest a couple of possible explanations in the context of the insights obtained in this study.

(i) The drastic difference might be a result of feeding regimes: if they were fed a percentage of their weight, a turtle with smaller weight would get less food, and less food would result in slower growth and smaller size.

(ii) It is possible that the juveniles, even though they are offspring of the same female (S. Cateau, pers.comm.), are offspring of different males, possibly resulting in different growth potentials. The adults in the reproduction program were individuals of unknown age and origin encountered in the wild. The females might be of Mediterranean origin because their length falls within the range for the Mediterranean population, and it was relatively constant during the duration of the reproduction program (data available starting from 2005). The males, however, are larger and some could be of the North Atlantic origin, especially considering that the North Atlantic males are often encountered in the Mediterranean [35]. The loggerhead turtles exhibit multi-paternity, and clutches carrying genetic markers from as many as five males have been documented [261]. Considering the insights obtained by this study, it would be interesting to see if faster growing turtles carry genetic markers specific for the Mediterranean region, while the slower growing ones carry genetic markers specific for the North Atlantic region. If so, the main source of difference would be the growth potential defined by the genetic (metabolic) characteristics.

The extremely good prediction for the maximum reproduction rate (Table 4.3), but the less satisfactory prediction for the clutch size as a functions of carapace length (Figure 4.7) implies that the allocation to reproduction was predicted well, but the calculations for the clutch size were not adequate. It is interesting that the model predicted, for the Mediterranean as well as for the North Atlantic population (see Chapter 3), a steeper trend than the data suggests, because the reproductive output of the Mediterranean loggerhead turtles, compared to that of the North Atlantic ones, includes shorter remigration intervals [26], fewer clutches per season [26, 81], yet more eggs per clutch [136, 77, 232, 204].

The calculation for the clutch size was performed in the simplest way, but it was also relatively rigid, assuming a consistent number of eggs per clutch, number of clutches per season, and length of the remigration interval within a population. Even though this approach did not affect the accuracy or quality of the prediction for total seasonal reproductive output, an alternative approach might be more suitable. The alternatives include: (i) measuring the total seasonal reproductive output rather than clutch size; (ii) allowing values of other parameters involved in the reproduction (such as k_J, the maturity maintenance) to be estimated, and (iii) introducing additional parameters (e.g., number of clutches as a function of length). Measuring the total seasonal output of individual loggerhead turtles over several seasons would provide the raw data, but such studies are often hindered by logistics [237, 232, 26]. The parameter k_J in most species has values close to or the same as the value (0.002 J/d) used in this study [126, 109]. Furthermore, when the parameter k_J was estimated on the data for the North Atlantic or Mediterranean loggerhead turtles simultaneously with other parameters, values close to 0.002 J/d (0.0015 to 0.0018 J/d) were obtained. The third option, i.e. the introduction of additional parameters, therefore might be justified. The additional parameter should have a biological meaning, so it can be introduced in a way which is consistent with the DEB theory. Ideally, it would be able to reproduce the observed variability in the number of eggs per nest [136, 77, 232, 204], balancing the available energy to account for a trade-off between the clutch frequency and the clutch mass [94].

Do posthatchlings just "waste to hurry"? Estimating parameters separately for the posthatchlings data sets, we obtained higher values for specific somatic maintenance, $[p_M]$, energy conductance, v, and (via $[p_M]$) the maximum assimilation, $\{p_{Am}\}$. The larger (compared to older life stages) value of $[p_M]$ and $\{p_{Am}\}$ for posthatchlings, which was the case for both the Mediterranean and the Atlantic population, might relate to the "waste-to-hurry" phenomenon to boost growth and account for faster movement [106], i.e. strategies to decrease the risk of predation [202]. The larger estimated value of parameter v means a faster mobilization rate of reserve, which implies a lower maximum reserve density if the specific assimilation rate would be constant: $[E_m] = \{p_{Am}\}/v$. However, by fixing the zoom factor in which the ratio of $\{p_{Am}\}$ and $[p_M]$ occurs ($z = \kappa\{p_{Am}\}/[p_M]$) to reduce degrees of freedom, and assuming that the proportion of the energy allocated to growth and maintenance (κ) does not change (see also Section 4.2.2.3), an increase in $[p_M]$ directly translates to an increase in $\{p_{Am}\}$. When $\{p_{Am}\}$ and v values of posthatchlings and adults are compared (Table 4.6), the difference in values of $\{p_{Am}\}$ between life stages is larger than the difference in v, resulting in a different maximum reserve density for the two life stages, with $[E_m]$ being larger for posthatchlings (Table 4.6). Because the maximum reserve density is positively correlated with a condition index (weight over cubed length), $[E_m]$ can be discussed in the context of condition indices calculated previously (Section 4.3.1). The values of the $[E_m]$ for adults and posthatchlings of both populations are indeed analogous to their condition indices, with the values similar between the same life stages of both populations, but higher for posthatchlings than adults of both populations (Table 4.6).

Table 4.6: A comparison of the primary parameters $[p_M]$, v, and $\{p_{Am}\}$ for posthatchlings and adults of the Mediterranean and the North Atlantic population, and the calculated maximum reserve density, $[E_m] = \{p_{Am}\}/v$, for each of the two life stages for each population. The calculated condition indices from Table 4.3.1 are also listed for comparison, as $[E_m]$ is positively correlated with the condition index. Units: $[p_M]$: J/d.cm^3, $\{p_{Am}\}$: J/d.cm^2, v: J/d, $[E_m]$: kJ/cm^3 (all referring to the surface area or volume of structure), condition index: kg/cm^3 (physical length cubed).

	North Atlantic				**Mediterranean**			
posthatchlings*	$[p_M]$	$\{p_{Am}\}$	v	$[E_m]$	$[p_M]$	$\{p_{Am}\}$	v	$[E_m]$
	17.1	1169.37	0.07141	16.37	22.86	1563.27	0.08582	18.21
condition index at hatching	0.2140				0.2385			
adults	$[p_M]$	$\{p_{Am}\}$	v	$[E_m]$	$[p_M]$	$\{p_{Am}\}$	v	$[E_m]$
	13.25	906.1	0.07084	12.79	13.6	933.1	0.072288	12.90
condition index at nesting	0.1413				0.1544			

'*' Individuals were captive reared.

A higher reserve density suggested by the results for the posthatchling stage might be beneficial during the first few days or weeks, as posthatchlings need to rapidly absorb (internalize) whatever is left of their yolk sac at the moment of hatching, and also need to sustain a prolonged period of swimming while getting accustomed to a new food source. The difference between the $[E_m]$ of the North Atlantic and Mediterranean hatchlings could be a consequence of the rearing conditions (e.g. different food quality or different conditions in the holding tanks), feeding conditions of the mothers at egg laying (North Atlantic posthatchlings were obtained from nests encountered in the wild, whereas the Mediterranean ones were part of a reproduction program in an aquarium), or a data artifact.

4.4.3 Interactions of physiology and the environment

Biphasic growth curve resulting from the change in food availability. We were able to test the influence of food availability on the growth pattern by simulating the change in food availability as the only difference between the two parts of the life cycle (relevant Sections: 4.2.2.2 and 4.3.3). The simulated conditions resemble those of the oceanic stage followed by the neritic stage, i.e. an ontogenetic shift connected with the recruitment to the nutrient rich habitats [20]. The assumption that the loggerhead turtles in the oceanic environment experience a substantially lower food availability resulting in half of the scaled functional response of the loggerheads in the neritic habitat, is not unrealistic. The nutrient quality of food and the costs of searching, ingesting, and digesting food were included in the scaled functional response (f) [109]. Food in the oceanic environment is less abundant, and has less energy per unit volume [80, 177], resulting in lower nutrition of individuals in the oceanic habitat 242, 80.

The model was again the standard DEB model, the parameters were those previously estimated for the North Atlantic (pars_{NA}) or the Mediterranean (pars_{Med}) population, and the ontogenetic shift was assumed to occur after a threshold size (30 cm CCL). A simple way to test whether different parameter values and/or constant food can reproduce the data well resulted in a biphasic growth curve obtained by two different parameter sets, and the classic von Bertalanffy growth curve obtained by the (pars_{Med}) parameter set, all suggesting that the combination of 'pars_{Med}' parameters and two fs results in the predictions that best fit the data.

Considering that the data was obtained by different methodologies, and was published and analyzed separately with von Bertalanffy growth models with different parameter values [39, 38], the excellent fit of a single DEB model demonstrated some of the possibilities and strengths of a mechanistic approach.

Studying the growth of posthatchlings. Predictions for the growth of posthatchlings obtained using the parameters specific to the posthatchling data captured the process of growth in a mechanistic way while accounting for changes in the experimental conditions. Studying the growth of captive reared posthatchlings from the Mediterranean and North Atlantic populations (relevant Sections: 4.2.2.3 and 4.3.4) made it possible to: (i) study the effects of (known) food availability and temperature on growth rates; (ii) compare the growth of the posthatchlings of Mediterranean and North Atlantic origin; (iii) compare the growth of the posthatchlings to the growth of older life stages.

A smaller variety of data and information was taken into account than the full DEB model would require, however while for the full DEB model the environmental conditions (food availability and temperature) often needed to be assumed (Section 4.2.2.1), for the posthatchling growth they were known, and could explicitly be accounted for. In combination with the mechanistic model, this made it possible to detect the differences in growth rates that would have otherwise been masked.

Conclusions based solely on empirical data analysis (e.g., fitting to a von Bertalanffy growth curve) would suggest that both Mediterranean and North Atlantic posthatchlings grow at a very similar rate (von Bertalanffy coefficients 5.45e-4 d^{-1} and 5.20e-4 d^{-1}, respectively). From this, one might conclude (considering both were reared in captivity) that the two populations are physiologically the same. However, the rearing temperatures were not the same: 23.5°C for the Mediterranean posthatchlings, and 27°C for the North Atlantic posthatchlings. Although the standard DEB model was simplified to the post-embryonic von Bertalanffy growth, the important difference was that the three parameters (r_B, L_b, and L_∞) have a particular physiological meaning and co-vary in a very special way when different food levels and temperatures are compared. When the von Bertalanffy growth rates at the reference temperature and same food availability were calculated, the difference between growth rates was evident, highlighting the physiological difference between the populations.

Parameter wise, the main differences that resulted in the different growth rates were values of the parameters $[p_M]$, v, and $\{p_{Am}\}$ (which was linked to $[p_M]$, see Section 4.2.2.3). The pattern is in accordance with the hypothesized acceleration in growth and development of posthatchlings (see the Discussion of the Chapter 3). Considering that the difference in salinity between the Mediterranean and the North Atlantic could cause differences in the somatic maintenance of the two populations due to the cost of the salt gland activity (see Section 4.4.2), the higher somatic maintenance of the posthatchlings compared to that of adults could also be a consequence of the salt gland activity, and the higher value for Mediterranean hatchlings might be linked to higher salinity of the Mediterranean sea. Nicholson and Lutz [161] suggested that the salt gland activity of hatchlings is higher than that of juveniles, which they attributed to a larger relative size of salt glands (0.3% body mass of hatchlings, compared to the 0.05% body mass of adults, [161]). Future research could aim to modify the standard DEB model to include the changes in parameter values, and to compare the new model predictions to the observations.

A note on growth models. Testing more (logistic, Gompertz, or von Bertalanffy) equations had been suggested [63], but often among these empirical (static) models, the von Bertalanffy growth curve is identified as the best fitting model (e.g. [173, 181, 39, 37]). Within a certain study, the size span represented in a sample is often limited, and the food availability and temperature are indirectly assumed constant. The authors' best fitting models, even though describing the studied data well (e.g. the model suggested in [39] or [181]), predict unrealistic values when extended to predict smaller carapace sizes than those which had been included in the analysis. This suggests that the whole growth curve cannot be represented by such a model.

Perhaps the most interesting static growth model has been suggested is the polyphasic growth model suggested by Chaloupka [40], albeit requiring six parameters to obtain a single growth curve of pelagic juvenile loggerhead turtles (eq. 1 in Ref. [40]). A simpler version, such as a biphasic growth resulting from the ontogenetic shift, i.e. recruitment to the neritic habitats noticed for loggerhead turtles of a certain size (40-50 cm SCL in the North Atlantic [13] and 30-35 cm CCL in the Mediterranean [38]), would be consistent with the good fit obtained by the von Bertalanffy model fitted to a specific size span which does not include the ontogenetic shift [38, 181, 14].

A very similar growth pattern was observed for yellowfin tuna, with juvenile growth rates prior to recruitment several times higher than expected based on the von Bertalanffy growth curve [62]. The authors do not provide a model, but refer to the growth pattern as the "multistanza growth", and conclude that the "mechanisms such as acquisition of sexual maturation, development of the swim bladder, as well as changes in habitat and prey with size/age could explain the growth phases" [62].

The major drawback in studying and comparing growth rates using static models such as the ones discussed, are the unavailability of data and the limitations of its use, and the limitations of the models used to calculate the growth rates. Because parameters are obtained by curve fitting on the available data, parts of the growth curves describing a size span with sparse data are not defined well. In addition, to keep the number of parameters relatively low, constant conditions need to be assumed.

Growth models obtained in this study are all dynamic (mechanistic or process) growth models, because their parameters have a direct link to the underlying physiological processes. The link makes it possible to obtain different growth curves by varying the factors that affect those processes. In this study, food availability and population-specific characteristics were varied, but other factors such as temperature can also be tested. Certain changes in physiology connected to recruitment had also been observed in loggerhead turtles [120, 138, 91]. The physiological changes can be explored as changes in the parameters values, such as those connected to maintenance ($[p_M]$), energy mobilization (v), or energy allocation (κ).

4.5 Conclusion

Even though one of the main motivations for this study was the size dimorphism reported between the loggerhead turtles from the Mediterranean and the North Atlantic populations, the Dynamic Energy Budget (DEB) approach made it possible to study additional properties that are connected not only to the size of the individuals, but also to the metabolism, energy allocation, and the implied properties.

The large susceptibility of the calculated von Bertalanffy growth rates to the environmental and physiological characteristics highlights the importance of knowing the experienced environmental conditions and the population of origin when comparing growth rates of individuals. Generally, an increase in temperature results in a higher von Bertalanffy growth rate and a higher absolute growth rate (expressed as change in length or mass over time). An increase in food availability results, somewhat counter-intuitively, in a lower von Bertalanffy growth rate, but yields a faster absolute growth rate.

The very good fit of the the biphasic growth curve (obtained by simulating the change in food availability) to the data published and modeled separately with two different growth models, consolidates the hypothesis of the polyphasic (or multistanza, [62]) growth suggested for the loggerhead turtles [40], and at the same time demonstrates the strength and possibilities of a mechanistic approach. Exploring other possible reasons for the growth pattern to exhibit multiple stages, such as the physiological changes related to the recruitment to neritic habitat or other developmental transitions [62, 113], will provide new insights into the biology and physiology of loggerhead turtles.

Our research demonstrated that the size dimorphism between the loggerhead turtles of the Mediterranean and the North Atlantic populations was probably influenced by the interaction of the environment and the population-specific (metabolic) characteristics of loggerhead turtles. The genetic separation between the two populations [31, 32, 181] implies that inheritance of the specific metabolic traits is possible. Different environmental (food, temperature, salinity) conditions that the individuals (belonging to the two populations) are experiencing, imply that certain ecological pressures are present. The difference in physiological characteristics, such as higher somatic maintenance and less energy required for maturation and maturity maintenance, could be adaptations to the environmental factors such as high salinity (the energetic cost of increased salt gland activity increasing the maintenance costs), and/or low food availability (reaching puberty at a smaller size and having more energy for reproduction).

Keeping in mind the observed effects of the environmental and the population-specific metabolic factors, the differences in growth, maturation, reproduction, and size between the North Atlantic and the Mediterranean loggerhead turtles, as reported in Refs. [181] and [232], can be fully reproduced by differences in four DEB primary parameters: $[p_M]$, $\{p_{Am}\}$, v, and E_H^p.

(i) The Mediterranean loggerhead turtles grow and mature faster than the North Atlantic loggerhead turtles in the Mediterranean sea, due to the larger assimilation and mobilization fluxes (regulated by the parameters $\{p_{Am}\}$ and v), but are smaller due to a higher value of the somatic maintenance rate ($[p_M]$);

(ii) The Mediterranean loggerhead turtles are smaller than the North Atlantic loggerhead turtles at first nesting, due to a lower value of the maturity-at-puberty parameter (E_H^p);

(iii) The smaller ultimate size of Mediterranean loggerhead turtles is a consequence of low food availability in the Mediterranean sea, but the reproduction output is comparable to that of North Atlantic individuals (and much larger than that of North Atlantic loggerhead turtles exposed to such low food levels) because more energy is available for reproduction due to a lower maturity maintenance (proportional to E_H^p).

(iv) In addition, a higher cost of maturity maintenance of North Atlantic fully grown adults provides a metabolic explanation for the observation that North Atlantic females do not nest in the Mediterranean.

The lower value of the maturity level at puberty (E_H^p) of Mediterranean loggerhead turtles revealed an interesting observation that, even though the energy invested into maturity was lower, the ratio of the energy and the structural volume at puberty was very similar to that of North Atlantic loggerhead turtles. The observation implies that the maturity thresholds might not be connected to the absolute value of the energy invested in maturation (E_H^*), but rather the ratio of the invested energy and the structural volume, a property which can also be considered maturity density ($[E_H^*]$). The applicability and evolutionary implications of the maturity density as a threshold have yet to be explored.

Chapter 5

Effects of environmental change and plastic ingestion on the energy budget of loggerheads

Abstract

Loggerhead turtles are vulnerable to natural and anthropogenic environmental pressures due to their longevity, global distribution, and their migratory way of life. Two significant environmental conditions, temperature and available food, have been correlated to growth, reproduction, and maturation of loggerhead turtles, often accounting for most of the observed variability. With the current and expected environmental changes, patterns in the environmental conditions could change as well, resulting in different food availability and temperature in habitats used by loggerhead turtles. Another important pressure is the plastic pollution (i.e. anthropogenic debris) present in all the oceans. Over 267 marine species have been reported to have ingested plastic. Sea turtles are considered particularly prone to ingestion of anthropogenic debris, and ingestion of plastic has been reported in as many as 76% of the studied loggerhead turtles. The problem has been recognized, but the effects of plastic ingestion on the energy budget and the life cycle has not yet been studied in detail. In addition, genetic variability present between loggerhead turtle populations might affect the metabolic responses to the environmental pressures.

We used the previously developed energy budget (DEB) model for loggerhead turtles of the North Atlantic and Mediterranean populations to study multiple aspects of environmental conditions independently, and to understand how each condition affects the relevant process and life history traits. The results suggest that the ultimate size (length and weight) is primarily affected by food availability, and that growth and maturation are primarily affected by temperature, whilst also showing correlation with available food. Reproduction is affected by both food and temperature, with the former influencing the size of the reproduction buffer, and the latter influencing the rate of the related processes (such as vitallogenesis). Length at puberty varied between scenarios, but only by a small proportion, suggesting that interindividual variability plays a larger role for length at puberty than the environmental factors do.

The effects of plastic ingestion were studied using the same DEB model, but using the framework of assimilation units. The severity of the effects on the energy budget (and consequently growth, maturation, and reproduction) resulting from ingestion of plastic and other inert debris depended on the amount of the ingested debris, and their residence time relative to that of food. When the residence time of debris was assumed to be equal to that of food, amounts of debris taking up more than 14% of the digestive system volume of loggerhead turtles substantially decreased their fitness and impeded reproduction. When the residence time of the debris was assumed to be three or more times longer than that of food, a very similar condition (decreased overall fitness and reproduction) resulted from only 3.4% of the gut volume being occupied by inert debris .

The insights gained by the study allow us to better understand what the driving sources of the observed variability in growth rates, maturation, reproduction, and size are, and to predict to some extent what the metabolic responses would be under the present environmental pressures and the predicted environmental changes.

5.1 Introduction

The loggerhead turtles are on the IUCN list of the endangered species, they are included in the CITES convention, and are protected by various national and international laws [236]. Due to their long life, long period required to obtain puberty and reproduce, migratory way of life, and global distribution, they are extremely vulnerable to natural and anthropogenic environmental pressures [41, 195, 256]. Protection measures require an understanding of the pressures, ecology, and biology of the species. A variety of anthropogenic and environmental pressures are significant despite the protection. In addition to the pressures present on land, be it natural (predators, nest infestations etc) or anthropogenic (pressures related to tourism), the abiotic and biotic conditions in the marine environment (food availability and temperature, natural predators, bycatch, etc.) also greatly determine the success of protection measures and their effect on the population dynamics therefore needs to be understood.

The number of predators feeding on an individual decreases as an individual increases in size: the faster the growth, the larger the chances of survival. Interindividual differences in growth rates can be caused by a variety of factors, ranging from genotype and environmental conditions experienced during the first year of life [223, 132], to environmental conditions (food and temperature) experienced during the whole life time [13, 12]. The variability in the observed growth rates of loggerhead turtles had been partially attributed to interindividual variability within [25] or between populations [181]. Most often however, it had been either partially [25, 181] or mostly [15, 17] attributed to the differences in experienced temperature and food abundance. Loggerhead turtles are a migratory species, and both males and females had been observed to follow certain types of temperature and food fronts [71], and/or exhibit fidelity to specific feeding areas [170, 180]. Growth can be up to 30% faster in a neritic habitat [215], a habitat characterized by food of higher energy content and higher temperatures [177], lending support to the hypothesis that food and temperature are the main causes of different growth rates.

A direct correlation between growth rates and reproduction output had already been suggested [17], but not quantified. Different habitats, characterized by food and temperature, can lead to drastically different adult sizes, and different reproduction patterns [82, 177, 80, 242]. The length of the remigration interval (period between two nesting seasons) had been found to correlate with the average sea surface temperature [216], and so did the length of periods between two clutch depositions within a single nesting season [203, 86, 144]. Large scale environmental fluctuations, such as the North Atlantic Oscillation and the El Niño Southern Oscillation had been shown to account for a large part of nesting variability [201, 190, 92]. In the context of the ongoing climate changes, global environmental oscillations might exhibit different patterns in the near future, with a different combination of changes in temperature and productivity. Studying “climate change effects on key habitats for sea turtles”, and “the effects of climate change on sea turtles at sea” had been recognized as two primary goals in the review “Climate change and marine turtles” [84], and several studies on the impact of climate changes on the distribution of loggerhead turtles exist (e.g. [256]). However, the mechanisms by which changes in food availability and temperature independently influence the time required to grow, and/or accumulate the required energy for maintenance and reproduction have not been specified or quantified. To do so, it is important to explore how each of the two environmental factors affects the energy budget and processes such as growth, maturation, and reproduction of loggerhead turtles.

Another global pressure is the increased amount of anthropogenic debris (around 80% of which is plastic, [152]) in the oceans [7, 50, 95, 101, 230, 76, 118, 200, 152, 243, 231]. Sea turtles are “peculiarly prone to eat plastic scraps and other buoyant debris and to tangle themselves in lines and netting discarded by fishermen” [30], especially in the oceanic habitat [30, 233, 36, 255], but also in transitional and neritic habitats [233, 140, 206, 207, 121, 27]. Six out of seven sea turtle species had been recorded already in 1997 [117] to ingest anthropogenic debris, and the incidence of anthropogenic debris encountered during necropsies had increased [157] since. Loggerheads are opportunistic predators and eat whatever resembles food, including plastics and other marine debris [75, 159, 233, 11]. Different proportions of loggerhead turtles with ingested anthropogenic debris have been reported, incidence ranging from 20 or 30% [75, 66, 121] to as high as 50 and 75% [36, 233] of turtles studied. The occurrence of debris ingestion will probably increase, as the size of the (plastic marine debris) particles is in most cases smaller than 2.5 mm and is decreasing [154, 51], making them easier to swallow. In some areas small plastic particles (microplastics) are more abundant than plankton [153]. High concentrations of floating plastic items, microplastic particles, and other marine debris have been recorded, some since 1970s, in all the world's oceans [46, 7], from the Arctic [167], the North [119, 154] and South [155] Atlantic ocean, the Pacific ocean [73, 51, 221], to the Mediterranean sea [47, 29, 225] and waters off Australia [191]. Anthropogenic marine debris had been identified as one of the research priorities for sea turtles [250], with an extremely high probability of ingestion and/or entanglement upon encounter [254]. Ingested plastic can block, damage, or reduce the volume of the digestive system [220, 75, 207], resulting in less ingested and/or digested food and even death [140, 36, 121]. Under the assumption that it does not cause lethal obstruction or damage to the digestive system, ingestion of marine debris effectively dilutes the ingested food. Food dilution studies on sea turtles are rare, as it is hard to experimentally conduct them for as long as a loggerhead turtle can live (65 years or more, [78]). The only laboratory study exploring the effects of food dilution on posthatchlings of loggerhead turtles [146] reported that the compensation for food dilution by feeding rates does not occur. There are no similar studies done on juvenile and adult individuals, and the sub-lethal consequences of ingestion of plastic might be even more severe than currently thought. So far, the problem had been recognized, but the effects of plastic ingestion had not been studied from a mechanistic approach, and the effects on the energy budget have remained unquantified.

In this study, we aim to decipher

(i) the effects of temperature and food availability, and

(ii) the mechanisms and consequences of plastic ingestion

on growth, maturation, and reproduction of loggerhead turtles. A set of realistic scenarios were tested, and the results discussed in the context of available data.

5.2 Methods

5.2.1 Effect of the environmental characteristics (temperature and food availability) on growth, maturation, and reproduction

The simulations were performed using the standard DEB model (see Section 2.1 in Chapter 3 for definitions and equations). A single (North Atlantic) population was studied in more detail to reduce variability introduced by differences between populations. Because the effects of food availability on the energy budget were studied, it should be noted that the assimilation flux (p_A), quantified as:

$$p_A = \kappa_X p_X = \{p_{Am}\} V^{2/3} f, \tag{5.1}$$

depends on the food *quality* and *quantity*. The coefficient κ_X is the assimilation efficiency, i.e. the proportion of the ingestion flux (p_X) which enters the reserve. The value of $\kappa_X = 0.8$ (a value consistent with the assimilation efficiency assumed for loggerhead turtles also by other authors [80]) is generally assumed constant, however this can depend on the type of food, as well as the abundance of food - changes in the gut residence time, often inversely correlated to the food abundance, can increase or decrease the proportion of assimilated food. The surface area specific assimilation rate, $\{p_{Am}\}$, can also be written as a product of a dimensionless food quality parameter (s_X), and a reference surface area specific assimilation rate ($\{p_{Am}^{\text{ref}}\}$), where $s_X = 1$ for standard food quality (see Section 2 in [114]). The half-saturation constant, K, used to calculate the scaled functional response:

$$f = \frac{X}{X+K} \tag{5.2}$$

is in fact also a function of the food type, because it depends on $\{p_{Am}\}$:

$$K = \frac{\{p_{Am}\}}{\kappa_X\{F_m\}}. \tag{5.3}$$

The parameter $\{F_m\}$ relates to the surface area of an organism, and incorporates the distinction between the food density expressed per unit of surface area of the environment or per unit of volume of the environment. For simplicity, we used a standard value of the maximum specific searching rate ($\{F_m\} = 6.5\,\text{l/d.cm}^2$, [127]), so the expression (5.3) resulted in the half-saturation coefficient with units of J/l (dimensions of energy per volume of the environment), which are also dimensions of food density. Whether K and food density should be expressed as energy per volume or energy per surface area of the environment depends very much on the feeding strategy of individuals: some might feed on pelagic organisms and search for food in the substantial part of the water column, whereas others might focus on the benthic communities. For the former, food density should be expressed with relation to volume, and for the latter with relation to surface area of the environment (see Section 2.1 in Ref.[109], p25). Because feeding strategy of sea turtles is not well defined on the "feeding in volume - feeding on surface area spectrum", it is not clear how exactly one should express food density. To avoid notational complexity, we follow the approach in Ref. [109] and scale the food availability by the half-saturation coefficient, arriving at a dimensionless scaled food density, $x = X/K$.

Considering that DEB primary parameters were known (Table 5.1), K could be calculated (equation 5.11). Values of the parameters κ_X and $\{p_{Am}\}$ were assumed to be constant through out the life cycle, therefore only changes in food *quantity* were taken into account, while assuming food of very high (and constant) quality that can be assimilated with 80% efficiency.

Table 5.1: The list of standard DEB model primary and auxiliary parameters used for the simulations. Symbols, values, and units for North Atlantic ('$pars_{\text{NA}}$') and Mediterranean ('$pars_{\text{Med}}$') population specific parameter values are listed. Parameter values that were estimated independently for each population are marked with bold font. Notation: square brackets, [ ], indicate parameters normalized to structural volume, and curly brackets, { }, indicate parameters normalized to structural surface area. More details are available in the online DEB notation document (`www.bio.vu.nl/thb/deb/deblab/`).

Parameter	Symbol	Value		
		$pars_{\text{NA}}$	$pars_{\text{Med}}$	Unit
Maximum searching rate	$\{F_m\}$	6.5	6.5	$1/\text{d.cm}^2$
Digestion efficiency (of food to reserve)	κ_X	0.8	0.8	-
Defaecation efficiency (of food to faeces)	κ_X^P	0.1	0.1	-
Maximum specific assimilation rate	$\{p_{Am}\}$	**906.1**	**933.1**	J/d.cm^2
Energy conductance	v	**0.0708**	**0.0723**	cm/d
Allocation fraction to soma	κ	0.6481	0.6481	-
Reproduction efficiency	κ_R	0.95	0.95	-
Somatic maintenance	$[p_M]$	**13.25**	**13.6**	J/d.cm^3
Maturity maintenance rate coefficient	k_J	0.002	0.002	1/d
Specific cost for structure	$[E_G]$	7847	7847	J/cm^3
Maturity at birth	E_H^b	**3.809e+**	**3.809e+04**	J
Maturity at puberty	E_H^p	**8.73e+0**	**5.713e+07**	J
Weibull aging acceleration	h_a	**1.85e-010**	**1.44e-10**	$1/\text{d}^2$
Gompertz stress coefficient	s_G	0.0001	0.0001	-
Reference temperature	T_{ref}	293	293	K
Arrhenius temperature	T_A	7000	7000	K
Shape coefficient	δ_{SCL}	0.3744	0.3744	-
Specific densities	d_V, d_E	0.28	0.28	g/cm^3

The changes in the environmental conditions were simulated for the North Atlantic loggerhead turtles (parameters '$pars_{\text{NA}}$' - column 3 of Table 5.1) as an increase or decrease in the average sea temperature, and an increase or decrease of the available food. The effects of temperature on the energy budget were explored for the range of sea temperatures that had been reported for the North Atlantic loggerhead turtles (between 16° C and 30° C, [83]).

For scenarios of different food levels, the scaled food availability ($x = X/K$) that resulted in the scaled functional response of 0.81 (approximation for the North Atlantic population) was considered to be the reference food availability (x_{ref}), and the food availability was then modeled to be 20, 30, and 100% higher, and 10, 20, 30, and 50% lower than the reference value. First the scaled functional response was calculated for each food level, and then the growth, maturation, and reproduction of the loggerhead turtles were predicted. The value of K, calculated using equation 5.3 and parameters listed in Table 5.1, was 174.25 J/l. Functional response, calculated using the scaled food density

$$f = \frac{x}{x+1}, \tag{5.4}$$

is equivalent to that in equation 5.2.

Food availability and temperature were assumed to be constant for the duration of a simulation, because loggerhead turtles keep their body temperature relatively stable (by following thermoclines [85]), and can also actively search for food to satisfy their energy needs. The model does allow exploring fluctuating environmental conditions, as well as changes in the average conditions at some point in the life cycle, but this was beyond the scope of this study.

After separately studying the effects of temperature and food availability on growth, maturation, and reproduction of loggerhead turtles from a single (North Atlantic) population, the metabolic responses of North Atlantic loggerhead turtles were compared to that of Mediterranean loggerhead turtles. The emphasis of Chapters 3 and 4 has been on estimating the parameter values (presented in Table 5.1) for the North Atlantic and the Mediterranean population (respectively), and comparing the populations with regards to the morphology and physiology (explored through the parameter values), while the environmental characteristics were merely a background setting. The emphasis in this study is on the environmental characteristics and the metabolic responses, i.e. model predictions obtained by using the previously estimated parameter sets. The example of North Atlantic loggerhead turtles living in the Mediterranean sea is a perfect case for a a physiology vs. environment study, because (based on the previous results that define what the influence of the environment on the predicted values would be) we can identify those predicted values that are a consequence of the physiology. The Mediterranean and North Atlantic loggerhead turtles in their main habitat (East Mediterranean and North Atlantic, respectively) experience the same temperature, but different food levels: the East Mediterranean was characterized by $T = 21.7^\circ$ C [133] and $f = 0.71$, and the North Atlantic by $T = 21.8^\circ$ C [83, 85] and $f = 0.81$. Differences in the metabolic responses (growth, maturation, and reproduction) between the North Atlantic loggerhead turtles *in the North Atlantic*, and Mediterranean loggerhead turtles *in the Mediterranean* that cannot be accounted for by the difference in *food availability* are therefore a consequence of physiological differences. At the same time, the North Atlantic loggerhead turtles *while in the West Mediterranean* are experiencing the same food level ($f = 0.71$) but different temperature than the Mediterranean loggerhead turtles in the East Mediterranean:

$T = 19^\circ$ C and $T = 21.7^\circ$ C (West and East Mediterranean, respectively, [133]). Consequently, differences between metabolic responses of those North Atlantic loggerhead turtles (living in the Mediterranean) and the Mediterranean loggerhead turtles, that cannot be accounted for as an effect of *temperature*, are probably a result of physiological differences.

DEB-related variables (energy in reserve, volume of structure, and energy invested into maturity) are studied alongside the more frequently reported properties (such as physical length, and relationship of weight and fecundity to length). Reproduction was assumed to occur every two years. All simulations were performed in MatlabR2011b.

5.2.2 Anthropogenic marine debris

Loggerhead turtles are opportunistic omnivores [177], and while feeding they also ingest anthropogenic debris, including tar, styrofoam, fibers, soft plastic, and microplastic particles [140, 233, 121, 131, 75]. The ingested anthropogenic debris (the majority of it being plastic and not undergoing any degradation in the digestive track [158]) has no digestible energy, but it does influence the assimilation by occupying volume in the digestive system. Therefore, the model needs to track both energy and volume. We use the concept of Synthesizing Units [107] and follow the assimilation units (AU) which assimilate energy from food. Following the notation in Ref. [108], the environmental density of food was marked with X, and that of anthropogenic debris (plastic particles) with Y. The assimilation units can either be processing food, blocked by plastics, or free i.e. not bound to any particle. The proportions (θ) of each group of AUs are therefore: θ_X (bound to food), θ_Y (bound to plastic), and θ_{-}(free). The proportions of the AUs add up to one: $\theta_X + \theta_Y + \theta_{-} = 1$. When we know the binding affinities, b_i, of substrates to the AUs, and the rates of substrate release, k_i, (where $i = X, Y$), the dynamics of the different proportions of AUs can be described as follows:

$$\frac{d\theta_X}{dt} = b_X \theta_{-} X - k_X \theta_X, \tag{5.5}$$

$$\frac{d\theta_Y}{dt} = b_Y \theta_{-} Y - k_Y \theta_Y, \text{ and} \tag{5.6}$$

$$\frac{d\theta_{-}}{dt} = -b_X \theta_{-} X + k_X \theta_X - b_X \theta_{-} Y + k_Y \theta_Y. \tag{5.7}$$

Steady states of the proportions (θ^*) can then be written as:

$$\theta_X^* = \frac{b_X}{k_X} \theta_{-}^* X, \tag{5.8}$$

$$\theta^*_Y = \frac{b_Y}{k_Y}\theta^*_- Y, \text{ and} \tag{5.9}$$

$$\theta^*_- = (1 + \frac{b_X}{k_X}X + \frac{b_Y}{k_Y}Y)^{-1}. \tag{5.10}$$

In order to calculate the energy assimilated by the assimilation units, we also need to know the conversion success of a substrate (X or Y) to reserve (E) (marked as y_{EX} for food, and y_{EY} for plastic), and the maximum surface-area specific ingestion rate ($\{J_{Xm}\}$ for food, and $\{J_{Ym}\}$ for plastic particles). Using the steady state proportions (equations 5.8 and 5.9), the assimilation mass flux (J_{EA}) for an animal of structural volume V can be written as:

$$J_{\text{EA}} = V^{2/3}\{J_{Xm}\}y_{\text{EX}}\theta^*_X + V^{2/3}\{J_{Ym}\}y_{\text{EY}}\theta^*_Y.$$

Because plastic has no digestible energetic value ($y_{\text{EY}} = 0$), the second term is equal to zero, and the assimilation is proportional to the fraction of AUs processing food only (θ^*_X). Substituting θ^*_- in equation 5.8 with the expression 5.10, and replacing the ratio of the binding affinity (b) and the release rate (k) for a substrate with the half saturation constant for that substrate (classic Holling type II response curve):

$$K_X = k_X/b_X, \text{ and } K_Y = k_Y/b_Y, \tag{5.11}$$

we obtain:

$$\theta^*_X = \frac{X}{X + K_X(1 + Y/K_Y)}.$$

The expression for the assimilation flux is then:

$$J_{\text{EA}} = \{J_{Am}\}V^{2/3}\frac{X}{X + K_X(1 + Y/K_Y)}. \tag{5.12}$$

Equation 5.12 is equivalent to equations 5.1 listed earlier, the only difference being the property of interest (energy or mass, respectively).

The equation for f, which was used for the simulations, can be written both using the absolute and the scaled food and plastic densities (cf. equations 5.2 and 5.4) as:

$$f = \frac{X}{X + K_X(1 + Y/K_Y)} = \frac{x}{x + 1(1 + y)}. \tag{5.13}$$

When we compare the two expressions for the scaled functional response ((5.2) and (5.13)), the only difference is the expression for the half-saturation coefficient:

$$K = K_X(1 + Y/K_Y).$$

The new half-saturation coefficient is larger, with the increase proportional to the concentration of plastic particles Y. This means that, compared to an environment where no plastic particles are present, the scaled functional response will be lower, even though the food density X did not change.

For simplicity, we assumed that the binding affinity and the release rate of the AUs for the ingested plastic particles are equal to those of food particles, leading to equal half-saturation constants ($K_X = K_Y$). This allowed us to calculate the concentration of plastic particles (Y) because the ratio of the AU processing food and AU processing plastic particles in the steady-state is directly proportional to the densities of food and plastic particles:

$$\frac{\theta_X^*}{\theta_Y^*} = \frac{X}{Y}, \text{ i.e. } Y = ({}^{\theta_Y^*}\!/{}_{\theta_X^*})X. \tag{5.14}$$

We assumed that the food density (X) corresponds to the scaled functional response of $f = 0.81$. Knowing the half saturation constant (K) for food from (5.3), we calculated using (5.2) which food density corresponds to $f = 0.81$, and marked it as X_{ref}.

Loggerhead turtles do not discriminate between prey and plastic items [159], resulting in the proportion of food and plastic entering the digestive system equal to X/Y. i.e. the proportion of food and plastic in the environment. The proportion of anthropogenic debris in the volume of the stomach contents of all loggerhead turtles in a study by Frick et al. [66] has ranged from 0 to 25.7%, but when the whole digestive system is considered it can be higher. The intestine contents in Tomás et al. [233] have contained a larger proportion of anthropogenic debris than stomach contents, with the “mean percentage of debris items with respect to the total 41.56%, (S.D. 28.59) and both types of items appeared mixed in the digestive tracts” [233]. Because the assimilation of nutrients occurs across the whole digestive system, values also higher than 25% of volume [66] were simulated in our study.

Assimilation units are linked to the surface area of the digestive system [109], not its volume. Taking 25% of the gut volume as a reference value for plastic ingestion, the reference ratio of the AUs ($r_{ref} = {}^{\theta_Y^*}\!/{}_{\theta_X^*}$) was calculated by converting the value expressed per gut volume (0.25) into a value expressed per surface area:

$$\frac{0.25}{V} = \frac{r_{ref}}{V^{2/3}} \rightarrow r_{ref} = 0.3969.$$

By inserting the calculated values for X_{ref} and r_{ref} into (5.14), we obtained the approximation for the plastic density in the environment, which was marked as Y_{ref}. We then used (5.13) to explore the effects that different (10, 20, 30, and 50% larger and smaller) plastic densities (Y) have on the scaled functional response.

Finally, because the residence time of plastic in the digestive system of sea turtles ranges from several weeks [140] to several months [131], whereas the mean passage time of food had been reported as 9 to 13 days [240], we assumed a lower release rate for plastic particles ($k_Y < k_X$), leading to a proportionally lower half-saturation constant ($K_Y < K_X$). We explored the effects of different K_Y values while assuming a certain density of debris (Y). Based on the observed ratios of residence times of food and of plastic items, it was assumed that the release rate of debris was 1.25 times slower (resulting in a residence time of 11 to 16 days), 2 times slower (residence time of 18 to 26 days, [140]), and 3, 5, and 10 times slower (residence time 27-130 days, [131]) than the residence time of food. The implications for the difference residence times on growth and reproduction were studied via the effect on the scaled functional response using (5.13). For all simulations the temperature was assumed constant ($T = 22^\circ$ C), and the parameters for the North Atlantic population (column 2 in Table 5.1) were used.

5.3 Results

5.3.1 Environmental characteristics (temperature and food availability)

The effects of changes in food density. The effects were substantial on the ultimate size (Table 5.2 columns 5 and 6, Figure 5.1 panels e and f) and reproduction (Table 5.2 column 7, and Figure 5.1 panels h and i). Effects on growth and maturation were smaller (Table 5.2 column 3, and Figure 5.1 panels e and g), except for the 50% decrease in food density, which exhibited a substantial effect on growth and maturation. Length at puberty was affected by the food density, but the effect was only marginal, with the largest reduction in size (5.7%) for the 50% lower (than reference) food density (Table 5.2 column 4).

The assumed reference (scaled) food density (marked as X_{ref}, or x_{ref} when scaled) resulted in a relatively high scaled functional response ($f = 0.81$). Because the functional response is a saturating function, a relative increase or decrease in food density did not result in the same relative increase or decrease of the scaled functional response f: a 50% *increase* in food density resulted in 6.79% increase of f, whereas a 50% *decrease* in food density resulted in a 15.93% decrease of f (more than a double relative difference). The difference in the ultimate length (relative to the reference length at $f = 0.81$) was equivalent to the relative difference in f (6.7% for the 50% higher and 15.9% for the 50% lower

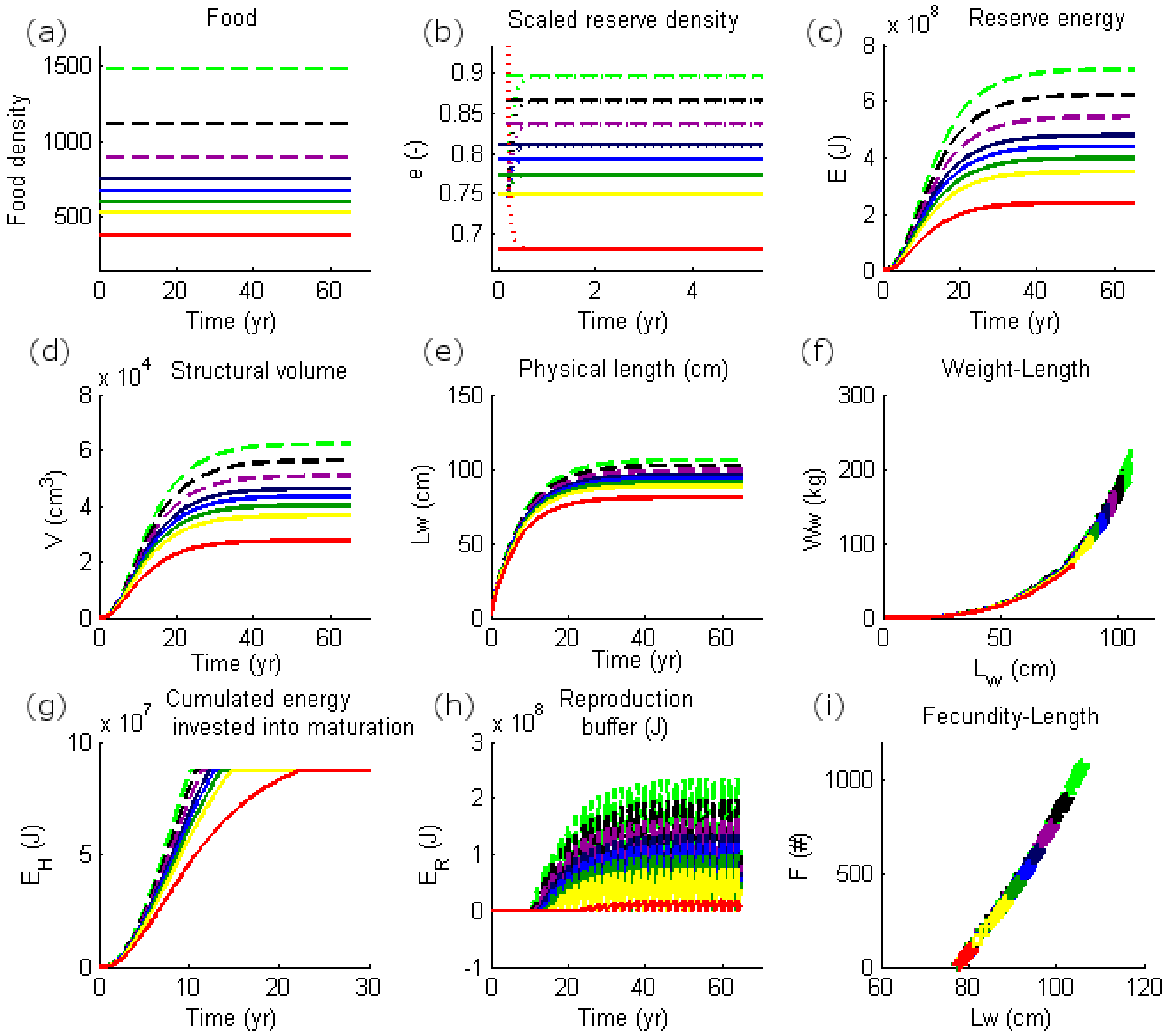

Figure 5.1: Model predictions for a set of food densities (relative to the reference food density giving a scaled functional response of 0.81) at a temperature of $T = 22°\text{C}$. Panel a: Food availabilities calculated as 20%, 50%, and 100% higher than the reference food level (dashed lines), and 10%, 20%, 30%, and 50% lower than the reference food level (full lines). Panel b: scaled reserve density (dotted line) in relation to the scaled functional response (full line), with the initial fluctuations in the scaled reserve density observable during the first short period. The scaled functional response of an individual is, at a constant feeding regime, equal to the scaled reserve density (ratio of the reserve density and maximum reserve density). Here an adjustment in the scaled reserve density of hatchlings as they start feeding can be observed. Panels c, d, g, and h: three of the main DEB state variables - reserve (panel c), structural volume (panel d), and energy invested into maturation (panel g), i.e. reproduction (panel h). The exact time when the plateau in the cumulative energy invested into maturation is reached corresponds to puberty, after which allocation to reproduction starts (see section 2.1 in Chapter 3 for more details). Panels e, f, and i: Observable equivalents of DEB state variables - length (panel e) is connected to the structural volume, weight (panel f) has contributions from structure, reserve, and the reproduction buffer, and fecundity (panel i) (calculated for reproduction events two years apart) is directly connected to the size of the reproduction buffer via energy allocated to reproduction. Colors and line-types in panels b to i correspond to the type and color of lines in panel a.

food density, Table 5.2 and Figure 5.1, panel e). The growth, maturation, and reproduction, however, did not scale with f in the same manner as the ultimate size. A higher food density did result in faster growth and maturation, but a 50% increase in food density resulted in 8% smaller age at puberty (length at puberty only slightly smaller than at lower food densities) and 43% larger fecundity, whereas the equivalent decrease in food density resulted in 80% higher age at puberty and 85% smaller fecundity (Table 5.2 and Figure 5.1, panels e, g, i).

Table 5.2: Model predictions for a set of different food densities at the temperature $T = 22°$ C. The food density (first column) is expressed as an increase or decrease relative to the reference food density that resulted in $f = 0.81$, see Section 5.2.1 for details. The reference values are indicated in bold font.

Change in food density (%)	Scaled funtional response (-)	Age at puberty (yr)	Length at puberty (cm SCL)	Ultimate length (cm SCL)	Ultimate weight (kg)	Max. fecund. (# egg)
+100	0.895	10.11	76.57	105.9	186.7 - 226.1	1068
+50	0.865	10.71	76.63	102.3	166.7 - 197.6	897
+20	0.836	11.38	76.7	99	145.8 - 173.5	747
-	**0.81**	**12.14**	**76.77**	**95.86**	**130 - 152.3**	**627**
-10	0.793	12.71	76.8	93.88	120.5 - 140.1	537
-20	0.773	13.52	76.88	91.52	111.3 - 126.4	448
-30	0.749	14.74	76.96	88.65	98.6 - 110.7	345
-50	0.681	22.30	77.21	80.57	69.6 - 72.9	89.07

The effects of temperature. Temperature was varied between 16 and 30° C. Temperature affects all metabolic rates; the effects were substantial for maturation (Table 5.3 column 2, Figure 5.2 panel g), growth (Figure 5.2 panels c, d and e), and reproduction (Table 5.3 column 6, Figure 5.3 panels h and i). The effect on length at puberty (Table 5.3 column 3) and ultimate length was negligible (Table 5.3 column 4, and Figure 5.3 panel e), and the effect on ultimate weight (Table 5.3 column 5, and Figure 5.3 panel f) directly reflected the fluctuations of the reproduction buffer (Figure 5.3 panel h).

The effects of temperature on rates depend on T_A, the Arrhenius temperature calculated in Chapter 3 as 7000 K. The relationship between the temperature and the rates was not linear: for example, a temperature difference of 2 degrees resulted in a difference of approximately 2 years of age at puberty only within a certain range (18 to 26° C), whereas outside of that range the age at puberty differed 1 year (between 26 and 30° C) or 4 years (between 16 and 18° C). The reproduction output was also affected by the temperature (Table 5.3 column 6, and Figure 5.3 panel i). The production of eggs includes various rates such as vitallogenesis, energy mobilization, chemical transformations etc [128, 49], implying that when food is abundant, the available time becomes the limiting factor. If individuals of the same size, experiencing the same food density, are exposed to different temperatures during the year, their reproduction output will be different (Figure 5.3 panel i).

Mechanisms of temperature effects on maturation, growth, and reproduction are important when studying the correlations between the global temperature changes or environmental oscillations and metabolic responses of sea turtles (e.g. [92, 203, 201]), and using the conclusions from those studies for conservation activities.

Table 5.3: Model predictions for a set of temperatures at the food availability resulting in a scaled functional response of $f = 0.81$.

Temperature (degree C)	Age at puberty (yr)	Length at puberty (cm SCL)	Ultimate length (cm SCL)	Ultimate weight (kg)	Max. fecund. (# eggs)
16	20	76.98	95.36	127.8 - 141.2	364
18	16.82	76.77	95.68	130.7 - 145.4	439
20	14.28	76.78	95.82	129.8 - 149	522
22	12.14	76.77	95.87	130.5 - 152.7	627
24	10.35	76.78	95.88	131.1 - 156.7	723
26	8.84	76.77	95.88	131.1 - 160.8	847
28	7.56	76.64	95.89	130.1 - 166.3	999
30	6.49	76.77	95.89	130 - 172.6	1154

Effects of physiological variability. When we compared the responses of different populations experiencing the same temperature but different food level (Mediterranean and North Atlantic individuals in their main habitat), an older age at maturity at a 3 to 5% smaller size and a smaller reproduction output were expected for the Mediterranean loggerhead turtles (see Table 5.2, and Figure 5.1). However, the Mediterranean turtles obtained maturity at a younger age (10.74 years, Figure 5.3 panel e), a substantially (15.5%) smaller size (65.1 cm SCL, Figure 5.3 panel c), and their reproductive output was several times higher than expected (around 500 eggs at 85 cm SCL, Figure 5.3 panel g).

When responses of different populations experiencing the same food level but different temperatures (2° C difference) were compared (Mediterranean and North Atlantic individuals in the East and West Mediterranean, respectively), Mediterranean individuals were expected to reach maturity at an age 2 years earlier than their North Atlantic conspecifics, at the same length, and reach approximately the same ultimate size with a slightly higher reproduction output (see Table 5.3, and Figure 5.2 for the predicted values). However, the results were again drastically different (see Figure 5.3), with the predicted age at puberty of individuals more than 10 years apart (10.74 years for the Mediterranean, and 24 years for the North Atlantic individuals), thedifference between a size at maturity of 16 cm SCL (65.1 cm SCL for the Mediterranean and 81.43 cm SCL for North Atlantic individuals), and a reproduction output three times higher for Mediterranean individuals (see Figure 5.3 panel g). The ultimate size was indeed the same in

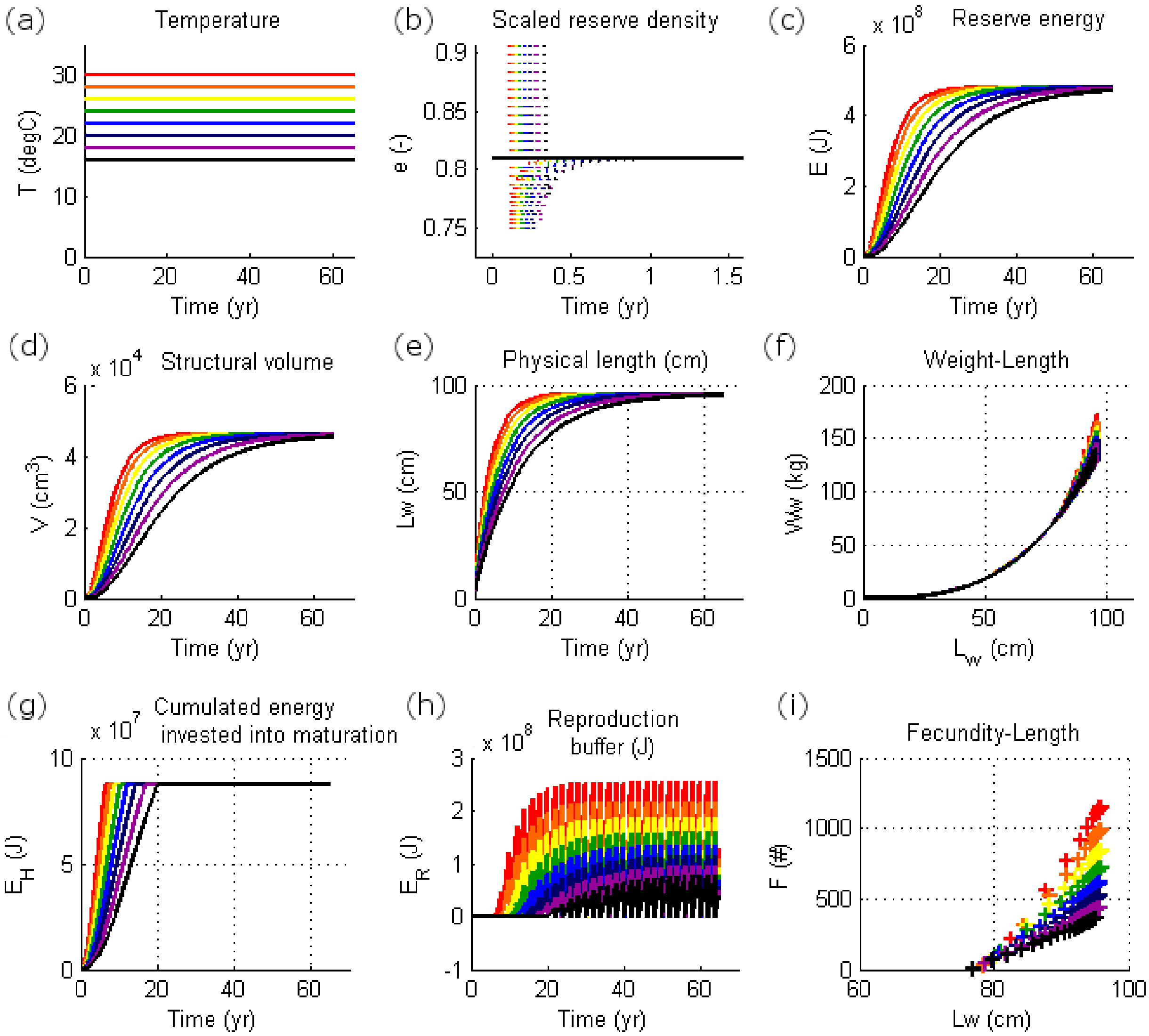

Figure 5.2: Model predictions for a set of temperatures (16° C, 18° C, 20°C, 22° C, 24° C, 26° C, 28° C, and 30° C) shown in panel a at the food availability resulting in a scaled functional response of $f = 0.81$. For description of panels b to i see description of Figure 5.1. The lines in panels b to i correspond to the color-coded lines in panel a.

both populations, and the growth rates were slightly lower for the North Atlantic individuals (see Figure 5.3, panel c).

One of the interesting results obtained during model simulations was the implication that (in the Mediterranean) North Atlantic individuals experience a slightly lower food level than Mediterranean individuals: the same scaled functional response ($f = 0.71$) can be a result of different food densities if the half-saturation coefficient is different (see equation 5.2). The half-saturation coefficient (equation 5.11) of Mediterranean individuals is higher than that of North Atlantic individuals as a consequence of a higher value of $\{p_{Am}\}$ (Table 5.1) . This is consistent with the observation that North Atlantic loggerhead turtles stay in the areas with lower productivity within the Mediterranean sea (north-western basin) , whereas the Mediterranean loggerhead turtles remain in the areas with higher productivity (south-eastern basin) [31, 192]. However, the fact that scaled functional responses of the individuals from two populations are equal, suggests that the difference in the productivity of the areas is not the cause of different growth and maturation rates which have been observed [181]. Indeed, when the same temperature was simulated for individuals from both populations, the growth rates were practically indistinguishable. The results would therefore imply that temperature (which is indeed different in eastern and western basin of the Mediterranean sea, [133]) is the main environmental driver for the observed difference in growth rates of individuals belonging to different populations.

The reproduction output (Figure 5.3, panels f and g) was higher than that expected in the context of previous results obtained under similar food density and temperature. This could be explained by a lower level of maturity at puberty specific for Mediterranean population (see Section 4.3.2 of Chapter 4), i.e. the difference in the cumulative energy that needs to be invested into maturation before reaching puberty (Figure 5.3, panel e). The level of maturity at puberty is correlated to the daily cost of maturity maintenance [109], which implies that the daily cost of maintaining maturity is lower for the Mediterranean loggerhead turtles, and they can allocate more energy into reproduction than the North Atlantic ones, resulting in more eggs at a certain body size. The fluctuation of energy within the reproduction buffer (Figure 5.3, panel f) simulates the reproduction (egg deposition) occurring every two years for all turtles. The amplitude of the fluctuations indicates how much energy was stored in the reproduction buffer between two nesting seasons, as the result of the available food (input of energy) and metabolic expenses (e.g. maintenance). More energy stored in the reproduction buffer resulted in more eggs, and consequently larger fluctuations in mass (Figure 5.3, panel d). The prediction of a higher age at puberty for the North Atlantic loggerhead turtles living in the Mediterranean (Figure 5.3, panel c) results from the need to accumulate the same amount of energy for maturation regardless of the food level (plateau of the curves), and is consistent with the observations [181]. The extremely low fecundity predicted by the model for the North Atlantic loggerhead turtles in the Mediterranean (black circles in panel g)

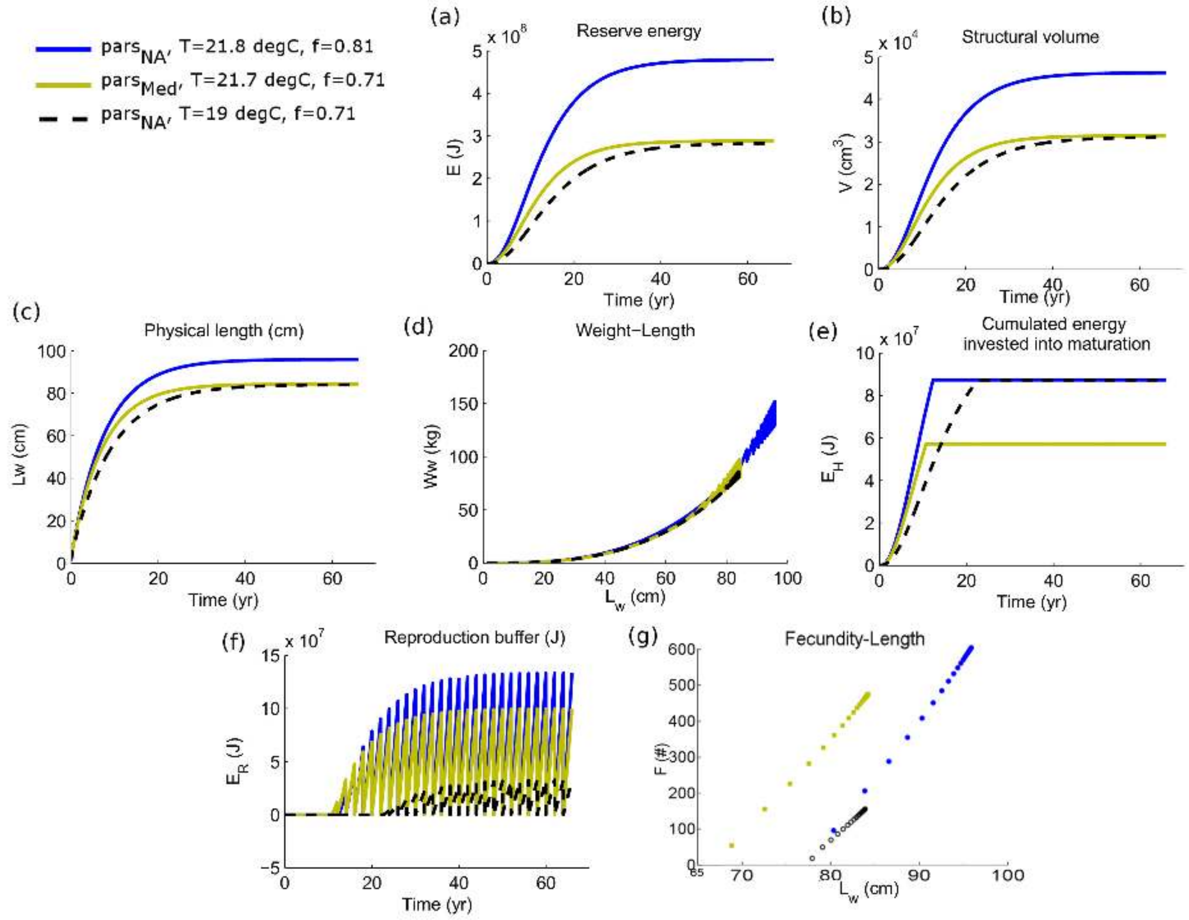

Figure 5.3: Model predictions for North Atlantic and Mediterranean loggerhead turtles to the age of 65 years. Legend in the top left corner provides a summary of simulation setups: *Full blue lines* in panels a to f and *blue* symbols in panel g are model outputs when using the parameter set specific to the North Atlantic population (pars_{NA}, Table **??**), and characterizing the environment with a constant temperature of 21.8° C [85] and food density resulting in a scaled functional response of $f = 0.81$. *Full yellow lines* in panels a to f and *yellow* symbols in panel g are model outputs when using the parameter set specific to the Mediterranean population (pars_{Med}, Table **??**), and characterizing the environment with a constant temperature of 21.7° C [166] and food density resulting in a scaled functional response of $f = 0.71$. These two cases simulate the North Atlantic and Mediterranean individuals in their primary habitats (North Atlantic and Mediterranean, respectively). *Dashed black lines* in panels a to f and *black* symbols in panel g are model outputs when using the parameter set specific to the North Atlantic population, and characterizing the environment with a constant temperature of 19° C [133] and food density resulting in a scaled functional response of $f = 0.71$. This case simulates North Atlantic individuals living in east Mediterranean sea. *Panels a and b* show change in two DEB state variables: reserve and structure, as a function of time. *Panels c and d* show observable quantities related to the two DEB variables: length as a function of time, and weight as a function of length; change in length is directly related to change in structure, whereas change in weight is related to change in structure and reserve, which includes also the reproduction buffer - dynimcs of the reproduction buffer are given in *panel f*. *Panel e* shows energy invested into maturation as a function of time; maturity is the third DEB state variable which cannot be directly observed, but obtaining the maximum level of maturity corresponds to puberty and denotes start of energy investement inot reproduction, which can be observed subsequently as nesting. *Panel g* gives a seasonal reproduction output (number of eggs) as a function of carapace length.

offers a reason for lack of observations of nesting North Atlantic loggerhead turtles in the Mediterranean [181].

5.3.2 Ingestion of non-digestible anthropogenic marine debris

In all cases, ingestion of marine debris resulted in a lower scaled functional response (f) (Tables 5.4 and 5.5 and Figure 5.4). As f accounts for the perceived food level, this means that individuals ingesting debris perceive less food, which results in slower growth and maturation, and a smaller reproduction output (Tables 5.4 and 5.5). At a scaled functional response of $f \leq 0.65$, North Atlantic loggerhead turtles cannot obtain enough energy to reach puberty or reproduce.

When the residence time of food and plastic debris was assumed to be equal, all densities of debris resulting more than 14% of the gut volume occupied by debris translated into a scaled functional response too low to sustain reproduction (Table 5.4 and Figure 5.4 panel a).

Table 5.4: Effects of plastic ingestion on growth and reproduction. Different (scaled) densities of plastic anthropogenic debris were simulated for a duration of 66 years, see Section 5.2.2 for details. In the *first column*, an increase or decrease in the (scaled) density of plastic is expressed, relative to the reference density equivalent to 25% [66] of the volume of the digestive system being taken by anthropogenic debris. In the *second column*, the ratio of steady states of assimilation units handling debris particles (θ^*_Y) and assimilation units handling food particles (θ^*_X) is displayed. In the *third column*, the percentage of the digestive system (volume) occupied by anthropogenic debris is displayed. In the *fourth column*, the ratio of the new half-saturation coefficient relative to the half saturation coefficient calculated for a control scenario without anthropogenic debris (K_{ref}, equation 5.3) is displayed. In the *fifth column*, the resulting scaled functional response (f) is given. In the *last three columns* the physical length (L_w), wet mass (W_w), and seasonal fecundity (F) of an individual (North Atlantic female) are given. The row containing data for the reference concentration of anthropogenic debris is marked with bold font, and the row containing data for the control scenario (no ingested plastic) is in bold italic font.

Change in debris density (%)	Steady state θ^*_Y/θ^*_X	Volume of digest. sys. (%)	Half sat. coeff. rel. to K_{ref}	Scaled funct. response, f	Length, L_w (cm SCL)	Weight, W_w (kg)	Fecund., F (#)
+50	0.595	45.93	3.54	0.546	64.670	31.91	0
+30	0.516	37.06	3.20	0.571	67.599	37.31	0
+20	0.476	32.86	3.03	0.585	69.165	40.45	0
+10	0.437	28.84	2.86	0.598	70.806	43.95	0
— Reference	**0.40**	**25.00**	**2.70**	**0.613**	**72.525**	**47.86**	**0**
-10	0.357	21.35	2.52	0.628	74.331	52.23	0
-20	0.317	17.89	2.35	0.644	76.228	57.13	0
-30	0.278	14.64	2.18	0.661	78.225	63.34	20
-50	0.198	8.84	1.85	0.698	82.548	80.18	122
-75	0.099	3.13	1.42	0.750	88.672	108.15	286
-100 (no debris)	***0.000***	***0.00***	***1.00***	***0.810***	***95.774***	***147.33***	***507***

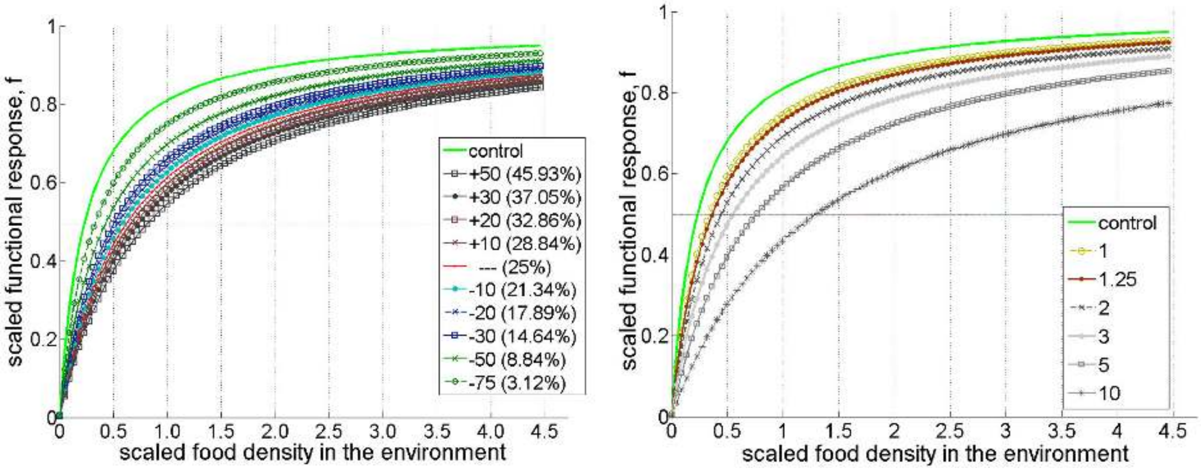

Figure 5.4: The change in the scaled functional response for a range of (scaled) food densities, depending on the concentration of anthropogenic plastic debris (panel a), or the residence time of debris (panel b). Food level is expressed relative to the food level that results in the scaled functional response of 0.81, see Section 5.2.1 for details. The change in the half-saturation coefficient can also be observed: the half-saturation coefficient of each curve can be read as the value on the x-axis for which the value on the y-axis is 0.5 (indicated by the horizontal line). *Panel a*: Numbers in the legend refer to an increase or decrease relative to the reference plastic debris concentration (the case of debris taking up 25% of gut volume). The numbers in brackets indicate the proportion of the gut (digestive system) taken up by plastic and other non-degradable anthropogenic debris for different scenarios. The residence time of marine debris was assumed to be equal to the residence time of food. *Panel b*: The numbers in the legend refer to the factor by which the residence time of debris is increased compared to the residence time of food. The percentage of volume taken up by debris was assumed to be 3.4%, similar to the mean volume of anthropogenic debris in stomach contents of all loggerhead turtles in a study by Frick et al. [66]. The curve marked as ”control” was plotted for a scaled functional response when no debris is ingested. See Section 5.2.2 for details.

When a longer residence time of debris (plastic) particles was assumed, even a “small” volumetric percentage of debris (3.4%) had substantial consequences on the scaled functional response (Tables 5.5 and Figure 5.4, panel b) and, consequently, the energy budget of loggerhead turtles. Residence time of several weeks [140], that is, 3 times longer than that reported for food [240], resulted in a scaled functional response too low to reach puberty and reproduce (Table 5.5). An average residence time of several months [131], that is 5 and 10 times longer than food particles, would most probably result in death as the sea turtles at the age of 66 years weighed 36 and 14.5 kg, respectively (last two rows of Table 5.5).

Table 5.5: Effects of plastic ingestion on growth and reproduction. Different residence times of anthropogenic debris, resulting in different half-saturation coefficients of debris and food ($K_Y \neq K_X$) were simulated. In the *first column*, the ratio of food and debris half-saturation coefficients is given, where the longer residence time results in proportionally lower half-saturation coefficient. The residence times are expressed relative to the residence time of food (based on values in Refs [240, 140, 131]). In the *second column* the residence time of debris was calculated in days. In the *third column* the ratio of the new (total) half-saturation coefficient relative to the half saturation coefficient calculated for a control scenario without anthropogenic debris (K_{ref}, equation 5.3) is given. In the *fourth column* the scaled functional response (f), see Section 5.2.2 for details. In the *last three columns* the ultimate physical length (L_w), ultimate wet mass (W_w), and maximum seasonal fecundity (F) are given.

K_X/K_Y	Residence time of debris (d)	Half sat. coeff. relative to K_{ref}	Scaled funct. response, f	Length, L_w (cm SCL)	Weight, W_w (kg)	Fecund., F (#)
1.00	9 - 13	1.45	0.746	88.225	105.94	273
1.25	11 - 16	1.56	0.731	86.519	97.74	226
2.00	18 - 26	1.90	0.691	81.775	77.00	103
3.00	27 - 39	2.36	0.644	76.203	57.06	0
5.00	45 - 65	3.26	0.567	67.060	36.27	0
10.00	90 - 130	5.52	0.436	51.581	14.48	0

5.4 Discussion

5.4.1 Is the energy budget realistic?

A large part of this study relies on the assumption that the estimated DEB parameters, and the resulting energy budget of the loggerhead turtles, are realistic. To assess and appropriately interpret the results, we should therefore first evaluate the validity of the energy budget, with emphasis on the parts of the budget most directly linked to the studied scenarios of different food levels, temperatures, and plastic ingestion. Hence, processes of assimilation (and ingestion), as well as processes of growth, maintenance, and reproduction (see Figure 5.5 and Section 5.2.1), are first discussed in more detail.

The diet of loggerhead turtles was simplified to food whose assimilation contributes to the energy budget equally regardless of age, i.e. life stage (modeled as a constant value of the surface-area specific assimilation rate, $\{p_{Am}\}$). For those loggerhead turtles that start feeding on markedly different food after recruiting to neritic habitats, additional assumptions may be needed to make simulations with more complex combinations of food densities possible. For example, in addition to temperature and food quantity, the model could be modified to include the change in food quality via a change in the parameter $\{p_{Am}\}$. Simplifying food to a single general food type has, however, a biologically realistic justification, adding to the applicability of the results. Namely, all

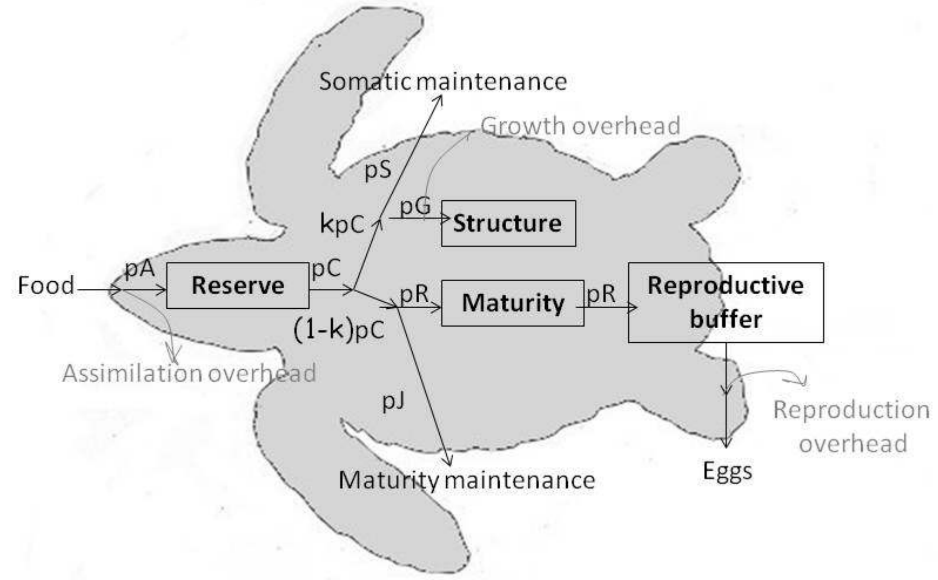

Figure 5.5: A schematic representation of the standard DEB model: p_A - assimilation, p_C - mobilization, p_S - somatic maintenance, p_G - growth, p_R - maturation/reproduction flux, and p_J - maturity maintenance. Modified from Kooijman [109].

loggerhead turtles spend a large part of their life in the oceanic habitat [23], and during that period the food quality does not markedly change. Some of the loggerhead turtles leave the oceanic habitats at a certain size or after obtaining puberty [177], but some of them remain in the oceanic habitats for the majority of their life [82, 80, 177]. Even though a range of food densities, scaled functional responses, and temperatures were simulated, these factors were considered constant throughout a simulation. The results therefore need to be considered in the context of this simplification, as they only *directly* relate to the loggerhead turtles that do not experience drastically different environmental conditions during their life.

Another assumption related to food was that assimilation efficiency is high ($\kappa_X = 0.8$). The assimilation efficiency that had been measured previously by various authors for pond turtles and sea turtles (feeding on food of similar protein content as the food of pelagic and neritic loggerhead turtles) was 80% (see [80]). However, there is an important conceptual difference between the assimilation efficiency which had been measured and reported by other authors (i.e. the difference between the energy content of the ingested food and the excreted faeces - the digestion efficiency) and the assimilation efficiency in DEB terms (i.e. the assimilation of energy from food to reserve, κ_X, or the cost of converting the food compounds into the form of the reserve - the specific dynamic action). The energy gain per unit of food in DEB is lower than the digestion efficiency because the energy needed for conversion of food into reserves needs to be paid as well [109], with the energy cost of feeding proportional to the feeding rate. A value of κ_X could have a different value than the one used in this study, as it is very sensitive to the type of food (for examples, see "Add my pet library", 110). The process of energy ingestion and assimilation of energy into reserve could be studied in more detail to obtain more accurate values for κ_X.

The maximum daily food intake that had been assumed in Ref. [80] (41% of body mass for the smaller 70 kg oceanic adults feeding on tunicates (pyrosomas), and 16% of body mass for the larger 90 kg neritic adults feeding on clams, [80]), would translate into a daily intake of approximately 8 897 kJ (19.3 kg) from pyrosomas or 28 454 kJ (3.7 kg) from clams (calculated based on the values presented in [80]). If we assume such large amount of ingested food (with the intake passage time of 9 to 13 days, [240]) is realistic, the calculated DEB assimilation flux (p_A) of 1205 kJ d^{-1} at 23° C would imply an extremely low (DEB) assimilation efficiency ($k_X \approx 0.1$). On one hand, a difference in the interpretation of the assimilation efficiency might account for the discrepancies in the energy budget as calculated by the DEB model compared to that calculated by Hatase and Tsukamoto [80]. On the other hand, while a low κ_X could be justified by an extremely low quality of food and by high costs of foraging, it would also imply an extremely high Food Conversion Ratio (FCR). For example, with an assimilation efficiency of $\kappa_X = 0.1$, a loggerhead turtle would require 110 kg of clams or 1 764 kg of pyrosomas to increase 1 kg in weight (within the range from 0.5 kg to 5.3 kg). The same turtle with an assimilation efficiency $\kappa_X = 0.8$ (a value used in the model) would need 13.83 kg wet mass (or around 4.1 kg dry mass when the average tissue density of 0.3 is assumed) of clam meat, or 462 kg wet mass (or around 46 kg of dry mass when the average tissue density of 0.1 is assumed) of pyrosomas for the same increase of 1 kg. A FCR of 4 for clam meat seems reasonable when compared to the values for other vertebrates [253], and FCR of 46 (or food conversion efficiency of 46% for dry mass, i.e. 0.5% for wet mass) seems consistent with the values reported for food conversion efficiency from krill to seals and from krill to whales [42]. Knowing the energy value of plankton (310 J kg^{-1} wet mass, with 100% edible parts) and clams (4940 J kg^{-1} meat wet mass, with 40% edible parts, all values from [80]), it is possible to calculate the amount of food a loggerhead turtle would need to daily ingest to satisfy its energy budget. Assuming the energy budget (determined by the parameters in Table 5.1) is correct, an adult loggerhead turtle of 150 kg would need to ingest around 4 to 5 kg of plankton daily, or 1.25 kg of clams, which seem to be reasonable values. Values of around 19 kg of plankton or 3.7 kg of clams daily [80] seem somewhat large, as for captive reared sea turtles, a daily food intake of 1% body weight satisfied the basal metabolic needs of juvenile loggerheads [163], and captive reared sea turtles were successfully reared when fed the commercial diet at a daily rate of 5% body weight while in the posthatchling stage, and 0.8% body weight when older [67].

Generally, the somatic part of the energy budget (the κp_C branch in Figure 5.5) seems realistic as the energy needs for growth and maintenance (FCR, and observed food intake for metabolic needs) can be satisfied by the energy that the model predicts is allocated for those processes.

The reproductive part of the energy budget (the $(1-\kappa)p_C$ branch in Figure 5.5) as predicted by the DEB model is also realistic: The egg energy value of 170-210 kJ [88] and the DEB predicted daily energy flux to reproduction (p_R) of 201.5 kJ d^{-1} (at 23° C), amounts to approximately one egg per day, or energy for 730 eggs allocated between nesting seasons that are two years apart. Allocation of that reproduction output into clutches results in 5 clutches of 146 eggs each, as observed in nature [237, 81]. Moreover, when the energy invested into maturity maintenance is integrated over two years between the nesting seasons, and added to the energy invested into reproduction during the same period, a value of around 300 MJ is obtained (127 MJ for maintenance and 147 MJ for reproduction investment, at the temperature of 23° C). The value is slightly smaller, but within the same order of magnitude as the reproduction costs (434 MJ, including migration and nest excavation)calculated for neritic Pacific loggerhead turtles [80]. The energy budget (as defined by DEB parameters) therefore seems realistic.

5.4.2 Effects of the environment (food density and temperature) on growth, maturation, and reproduction of loggerhead turtles

Processes such as growth, maturation, and reproduction, as well as life history traits such as ultimate size, age and size at puberty, are a result of abiotic (environmental characteristics) and biotic (individual characteristics) factors. A process model such as a DEB model makes it possible to separate the characteristics (and effects) of the environment from the characteristics of the individual (Chapter 4). Furthermore, because the DEB model is a mechanistic model, it was possible to independently explore the energy budget at different food densities and different temperatures, and use the gained knowledge to interpret the results of a more complex scenario (Section 5.3.1). While exploring the effects of the environment on growth rates, it became clear that growth rates derived from capture-mark-recapture data (e.g. [38]), growth marks on the bones (e.g. [25, 39]), or length frequency analysis (e.g. [34, 13]) should not be directly compared unless it is known that the individuals experienced the same or similar environmental conditions. Direct comparison could be misleading because different environmental conditions might result in the observed growth rates being similar between individuals belonging to different populations and/or life stages [15, 17].

The temperature and food availability presented in the section 5.3.1 represented a somewhat arbitrarily chosen, yet realistic range. The temperature range was between 16 and 30° C, based on data presented in [85, 83]. The loggerhead turtles in the North Atlantic rarely experience sea temperatures outside this range, even during winter [83]. The body temperature of juvenile, subadult and moderately active adult chelonid turtles corresponds to the surrounding water temperature [183], but the adults are more efficient than juveniles in keeping their body temperature close to optimal values ([91, 85]). Therefore, juveniles might experience even lower temperatures than 16° C during winter, which might slow down their growth and maturation.

One of the results obtained by studying the effects of different (scaled) food densities was an insight into the relation between a difference in food density (a property of the environment) and a difference in the scaled functional response (the environment as perceived by the individual) directly resulting in differences in observable quantities (age and size at puberty, ultimate size, and fecundity) (Table 5.2 and Figure 5.1). Explored food densities ranged from those resulting in a very high scaled functional response ($f = 0.9$) to those barely sustaining reproduction ($f = 0.68$). Values outside this range are probably rarely present in nature, as the maximum scaled functional response ($f = 0.999$) is reached at extremely high food densities (23 times, or 2222.22% higher than the value taken as the reference), and a lower scaled functional response is evolutionary unfavorable, as it does not support reproduction (see Table 5.4). Keeping in mind the implications of the saturating relationship of food density with the scaled functional response is important for populations living at lower food densities (e.g. individuals in the Mediterranean sea, or in the oceanic habitats) as at the lower end of the food density range, a smaller reduction in food availability always results in a larger decrease of the scaled functional response due to the nonlinear nature of the relationship. It is important to keep in mind that food intake is a saturating function of food density when studying the correlation of primary productivity with growth of loggerhead turtles, or constructing population models and conservation methods based on such correlations.

While the predicted growth rates can be more or less directly compared to the growth rates observed in an environment similar to one assumed in the simulations, the reproduction output needs to be evaluated with respect to the observed [88, 232] trade-off between the number of clutches per season, eggs per clutch, and remigration intervals. We simplified the model by assuming that nesting occurs every two years if there is enough energy stored in the reproduction buffer. The simplification resulted in predictions for an extremely small (e.g. 90 eggs, Table 5.2) or large (e.g. 1154 eggs, Table 5.3) reproduction output in a nesting season. Even though nests of less than a hundred, and nests of more than 200 eggs have been observed [151, 136, 88, 204], it is more likely that those loggerhead turtles with more energy available will have shorter remigration intervals (i.e. reproduce every year), whereas those with less energy available will have longer remigration intervals (i.e. reproduce three or more years apart). This is consistent with the previously found correlation between remigration intervals and sea surface temperature [216], and is one of the possible explanations for remigration intervals having a modal value of 2-3 years, yet ranging from 1 to 7 years [26].

Our analysis suggested that variability of food abundance has a much stronger effect on reproduction than variability of temperature. In the previous studies, a higher temperature at *breeding* sites had been found to correlate to the shorter period between two clutch depositions within a single nesting season [203, 86, 144], probably by influencing the processes of vitallogenesis [128]. However, there was no observed correlation to the number of clutches per nesting season [144]. This is consistent with the assumption that the energy is invested into the reproduction buffer throughout the year [109], and not just at breeding sites, implying that at a higher food level more energy will be continuously invested into the reproduction buffer. In the context of continuous investment into reproduction, the mentioned observation is also consistent with the previously observed [145] correlation of the nesting abundance and temperature at *feeding* sites, where temperature could positively correlate with food abundance [177, 17].

In addition to the scenarios of several constant temperatures and food densities tested in this study, a temporary increase or decrease in temperature or food availability could be simulated. Compensatory growth had been observed [193] in sea turtles experiencing an increased food level after a period of decreased food availability, but the consequences of the compensation on the energy budget later in life are unknown [149], and could be studied using the DEB model presented here. The frequency and length of the migrations could also be integrated into the model through influence on the energy budget in proportion to the traveled distance [80, 109] . This might give a more realistic range of predictions for growth and maturation, and account for some of the observed intrapopulation variability in the growth rates, size and age at sexual maturity.

The environmental conditions and scenarios explored in this study concentrated on the loggerhead turtles in the marine environment, i.e. while they are at sea. However, environmental changes affect, in addition to marine habitats, also the nesting beaches. Loss of nesting beaches due to coastal land loss [55], as well as change in survivability [227, 176] and proportion of hatchling sexes [84, 256, 227] due to changes in the incubating conditions, can additionally influence the dynamics of the loggerhead turtle populations, but were outside the scope of this study.

A number of implied properties could also have been studied using the DEB model. Examples of such additional properties are: daily energy budget at different life stages, i.e. expenses for maintenance and other metabolic processes at different temperatures; intensity of food density fluctuations that an energy buffer (reserve) can handle without affecting the budget; food density (i.e. scaled functional response) at which all available energy is required by the processes of maintenance, resulting in no growth or reproduction, etc.

5.4.3 Anthropogenic pressure: plastic and other non-digestible marine debris

We have explored the long-term effects of plastic (anthropogenic debris) ingestion under two hypotheses: (i) the ingested debris pieces have the same residence time as food, and (ii) the ingested debris pieces have a longer residence time than food. Different amounts and residence times of the ingested debris were simulated. Plastic was considered to be an inert material, not undergoing any degradation. Degradation in the digestive track tested for some types of plastic labeled as biodegradable proved to be negligible after almost two months [158], so the simplification was justified.

The simulated environmental densities of the debris resulted in 0 to 50% of the gut volume being taken up by debris, with the density that resulted in 25% of the gut volume being taken up by the plastic debris taken as a reference density. Even though most reported values for stomach volume percentage occupied by debris have been below 25% (mean value of 3.2%, [66]), three important points are (i) that the values up to 25% of stomach contents have been recorded in the same study [66], (ii) that the debris load (as percentage of volume) of the whole digestive system is higher than that of a stomach [233], and (iii) that marine debris "enters the digestive system in similar proportion as prey items" [233], suggesting that 50% of a full digestive system might consist of non-degradable marine debris. Values higher than 50% of gut volume are not likely, as this much debris inhibited 65% of the assimilation units (Table 5.4 column 2), and would probably result in death by starvation. Anthropogenic debris had often been reported as percentage of dry mass of gut contents, making up on average 1.7% [36] or 2.2% [121] of total gut content's dry mass. The proportions might seem lower than proportions used in this study, but the dry mass of plastic is relatively small compared to the volume (or surface area) it occupies: dry weight of plastic in a study by Lazar and Gračan [121] had ranged from below detectable limits to 0.71g (mean dry weight 0.08g), but the length of items ranged from 1 to 16 cm. In fact, 73% of loggerhead turtles which had ingested less than 0.01g of plastic, had ingested 1 to 3 pieces of anthropogenic debris of length 1 to 6 cm (mean length 3.1 cm) [121]. A light but large piece of plastic (e.g. a plastic bag or a sheet of plastic) can cause more damage than a heavier smaller item, due to a larger surface area.

The results suggested that the longer residence time of the debris has even more deleterious effects than the increased concentration of the debris. It should be noted here that the type of simulations assumed that the individuals are constantly exposed to a certain concentration of debris which all had a same residence time. On one hand, the reality might be less grim because sea turtles probably experience periods when no plastic or other anthropogenic debris items are present in their digestive system, if for no other reason then because the turtles do not feed while at breeding grounds [49]. When exploring the stomach and esophagus of green turtles, Bugoni et al. [27] had not found a significant correlation between the curved carapace length of the turtles and the weight of plastic pieces, supporting this hypothesis. On the other hand, Tomás et al. [233], who have analyzed the whole digestive system of loggerhead turtles, have reported a correlation between CCL and volume of (natural and anthropogenic) debris ingested by (juvenile) loggerhead turtles. A possibility that more debris is present in parts of the digestive system not analyzed in the study has been acknowledged also by Bugoni et al. [27], who suggested that the amount of debris in the whole digestive system might be higher. A positive correlation of carapace size and amount of debris in the digestive system implies that sea turtles accumulate debris through out their life, which would be consistent with an overall longer residence time of debris. The effect of the longer residence time therefore needs to be taken into account when reporting and studying the ingestion of plastic and other anthropogenic debris.

In all tested scenarios, the model predicted that chronic exposure to food effectively diluted by plastic (resulting in a lower scaled functional response), will result in smaller length, smaller weight, and lower (or no) reproduction (Tables 5.4 and 5.5). Slower increase in weight [199] and decreased formation of fat deposits influencing fitness and reproduction [44] as a consequence of plastic ingestion have been reported for seabirds as early as in the 1980s, and same consequences of plastic ingestion have been hypothesized to be responsible for the lack of correlation between the weight of ingested plastic and the weight of an individual [260]. Energy reserves have been shown [259] to be 50% smaller in a marine worm "from a combination of reduced feeding activity, longer gut residence times of ingested material, and inflammation." All these types of effects have been reported for sea turtles as well [140, 146, 220]. Younger (pelagic) turtles have been considered more susceptible to food dilution since their gut has a smaller capacity and their prey is of poorer nutritional quality [233, 146]. However, with the recent insights into the dispersion and interaction of microplastic particles [229, 43, 33], one should wonder whether food of neritic stage sea turtles really is better in nutritional quality. Thompson et al. [229] tested in a laboratory three species of benthic organisms (from the same groups of organisms that loggerhead turtles feed on, [122]) and all three species ingested plastic. Graham and Thompson [74] showed that deposit feeding organism ingest plastic particles (even preferentially) also in the field, anthropogenic debris (plastic filaments) had been found in bivalves [196] and fish [19] that sea turtles feed on [252, 177, 121, 66].

While chronic exposure results in reduced size and lowered fitness, acute exposure might result in death by starvation. Starvation might occur either because of obstruction or blockage of the digestive system (esophagus or the intestinal tract) [75], interruption of gastric secretion and processes [50, 117], or because an individual of a certain (larger) size had accumulated so much debris in a recent relatively short period that it cannot assimilate or mobilize from its reserves enough energy to pay the metabolic costs [140].

In addition to the studied sub-lethal consequences of food dilution caused by plastic ingestion, and the discussed sub-lethal and lethal consequences of the obstruction or damage to the digestive system and death by starvation, the ingested plastic particles can also transfer toxic chemicals [141]. Toxic contamination by plastic ingestion was outside the scope of this study, but it does cause an additional threat to sea turtles, as their tissues had been shown to contain elevated concentrations of toxic elements and compounds which are also transferred into the eggs (e.g. [123, 2, 100]).

5.5 Conclusion

The energy budget model that had been developed previously for the loggerhead turtles of the North Atlantic and the Mediterranean population (Chapters 3 and 4, respectively) can not only be used to study the differences between the populations, but also to study the metabolic responses (growth, maturation, and reproduction) to different environmental stimuli that are generally hard to study independently for longer periods. Evaluation of the developed energy model (comparing the predicted and observed energy ingestion and expenditure) provided convincing arguments that the model itself and the calculated energy budget are realistic.

Using the DEB model we studied the influence of temperature and food availability on the energy budget of loggerhead turtles. The food availability substantially affected the ultimate size (length and weight), and reproduction of individuals, moderately affected growth and maturation (age at puberty), and had negligible effect on length at puberty. The temperature substantially affected growth and maturation, moderately affected reproduction, and had negligible effect on ultimate size and length at puberty. Results obtained from the simulations can serve as a general guide for estimating the influence of temperature and food availability on processes (growth, maturation, and reproduction) and life history traits (size and age at puberty, ultimate size, fecundity, etc) of loggerhead turtles.

The effects of plastic ingestion were studied using the concept of Synthesizing Units [107], focusing on assimilation units and food availability presented as a scaled functional response of an individual. Using this approach (similar to studying enzymes and their inhibition), we obtained a modified half-saturation coefficient which resulted in a scaled functional response as a function of debris density or debris residence time. Lower scaled functional response is effectively equivalent to a lower food availability in an environment. When we assumed that the ingested debris has the residence time equal to that of food, our results implied that loggerhead turtles that have approximately 14% of their gut volume occupied by debris will probably not reproduce, and those with more plastic debris in their gut might not acquire enough energy during their life time to reach puberty. When we assumed that the residence time of the ingested debris is three or more times longer than that of food, a smaller percentage (3.4%) of the gut volume occupied by plastic already resulted in the inability to sexually mature and reproduce. Studying and defining the mechanisms by which ingestion of inert anthropogenic debris (predominantly plastic, but also styrofoam, filaments, etc) affects the energy budget made it possible to understand, predict, and *quantify* the effects of marine debris ingestion on growth, maturation, and reproduction of loggerhead turtles.

Results suggest that the consequences of ingesting marine debris, even when studied only from the food dilution aspect, can substantially reduce the fitness of individuals, and impede the conservation measures. Insights gained by this study are applicable to other species that have similar metabolic traits. In addition, the approach presented here can be used for any other species. Prevention of new and reduction of existing marine litter are a part of many strategies and international policies targeting the issue of marine litter (e.g. the Honolulu strategy [59], and see [135, 3] for an overview of policies). Considering that the production of plastic has been steadily growing over the past decades, that a large proportion of disposed plastic ends up in the oceans, and that the reports of marine litter ingestion are increasing, reducing the amount of anthropogenic marine debris should be included in conservation efforts for protecting the marine species and habitat,and become a priority for all countries that are in contact with the marine environment.

Chapter 6

General Conclusion

The overall aim of this research was to study the environmental (food density and temperature) and anthropogenic (plastic pollution) pressures on loggerhead turtles, and to provide new insights into the biology and ecology of loggerhead turtles. I focused on the processes important to the life cycle and ecology (growth, maturation, and reproduction), and thus the protection of loggerhead sea turtles, and my methods included using obtained experimental and collected literature data, and developing a life cycle model based on the Dynamic Energy Budget (DEB) theory. By far the most shocking result was the effect of plastic ingestion on the energy budget of loggerhead turtles, however several other results deserve attention as they provide valuable insight into the biology and ecology of loggerhead turtles.

Making sense of conflicting data. By comparing two neighboring regional subpopulations of the North Atlantic loggerhead turtles, and three life stage subsets, I was able to identify that the reported [28] inconclusive differences in the expressions used for relating different types of length and width measurements are mostly due to the differences in the carapace shape of small loggerheads (posthatchlings and juveniles before moving to a neritic habitat) compared to that of larger juveniles, and adults. Generally, although the results of Chapter 2 suggest that growth is slightly more preferential in length after a certain size (possibly connected to recruitment to neritic habitats), the calculated $\leq 5\%$ deviation from isomorphic growth is small enough to be disregarded for the purpose of mathematical approximations, as any model is, due to necessary assumptions and simplifications, at most a sketch of the reality. It was therefore concluded that a single set of expressions can be used for the whole size span (life cycle) in most practical applications. However, using the correct type of the expression to convert the measurements is crucial for methods that completely rely on two measurements of size to maintain the same ratio throughout the life cycle, such as those using the diameter and marks on a bone intersection to deduce the carapace length and age of sea turtles (e.g. [264, 214]). It would be interesting to see whether the change in the relationship of bone diameter-to-carapace length [214] occurs simultaneously with the slight change in shape throughout ontogeny which was suggested by results in Chapter 2. Furthermore, using the correct relationship of width and body depth to length is important while planning protection measures that rely on the size *and* shape of the individuals, such as openings in fishing nets (Turtle Excluder Devices or TEDs) that are aimed to reduce the largest cause of mortality, fisheries bycatch [57].

DEB parameters and model predictions Focusing first on the North Atlantic (Chapter 3), and then on the Mediterranean population (Chapter 4), the estimated set of parameters characterized the whole life cycle of loggerhead turtles in a satisfactory way. The parameter values were consistent both in the context of the species (when two populations were compared) and in the context of other two sea turtles (Kemp's ridley and leatherback turtle) for which the DEB parameters have previously [105, 179] been estimated and uploaded to the "Add my pet library" (see Chapters 3 and 4). The energy budget and the predictions for loggerhead turtles were realistic when compared directly to the observations about growth and reproduction (see Chapters 3 and 4), and when the estimated parameters were used to infer the food conversion ratio, and the daily energy need and expenditure of a fully grown adult (see Chapter 5).

Deviations of model predictions from observed data Because of such a good fit with so many observations, deviations of model predictions from data, where present, received more attention. For example, the length and weight at hatching were overpredicted, growth of posthatchlings was underpredicted, and the age at puberty was at the lower end of the reported (estimated) values for wild individuals, while newer estimates mostly suggest values closer to the higher end of the range (e.g. [209, 5]). Analysis of these deviations suggested interesting patterns and changes in parameter values that would have been hard to identify with a different approach. It would be worth further exploring: (i) the embryo phase, to identify the possible reasons for the mismatch between the observed and predicted size at hatching (such as metabolic acceleration, [113]); (ii) the posthatchling growth, to validate current results (suggesting increased assimilation, somatic maintenance, and reserve mobilization, with the combination resulting in an increased maximum reserve density), and to compare the predictions with the growth patterns of other species under similar environmental and evolutionary pressures; (iii) the growth of juveniles, to gain further understanding into the extent to which it deviates from the most often assumed von Bertalanffy growth (as suggested by Ref. [40] and discussed in Ref. [38]), and to identify the most important causes of this growth pattern, be it the conditions in the oceanic and the neritic habitat, or a change in physiology of loggerhead turtles.

Comparison of the North Atlantic and the Mediterranean population Developing and applying the model made it possible to explore the variability of individuals within populations and among populations. Comparing the Mediterranean and North Atlantic individuals (Chapter 4) on the basis of size (length, weight, and the ratio of the two, i.e. the condition index) and physiology (that is, model parameters) I simultaneously explored the directly observable and more "hidden" characteristics. An interesting result was a similar maximum reserve density of adults from both populations, coinciding with the calculated similar condition indices of the adults from both populations, as well as the higher maximum reserve density of the posthatchlings, coinciding with higher (compared to adults) condition indices of hatchlings from both populations. It is however necessary to calculate the condition indices while accounting for the interindividual variability for any firmer conclusions. It would also be interesting to further study other possible metabolic adaptations of the Mediterranean individuals, such as those to the higher salinity of the Mediterranean sea. The energetic cost of osmoregulation has not been studied in detail, and it is yet to be determined how important the difference of few ppt (between 36 and 39 ppt) is. Identifying the level of maturity at puberty as the main physiological difference between the two populations, and the one most responsible for different size at puberty, would be impossible without a mechanistic model that includes maturity, and also puberty as one of the maturity switches. Switches happening at a certain maturity density rather than maturity level might become an important factor for other populations of other species that have been genetically isolated for a longer period, and have been exposed to different environmental pressures.

Influence of temperature and food availability on the energy budget of individuals The same model which was used to compare the differences between individuals and populations was used to study the influence of selected global and local pressures on the loggerhead turtle (Chapter 5). Knowing the relationship between the food density and temperature on one side, and the response of an individual (growth, development, reproduction) on the other, is a valuable tool for understanding the observed differences between individuals and populations. In this study mostly constant conditions were simulated, directly relating to individuals that spend most of their lives in a similar type of habitat. The formulation and the mechanistic nature of the model allow, however, simulations of fluctuating conditions, as well as prolonged periods of stable yet different conditions (such as a long period in the oceanic zone followed by transitions between the oceanic and neritic zone), which could be explored further. It would be interesting to validate the model predictions on populations for which adults with bimodal feeding strategies have been observed, such as the population nesting in Japan [80] and in Cape Verde [82, 85].

Effects of plastic ingestion on the energy budget of the individuals The effects of plastic ingestion on the energy budget and processes of growth, maintenance, maturation, and reproduction of loggerhead turtles were greater than I initially assumed. The results suggest that the effect of ingested plastic taking up just 3% of the gut volume but with a (3 times) longer-than-food residence time, can have tremendous effects on the life cycle of a loggerhead turtle, and consequently on the whole population (as the turtle would not reproduce). Two major hurdles in studying the effect of plastic were that (1) representative control data is lacking, and (2) the mechanism by which plastic ingestion affects the energy budget was not known. To overcome the first issue, I assumed the data used for DEB parameter estimation describes individuals which had not ingested any plastic. For the second issue, I used the concept of Synthesizing (i.e. Assimilation) Units, and arrived at the kinetics similar to that of enzymes when in an environment with inhibitors. Several other assumptions were needed in the process: for example, I assumed that most of the individuals in the wild were without any plastic in their digestive system (assumed "control" value of $f = 0.81$), and that they were exposed to more or less constant environmental conditions resulting in a von Bertalanffy growth. Keeping in mind the previous results (namely biphasic growth), the growth curve might look markedly different, resulting in a later age at puberty, suggesting that the effects of plastic ingestion are even more serious. Also, individuals normally exposed to a higher food level than that simulated as chronic exposure to a certain plastic density, would grow to a larger size than was the ultimate size in the simulation. A larger size includes a higher maintenance cost, suggesting that when exposed to a high load of plastic the loggerhead turtle could not acquire enough energy to satisfy its energy need, and would die of starvation. Now that a mechanism of modeling plastic ingestion has been defined, simulating such an acute exposure to plastic is one of the possible future studies.

Another important point identified by this approach is the plastic ingestion analysis, as there is currently no standardized way of reporting the occurrence of ingested anthropogenic marine debris. Debris is often reported as absolute volume or mass (wet and/or dry), or the proportion of stomach contents, sometimes jointly for all individuals in a study (e.g. [121, 233, 75]). While all these reports are valuable, a more unified approach, ideally reporting the percentage of total gut occupied by debris, would provide even more information needed to mechanistically study the effects of plastic ingestion.

Outlook Finally, while all of the results, and especially the model obtained in this study are extremely useful, they are all, like DEB theory, individual-based. Luckily, like DEB theory, they are applicable in many forms. One of the applications is an individual-based population model (e.g. [139]), which could account for interindividual variability in some parameter values. The model, in combination with rules for interaction between individuals and models for the environment, also specifies the behavior of populations. Oneadditional possibility is a multi-species parameter exploration, where a number of interesting properties could be studied with respect to the parameter values of loggerhead and other sea turtles in relation to parameter values of other reptiles or even other animal groups. Linking some traits, e.g. temperature dependent sex determination present in many reptile groups [265] to a specific combination of parameter values would surely be something only a theory applicable on a large number of species and groups can yield.

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Appendix A - Description of data obtained from Sidonie Cateau

Various aquaria and rearing facilities were contacted for data, and I am greatly thankful to Sidonie Cateau for responding to my request and sharing her data. Data sets were obtained in the Marineland (Antibes) rearing program and represent captive-reared individuals. The data include measurements of two loggerhead turtles, named 'T3' and 'T4', that hatched in 2010 and were measured until they were 4 years of age, and of 22 loggerhead turtles, their name a 4-number sequence, that hatched in 2011 and were measured until they were 3 years of age. For the purpose of the parameter estimation, I used data both turtles hatched in 2010, and data for twelve or six (depending on the data set) turtles hatched in 2011. Some of the turtles were kept in the Marineland during the whole period, and some were transferred to different rearing facilities, which is denoted by the name of the dataset.

The datasets used for the analysis were:

- Weight vs. age (tW) - Data consisted of measurements of 14 individuals, 12 of which hatched in 2011, and two of which hatched in 2010; between 30 and 40 data points per individual, age ranging from birth until 3 (if hatched in 2011) or 4 (if hatched in 2010) years of age were available. All individuals hatched in 2011 were reared at Marineland ($T = 23.5^\circ$ C) until they were approximately 1.7 years of age. Subsequently, some were transferred to different facilities: Le Croisic ($T = 22^\circ$ C, datasets: $'tW_{2207}'$, $'tW_{2215}'$), Oceanopolis ($T = 25^\circ$ C, datasets: $'tW_{2202}'$, $'tW_{2213}'$), Nausicaa ($T = 26^\circ$ C, datasets: $'tW_{2217}'$, $'tW_{2218}'$), La Rochelle ($T = 26^\circ$ C, datasets: $'tW_{2226}'$, $'tW_{2302}'$), while the others were kept at Marineland ($T = 23.5^\circ$ C, datasets: $'tW_{2211}'$, $'tW_{2222}'$). Two individuals hatched in 2010 were kept at Marineland for the whole duration ($T = 23.5^\circ$ C, datasets: $'tW_{T3}'$, $'tW_{T4}'$).

- Length vs age (tL) - Data consisted of measurements of six individuals hatched in 2011, but measured at age ranging between 1.7 years and 3 years. In total, 23 and 32 points for two individuals transferred to La Rochelle, and 5 data points for each of the other four turtles were available. All individuals were reared at Marineland ($T = 23.5^\circ$ C) prior to transfer (coinciding with the first data point in each data set). The facilities where the measurements were taken: Le Croisic ($T = 22^\circ$ C, dataset: $'tL_{2207}'$), La Rochelle ($T = 26^\circ$ C, datasets: $'tL_{2226}'$, $'tL_{2302}'$), and Marineland ($T = 23.5^\circ$ C, datasets: $'tL_{2210}'$, $'tL_{2211}'$, $'tW_{2222}'$).

- Weight vs. length (LW) - Data consisted of measurements for five individuals included in the previously described datasets (tW, tL), where both length and weight were taken simultaneously. These data were used to construct the LW datasets: 'LW_{2207}', 'LW_{2211}', 'LW_{2222}', 'LW_{2226}', 'LW_{2302}'.

Data was plotted in Chapter 4 as follows:

Figure 4.5: Weight as a function of age. Panel (a): juveniles hatched in 2010 ('tW_{T3}', 'tW_{T4}'), data plotted as triangles. Panel (b): juveniles hatched in 2011, data from Marineland and Oceanopolis (datasets: 'tW_{2202}', 'tW_{2213}') plotted with symbols 'x', and data from Marineland and Nausicaa (datasets: 'tW_{2217}', 'tW_{2218}') plotted with symbols '+'. Panel (c): juveniles hatched in 2011, data from Marineland and Le Croisic (datasets: 'tW_{2207}', 'tW_{2215}') plotted with symbols '□', and data from Marineland (datasets: 'tW_{2211}', 'tW_{2222}') plotted with symbols '*'. Panel (d): juveniles hatched in 2011 and later moved to La Rochelle ($T = 26^\circ$ C, datasets: 'tW_{2226}', 'tW_{2302}'), data plotted with symbols 'o'.

Figure 4.6: Length as a function of age, and the relationship of weight and length, all data for juveniles hatched in 2011. Panel (a): data from Le Croisic 'tL_{2207}' plotted with symbols '□', and data from Marineland (datasets: 'tL_{2210}', 'tL_{2211}', 'tW_{2222}') plotted with symbols '*'. Panel (b): data from La Rochelle (datasets: 'tL_{2226}', 'tL_{2302}') plotted with symbols 'o'. Panel (c): the relationship of weight to length, the symbols correspond to those in panels a and b, and Figure 4.5.

Appendix B

Summaries

Summary

My main motivation for starting this journey, which resulted in (but does not end with) a doctorate of science, was to find out how much damage we are doing by allowing the plastic waste to enter the marine ecosystem. It is common knowledge that plastic takes a very long time to degrade; after all, the durability of plastic items is what made plastic so useful, and consequently, so ubiquitous! So, where exactly does all that plastic go? Does it sink? Does it just float in the oceans, swirling around in the ocean currents forever? Does it get ingested by marine organisms? And if so, what happens then?

There are reports of almost three hundred different species of marine organisms interacting with our plastic waste. Getting entangled in it, eating it, or using it as a transportation device to arrive to new habitats, where they sometimes thrive so successfully that they "squeeze out" native species. And while being attached to a piece of floating plastic to find a new ecosystem does sound like a promise of a fresh new start, being entangled by a discarded fishing net, or starving to death after eating too much plastic, certainly does not sound so inviting. The second scenario is, however, more common, and is the one experienced by sea turtles.

Sea turtles are remarkable creatures. They have existed in the form we see today for over 150 million years - this means they have coexisted with, and by far outlived the large dinosaurs! They have fascinated humans from the early civilizations, but the fascination did not help them - all seven species of sea turtles that currently exist are on the IUCN list of endangered species, and most populations are declining despite the protection. The most vulnerable species for plastic are the long-lived ones, because their potential the adapt to changing environments across generations is most limited. Sea turtles fall into this category.

Loggerhead sea turtles are present throughout the temporal zone of all the world oceans, evolving into multiple populations and local subpopulations. They live longer than 65 years, and their sex is determined during the last third of their 60-days long embryonic development by the temperature during incubation. During their life they increase in size 25 times: from a 4 cm long and 20 g "heavy" hatchlings that exit the nest, to adults weighing over 100 kg with a carapace length of 100-130 cm that return to lay eggs at the same beach where they hatched. These two moments (hatching and nesting) are also the only two moments in their long life when loggerhead turtles have contact with the land environment. Consequently, beach and offshore (coastal) sea were for a long period the only two habitats in which loggerhead turtles could be observed. The remaining period

- be it 5 years, a decade, two, three or more - were referred to as "the lost year(s)" (a term coined by Archie Carr in 1986), and remained a mystery for a long time.

Advances in science since have made it possible to study the sea turtles and uncover many of the mysteries. The life cycle of loggerhead turtles had been roughly divided into three life stages: embryo, juvenile, and adult, and now it was possible to include observations about the ecology and define life-stages in more detail. Within the juvenile stage, one can differentiate between the hatchling (individual that has just hatched and is moving towards the open sea), posthatchling (a slightly older individual, up to 15 cm carapace length), oceanic juvenile (individual larger than 15 cm carapace length that mostly resides in the oceanic habitat feeding on plankton and other pelagic organisms), and neritic juvenile (individual larger than 30-50 cm carapace length that mostly resides in the neritic habitat feeding on benthic organisms). The transition from the oceanic to neritic habitats (assumed rapid and called the recruitment to neritic habitat) occurs for most individuals when they reach a certain size or developmental stage, but sometimes the transition is longer, or doesn't happen at all, resulting in adults feeding in oceanic habitats.

Many studies have been performed, and much literature has been published, but the focus of a study has most often been a specific life trait or a specific life stage. Due to the (i) different use of habitat, (ii) different sampling (such as taking different measures of carapace, and then devising expressions to translate between them; calculating growth rates from capture-mark-recapture data or growth marks visible on bones) and (iii) different analytical techniques (such as studying the change in length, or the change in mass, and fitting different growth models), reported data was not only disjointed, but was often conflicting. Most conflicts related to growth rates and growth models reported for different populations and life stages, lack of agreement whether to use the minimum or the average carapace length of nesting females within a population as "length at puberty", and the estimates of age at puberty ranging from 6 years to 38 years. Furthermore, several authors pointed to significant differences between populations, the most obvious being the size difference between Mediterranean adults compared to adults from other populations, but also differences present within a population, such as different growth rates and different expressions used to convert one measure of carapace length into another.

For me to solve the mystery of the "lost plastic" and its effect on the loggerhead turtles, I needed to know a lot more about the biology and ecology of loggerhead turtles: How long does it exactly take for a loggerhead turtle to mature? Why do some loggerhead turtles grow faster than others? Why are loggerhead turtles in the North Atlantic larger than those in the Mediterranean sea? Do larger turtles also reproduce more? Are size and reproduction results of environmental or physiological characteristics? How will loggerhead turtles cope with global environmental changes? Or, more precisely, how do available food, salinity, or temperature influence processes such as growth, maturation, maintenance, and reproduction? And - if I want to know the effect of ingested plastic on those relevant processes fueled from the energy budget - how much energy does a loggerhead turtle need daily for the processes, and how much energy can it obtain?

Defining and following an energy budget of a loggerhead turtle was the most logical approach to take, one that would provide answers to most, if not all of my questions, as any effect of plastic ingestion on a species must become visible as an effect on the energy budget and/or life span. I chose the Dynamic Energy Budget theory as the path to my "Holy Grail": the DEB model of a Loggerhead Turtle. It had everything: observance of the laws of thermodynamics, several types of homeostasis that any system (from cells to individuals and ecosystems) tries to obtain and keep, the effects of food and temperature on the energy budget, the interaction of the energy budget with processes such as growth, maintenance, maturation, and reproduction. Additionally, it was and is the most consistent theory currently available.

Generally, mass is more informative than length when defining energetics, but as the same curve was successfully fitted for the relationship of length and mass across the whole size span of individuals from several different populations (Wabnitz and Pauly, 2008), I focused on length. The reported differences in expressions for converting carapace lengths were my chosen starting point, because differences in conversion expressions for the same two types of measurements suggest that the shapes of individuals differ among life stages and possibly even populations. The differences can have important implications for modeling the energy budget, as the shape (structural) homeostasis is one of the assumptions of DEB theory. Change in shape (deviations from isomorphy) can easily be accounted for by modifying the shape coefficient (δ_M), but first their significance needs to be analyzed. Focusing on the North Atlantic population for which the (inconclusive) difference in conversion expressions was reported, I compared the data from two different regions ('north' and 'south') of the North Atlantic, and three different life stages ('posthatchlings and oceanic juveniles', 'neritic juveniles', and 'adults'). The results suggested that there are no significant differences when the same life stages of different regions are compared, but that one should be careful when extrapolating shape-dependent conclusions from the smallest ('posthatchlings and oceanic juveniles') to larger life stages, and vice versa. Still, the noted differences in shape were not significant enough to require additional shape coefficients for different life stages, as the deviation from an ideally isomorphic organism was only around 5%. This conclusion implied that I can use the standard (simplest) form of the DEB model.

Developing and formalizing a complete life cycle DEB model of a loggerhead turtle was the second step. The standard DEB model describes an individual by following the dynamics of three compartments: structure, reserve, maturity, and (after puberty) the reproduction buffer. The first two (structure and reserve) can be indirectly measured as length and/or mass of an individual, whereas the third one (maturity) is formally quantified as the cumulative investment of reserve for increasing in complexity. The dynamics of each compartment is unique, and fully specified by the parameters of the model which are estimated simultaneously. The starting hypothesis was that differences between populations (North Atlantic and Mediterranean), and effects of plastic ingestion on the energy budget will be visible as changes in parameter values or as changes in predictions of the DEB model. These values must, therefore, be determined first. The procedure of parameter estimation uses all available life-history data (such as length and age at birth and puberty), and other type of data (growth curves, reproduction output etc.) at the same time to arrive at the most realistic set of parameter values of the DEB model. Due to large variations within a single population, analyzing more than one population simultaneously was not a viable option. While focusing on the North Atlantic population - the largest (and probably the best studied) population of loggerhead turtles in the world, I obtained the values of all primary parameters of the DEB North Atlantic loggerhead model. The model had a very good fit with the observed data used as input, ranging from prediction for incubation duration, length and weight growth rates, to length at puberty, and reproduction output. Furthermore, by obtaining the parameter values that specify the whole life cycle of loggerhead turtles, I was also able to study the daily energy budget of the same loggerhead turtles. The results suggested that while the young posthatchlings use most of their energy for maturation and growth, a fully grown adult uses almost three quarters of the energy budget for (somatic and maturity) maintenance. In addition, I could explore effects of mothers' feeding conditions on the embryo's energy budget: while at the food level resulting in the maximum food intake, the embryo needs to use less than half of the initial energy in an egg for development and growth, but at 20% lower food level it needs to use more than half. This directly translates to the amount of reserves (yolk sac) left at hatching, and thus possibly the survival of embryos.

However, as often happens, the predictions that did *not* have an excellent fit with data were the more interesting ones. Namely, while the duration of incubation was predicted reasonably well, the size at hatching was overpredicted. The age at puberty was predicted to be at the low end of the range reported for loggerhead turtles (around 13 years), whereas most of the more recent studies point to the high end of the reported range (20 or more years). Are the loggerhead turtles allocating to reproduction much sooner than is currently thought? Or was the assumption that the loggerhead turtles adapt to their environment (resulting in more or less constant conditions throughout their life-cycle), and grow similar to what the von Bertalanffy model predicts, an over-simplification? An interesting result was also the reported growth of posthatchlings, which appears to be faster than the model was able to reproduce. Is it possible that the posthatchlings grow faster because they have a metabolism even faster than the standard model predicts? This pattern has been recognized as "waste to hurry" in species that need to grow fast even at the expense of wastefully using their resources. Evolutionarily it would make sense that small posthatchlings (hatching during summer) maximize their growth to use the available resources and arrive to a larger size less appealing to predators. But faster growth, as well as the other noted peculiarities, may have been related to biases in the data, such as a higher food quality of posthatchlings compared to that of adults. Studying another set of data for a different population would therefore help to confirm or dismiss the hypotheses.

Studying the Mediterranean population, which is much smaller in several ways (smaller number of smaller individuals living in a smaller habitat), was the third step in my work. To obtain a first insight into the extent and possible reasons for reported size differences, size data (length and weight) from each population were analyzed and compared at the two most distinct moments of the loggerhead turtle life cycle: hatching and nesting. The size of eggs was taken into account as well, as it has previously been found to account for most of the size difference between hatchlings of different populations. The average size of hatchlings and nesting adults was indeed substantially different between populations. Surprisingly, the ratios of the average weight and cubed length (the condition index) were not. The condition index did, however, differ between life stages. I discussed various possible reasons for the size difference, from incubating environment of embryos to food abundance experienced by juveniles and adults. None of those pressures could, however, result in such large differences in size at nesting and simultaneously supported the observed reproduction output. The answer to that puzzle was revealed only by estimating the parameters of a DEB Mediterranean loggerhead turtle model, and studying the implications. Whereas maturing earlier at a smaller size in an environment with less food was not intuitive, the model suggested that the main explanation for it was the lower level of maturity that the Mediterranean loggerheads need to obtain to reach puberty. This implied two things: (i) the Mediterranean loggerhead turtles need to cumulatively invest less energy to reach puberty, suggesting they can reach puberty earlier and at smaller size than the North Atlantic ones, and (ii) the Mediterranean loggerhead turtles need to maintain a lower level of maturity (via maturity maintenance), suggesting a larger part of their energy budget can be allocated to reproduction. The predicted properties (such as size and age at puberty being smaller than, but having similar reproduction output to that of the North Atlantic loggerheads) were consistent with the observations, and the underlying mechanistic explanation was consistent with the DEB theory. The size of the hatchlings was slightly overpredicted, and the predicted age at puberty was close to the lower end of the range estimated by other authors, as was the case with the North Atlantic loggerhead turtles. The growth of posthatchlings, analyzed now in more detail and simultaneously for both populations, indeed confirmed a metabolic acceleration during the observed period. The same analysis also emphasized potential problems in analyzing the observed growth rates, as the faster growth of Mediterranean posthatchlings (compared to that of the North Atlantic ones) was evident only after the growth rates were calculated for a reference temperature and food level. In addition, using the same DEB model, I simulated a substantial change in food availability during the life of loggerhead turtles, and explored effects of the changes on growth. The resulting growth curve suggested biphasic growth, similarly to that proposed by very few authors while the others were using classic (monophasic) growth models. Biphasic or even polyphasic growth would indeed result in a greater age at puberty, consistent with the estimations at the higher end of the reported range, and is a pattern worth further exploring. Arriving at such a distinct growth pattern was interesting, but I was not sure whether only food was responsible for the differences, or should also temperature be included? And what exactly are the effects of one or the other on the whole energy budget and its underlying processes?

The most recent part of my journey (and the last part of this thesis) explores, first independently and then simultaneously, effects of food and temperature on the energy budget. Experimentally, it is very difficult, if not impossible, to keep conditions completely constant throughout the life of a loggerhead turtle (65 years), and it is even more difficult to do this for as many turtles as are needed to study all the combinations of food and temperature we desire to test, hoping that loggerhead turtles in our study are good representatives of the species. One of many strengths of using a mechanistic modeling approach is precisely an opportunity to test such scenarios. Focusing again first on the North Atlantic population, I simulated realistic ranges of food densities and temperatures experienced by loggerhead turtles. The effects of food density differences were present on growth rates, but were the strongest on the ultimate size of adults. The effects of temperature were most evident in the growth and maturation rates. Both environmental factors substantially affected the reproduction output. The length at puberty was hardly affected by either of the tested environmental factors, corroborating the conclusion of some authors that, even though variability in length at puberty is present, compared to age and decrease in growth rates (also suggested as indicators of attained puberty), it is one of the least variable observable properties. The results also consolidated the conclusions of an intrinsic (physiological) difference that allows the Mediterranean loggerhead turtles to reach puberty at a smaller size. The model for the Mediterranean loggerhead turtles was then used as well to compare the responses of Mediterranean and North Atlantic loggerhead turtles to the conditions present in the Mediterranean environment, and explore to what extent organisms with different physiology can respond to similar environmental conditions. This is important because individuals of both populations are often encountered in the Mediterranean. Recently their growth and maturation rates in the Mediterranean have been reported separately for individuals of different origin, providing a good validation tool for my simulations. Results obtained using the DEB models were in agreement with the published results and conclusions, successfully reproducing the faster growth and earlier maturation of Mediterranean loggerhead turtles. In addition, it became clear why loggerhead turtles of the North Atlantic origin are generally not observed nesting in the Mediterranean, as the model predicted their reproduction output would be extremely low in the simulated environment.

Lastly, the global environmental pressure that has set all the wheels in motion - the anthropogenic debris and the effects of its ingestion on the energy budget - were studied.

The effect on the energy budget was modeled in the context of Synthesizing Units, or more precisely, assimilation units (AUs) that are normally responsible for converting the ingested food into reserves and providing energy for all required processes (growth, maintenance, and maturation or reproduction). Simply put, the AUs can either be busy with processing particles (extracting energy from them) or free to accept new particles. When an increasing proportion of food particles becomes replaced by plastic (or other inert debris) particles, an increasing proportion of the busy AUs are processing particles that have no energy gain. First I assumed that the processing time of plastic particles is the same as that of food, and I quantified long-term effects resulting from ingestion of reported quantities. The reported proportion of stomach volume taken up by plastic was on average 3% of the stomach contents (ranging from 0 to 25%), but is probably higher when the whole digestive system is considered because the proportion of plastic debris is higher in the intestine contents compared to the stomach contents. Then, bearing in mind that the gut residence time of plastic debris has been reported as being up to several times longer than that of food, I simulated a proportion of ingested plastic at 3%, requiring more processing time. Therefore, first I simulated a range of realistic values of ingested plastic with the same residence time as food, and then I simulated a range of different residence time of ingested plastic taking up 3% of gut volume. The effect of ingested plastic, to my scientific excitement and moral dismay, turned out to be substantial. The ingested plastic effectively had the same consequences as a reduction of food intake, resulting in slower growth (i.e. higher predation risk), smaller ultimate size, and a smaller reproduction output. When equal residence times were assumed, already 14% of volume of the digestive system taken up by plastic caused such a low reproduction output, that it is realistic to assume that the loggerhead turtles would not reproduce at all (a similarly low reproduction output was predicted by the model for the North Atlantic individuals residing in the Mediterranean, nesting of which is indeed rarely observed). Should the plastic take up even more of the digestive system volume, it becomes impossible for loggerhead turtles to reach puberty. When a residence time three or more times longer than that of food was assumed, the same effect occurred already at a 3% volume proportion. In nature, the proportion of ingested plastic is not constant, nor do all the ingested particles have the same residence time. Equally realistic scenarios are (i) loggerhead turtles can tolerate a short (acute) exposure to a load even higher than 14% and recover, and (ii) ingestion of even smaller amount of debris will result in death by starvation, as the individuals normally ingesting more food have grown to a larger size, requiring more energy for maintenance, which now cannot be paid due to insufficient energy being available.

Completion of the work carried out as part of this thesis has provided many valuable insights. To my satisfaction, many questions have been answered, but also more have arisen - and those I wish to pursue further. For example, why was the size of the hatchlings consistently overpredicted? Can the “waste to hurry” pattern explain faster growth of the posthatchling, and does it hold for all populations of loggerhead turtles, or even all species of sea turtles that share similar environmental pressures? Is the combined effect of pelagic environment with lower food density and lower temperature, and the neritic environment with higher food density and temperature, resulting in a biphasic growth curve for most juveniles? Can these two patterns, one driven by metabolism ("waste to hurry") and the other by the environment (changes in food density and temperature) simultaneously explain the hypothesized polyphasic growth and the mismatch between the reported (20-30 years) and predicted (13-15 years) age at maturity? Would studying the effect of plastic ingestion on *those* growth curves predict an even larger age at puberty and even grimmer scenarios for the future of loggerhead turtle populations? And finally, could this improved understanding of biology and ecology of this magnificent species, and the detrimental effects that plastic waste has on our environment, explain why some loggerhead turtle populations are still declining despite the protection, and motivate us to change our behavior?

Sažetak

Uvod

Glavna motivacija dok sam započinjala ovo putovanje, koje je rezultiralo (ali ne završava) doktoratom znanosti, bila je doznati koliko štete uzrokujemo dopuštajući da plastičan otpad dospijeva u morski ekosustav. Opće je poznato da je plastici potrebno jako puno vremena za raspad; naposljetku, izdržljivost plastike je ono što je i učinilo plastiku tako korisnom i, posljedično, sveprisutnom! Dakle, gdje točno odlazi sva ta plastika? Da li tone? Da li pluta po oceanima, putujući vječno morskim strujama? Da li je pojedena od strane morskih organizama? I ako da, što se događa onda?

Postoje izvještaji o interakciji gotovo tristo različitih vrsta morskih organizama s našim plastičnim otpadom. Zapetljavaju se u njega, jedu ga, ili ga koriste kao transportno sredstvo kako bi stigle u nova područja, gdje su ponekad toliko uspješne da "istisnu" autohtone vrste. I dok biti pričvršćen na komad plutajuće plastike u potrazi za novim ekosustavima možda zvuči kao obećavajući novi početak, biti zapetljan u odbačenu ribarsku mrežu, ili umrijeti od izgladnjivanja zbog previše zabunom pojedene plastike, zvuči puno manje primamljivo. Drugi scenarij je, međutim, puno učestaliji, i njega proživljavaju morske kornjače.

Morske kornjače su fantastična stvorenja. Nepromijenjenoga izgleda, postoje već preko 150 milijuna godina - što znači da su živjele u isto vrijeme, a onda i daleko nadživjele, velike dinosauruse! Fasciniraju ljude već od ranih civilizacija, ali fascinacija im nije pomogla, jer se svih sedam vrsta morskih kornjača koje trenutno postoje nalazi na IUCN listi ugroženih vrsta, a brojnost većine populacija je u opadanju usprkos zaštiti. Učinkovitost zaštite ovisi i o tome koliko se vrste mogu prilagoditi pritiscima prisutnima u okolišu. Plastika predstavlja najveću opasnost za vrste koje dugo žive, jer je mogućnost takvih vrsta da se prilagode promjenama u okolišu ograničena. Morske kornjače se kao dugo živuće vrste nalaze u ovoj kategoriji.

Glavate želve (*Caretta caretta*, Linnaeus 1758) prisutne su u umjerenoj klimatskoj zoni svih svjetskih oceana, evoluirajući u nekoliko populacija i lokalnih subpopulacija. Žive duže od 65 godina, a njihov spol određen je temperaturom inkubacije tijekom zadnje trećine embrionalnog razvoja (koji traje oko dva mjeseca). Tijekom svog života, mogu narasti do veličine čak 25 puta veće od one pri izlijeganju: u prosjeku 4 cm dugačka i 20 g "teška" kornjačica koja izađe iz gnijezda, može postati odrasla jedinka teška preko 100 kg s oklopom dugačkim 100 do 130 cm. Ženke dolaze položiti jaja na istu plažu na kojoj su se izlegle, zbog čega ponekad preplivaju stotine kilometara. Ova dva trenutka (izlijeganje iz jaja i polaganje jaja) su jedina dva trenutka tijekom njenog dugog života, kada kornjača ima doticaj s kopnenim staništem. Posljedično, plaža i obalno more su dugo vremena bila jedina dva područja gdje su ljudi mogli promatrati želve. Preostali period - bilo to 5 godina, desetljeće, dva, ili više - nazvan je "izgubljene godine" (naziv je skovao Archie Carr 1986) i ostao je tajna dugo vremena.

Od tada je znanost napredovala, te je razvojem novih metoda postalo moguće sveobuhvatnije istraživati morske kornjače i razotkriti neke tajne. Životni ciklus morskih kornjača je dugo vremena bio u grubo podijeljen u tri životna stadija: embrio, seksualno nezrele (juvenilne) jedinke, i odrasle (adultne) jedinke. Rezultati dobiveni razvojem novih metoda i primjenom ekoloških istraživanja omogućili su detaljnije definiranje ontogenetskih životnih stadija. Unutar seksualno nezrelog (juvenilnog) stadija, razlikujemo (i) tranzicijski period izleglih kornjačica u neritičkoj provinciji (duljina oklopa želvi manja of 15 cm), (ii) pelagičku razvojnu fazu u oceanskoj provinciji (želve duljine oklopa između otprilike 15 i 40 cm koje se hrane planktonom i drugim slobodno-plivajućim tj. pelagijalnim organizmima), te (iii) neritičku razvojnu fazu u neritičkoj provinciji (želve oklopa većeg od 30-50 cm koje uglavnom borave u priobalnom moru i hrane se pridnenim i za dno pričvršćenim, tj. bentičkim organizmima). Ontogenetska promjena staništa (prijelaz iz otvorenog u priobalno more) događa se za većinu jedinki kada dosegnu određenu veličinu ili razvojni stadij, zbog čega je smatrano da ontogenetska promjena staništa kratko traje. Međutim, ova promjena staništa je ponekad postepena, ili do nje uopće ne dođe, rezultirajući time da se i neke odrasle jedinke hrane u staništima otvorenog mora.

Iako je provedeno mnogo studija i objavljeno mnogo literature na tematici glavatih želvi i morskih kornjača općenito, fokus određene studije je najčešće bila određena karakteristika ili određen životni stadij. Zbog (i) razlika u korištenju staništa, (ii) razlika u metodama uzorkovanja (primjerice postoji nekoliko načina mjerenja duljine oklopa, i nekoliko formula za preračunavanje jedne duljine u drugu; također, postoje različiti načini mjerenja brzine rasta (npr. markiranje i ponovni nalazi ili brojanje godišnjih prirasta na kostima), te (iii) različitih metoda analize podataka (primjerice proučavanje promjena duljine ili težine, te konstrukcije opisnih modela), objavljeni podaci nisu bili samo nepovezani, nego često i kontradiktorni. Većina neslaganja odnosila se na brzine rasta i modele rasta koji su bili objavljeni za različite populacije i životne stadije, na dogovor oko toga treba li kao "duljinu oklopa kod dostizanja spolne zrelosti" koristiti najmanju izmjerenu duljinu oklopa unutar određene populacije ili prosječnu duljinu oklopa pri gniježđenju za tu populaciju, te procjene za dob pri dostizanju spolne zrelosti, koje se kreću od 6 do 38 godina. Nadalje, nekoliko autora upozorilo je na bitne razlike među populacijama, od kojih je najočitija razlika između odraslih jedinki Mediteranske i drugih populacija, ali i na bitne razlike unutar iste populacije, kao što su različite brzine rasta i različite formule za preračunavanje jedne mjere oklopa u drugu.

Kako bih mogla riješiti tajnu "nestale plastike" i utjecaja plastike na glavate želve, morala sam znati još više o ekologiji i biologiji glavatih želvi: Koliko dugo im zaista treba kako bi dosegle spolnu zrelost? Zašto neke glavate želve rastu brže od drugih? Zašto su glavate želve u Sjevernom Atlantiku veće od onih u Mediteranu? Da li se veće želve također i više razmnožavaju? Jesu li veličina i razmnožavanje posljedica okolišnih ili fizioloških čimbenika? Kako će se glavate želve nositi s okolišnim promjenama? Ili, konkretnije, kako dostupnost hrane, salinitet, i/ili temperatura mora djeluju na procese kao što su rast, sazrijevanje, održavanje, i reprodukcija? I - ukoliko želim doznati kako pojedena plastika utječe na te bitne procese koji su dio energijskog proračuna jedinki - koliko je uopće energije potrebno glavatoj želvi, i koliko energije može prikupiti u određenom vremenskom periodu?

Metode i rezultati

Definiranje i praćenje energijskog proračuna glavate želve bio je najlogičniji pristup, onaj koji će pružiti odgovore na većinu, ako ne i sva moja pitanja, jer bilo koji utjecaj pojedene plastike na vrstu mora biti vidljiv kao utjecaj na energijski proračun i/ili životni vijek te vrste. Odabrala sam teoriju dinamičkog energijskog proračuna (eng. *Dynamic Energy Budget*, DEB) kao stazu koja će me dovesti do mog “Svetog Grala”: DEB modela glavate želve. Pristup ove teorije je sveobuhvatan: praćenje zakona termodinamike, nekoliko tipova homeostaze (ravnoteže) koju svaki sustav (od stanice preko jedinke do ekosustava) pokušava postići i održati, utjecaj hrane i temperature na energijski proračun, međuovisnost energijskog proračuna i procesa kao što su rast, održavanje, sazrijevanje,i reprodukcija. Dodatno, DEB teorija bila je i ostala jedna od najbolje razrađenih i dosljednih dostupnih teorija.

Općenito kada govorimo o energiji, masa je informativnija od duljine. Međutim, kako je samo jedna krivulja bila dovoljna za uspješno opisivanje odnosa duljine i mase za cijeli veličinski raspon jedinki iz nekoliko različitih populacija (Wabnitz and Pauly, 2008), fokusirala sam se na (dostupnije) podatke o duljini. Neslaganje u izrazima za preračunavanje mjera duljina oklopa bilo je početna točka mog istraživanja, jer neslaganje u izrazima koji preračunavaju iste dvije mjere oklopa navodi na zaključak da se oblik oklopa, odnosno želvi, razlikuje među različitim životnim stadijima i/ili populacijama. Razlike tog tipa mogu imati značajne implikacije kod modeliranja energijskog proračuna, jer je postojanost oblika životinje kroz životni ciklus (tzv. strukturna homeostaza ili izomorfija) jedna od pretpostavki DEB teorije. Eventualnu promjenu oblika (odstupanje od izomorfije) moguće je vrlo jednostavno uključiti u model putem promjene u koeficijentu oblika (δ_M), ali prvo je potrebno utvrditi koliko je promjena značajna. Koristeći podatke za sjeverno-atlantsku populaciju glavatih želvi za koju su nedosljednosti u izrazima za preračunavanje i pronađene, usporedila sam dvije različite regije (’sjevernu’ i ’južnu’) Sjevernog Atlantika, i tri različita životna stadija (’kornjačice i spolno nezrele jedinke otvorenog mora’, ’spolno nezrele jedinke priobalnog mora’, i ’odrasle jedinke’).

Rezultati su upućivali na to da nema značajnih razlika kada se uspoređuju jedinke istog životnog stadija koje žive u različitim regijama, ali da treba biti oprezan kada se rezultati i zaključci vezani uz oblik donose na temelju analize najmanjih jedinki (životni stadij 'starijih kornjačica i spolno nezrelih jedinki otvorenog mora'), a primjenjuju na starijim životnim stadijima ('spolno nezrele jedinke priobalnog mora' i 'odrasle jedinke'), i obrnuto. Ipak, primijećene razlike u obliku nisu bile dovoljno značajne da bi opravdale uvođenje dodatnog koeficijenta oblika za pojedine životne stadije, jer je odstupanje od izomorfije bilo manje od 5%. Ovakav zaključak implicirao je i da mogu koristiti standardni (najjednostavniji) oblik DEB modela u daljnjoj analizi.

Razvoj i formalni opis DEB modela čitavog životnog ciklusa glavate želve bio je drugi korak. Standardni DEB model opisuje jedinku prateći dinamiku tri odjeljka: "strukture", "rezerve", i "zrelosti", od čega je zadnji odjeljak povezan uz sazrijevanje i (nakon dostizanja spolne zrelosti) razmnožavanje. Prva dva odjeljka ("struktura" i "rezerva") mogu se direktno izmjeriti kao duljina i/ili masa jedinke, dok je treći odjeljak ("zrelost") formalno definiran kao uzastopno ulaganje energije kako bi se povećao stupanj složenosti organizma. Dinamika svakog pojedinog odjeljka je jedinstvena, i potpuno je određena parametrima modela koji su procijenjeni istovremeno. Početna pretpostavka bila je da će razlike među populacijama (sjeverno-atlantskom i mediteranskom), i utjecaj pojedene plastike na energijski proračun, biti vidljivi kao promjene u vrijednostima parametara ili kao promjene u predikcijama DEB modela. Prvo je dakle potrebno odrediti vrijednosti parametara. Postupak određivanja vrijednosti parametara (metoda kovarijacije) koristi istovremeno sve dostupne podatke o biologiji vrste (kao što su veličina i starost pri izlijeganju i dostizanju spolne zrelosti, te krivulje rasta, podatke o razmnožavanju, itd.) kako bi se ustanovio najizgledniji set vrijednosti parametara DEB modela. S obzirom na to da je unutar svake populacije prisutna velika varijabilnost u podacima, analiziranje više od jedne populacije istovremeno nije bila izgledna opcija. Koristeći prvo podatke za sjeverno-atlantsku populaciju - najveću (i vjerojatno najbolje izučavanu) populaciju glavatih želvi na svijetu - odredila sam set svih primarnih parametara za DEB model sjeverno-atlantske glavate želve. Model je pokazao jako dobro poklapanje s podacima koji su korišteni za određivanje parametara, i to od predviđanja trajanja inkubacije, brzine rasta u duljinu i težinu, do duljine oklopa pri dostizanju spolne zrelosti, i razmnožavanja. Dodatno, to što sam odredila vrijednosti parametara koji određuju čitav životni ciklus glavate želve, omogućilo mi je i da proučavam dnevni energijski proračun glavate želve. Rezultati su ukazivali da, dok kornjačice i mlade kornjače dnevno većinu energije ulažu u sazrijevanje i rast, odrasla jedinka koja je već dosegla punu veličinu koristi čak tri četvrtine energije na troškove metabolizma i održavanje stupnja zrelosti odnosno kompleksnosti. Također, istražila sam kako uhranjenost majke utječe na energijski proračun embrija: dok je hrane u okolišu dovoljno da majke mogu jesti do sitosti, embriju je za rast i razvoj dovoljno i manje od polovice početne energije dostupne u jajetu. Međutim, kada je hrane u okolišu manje, rezultirajući u 20% manjem normaliziranom unosu hrane, tada embrio za iste procese mora iskoristiti više od polovice početne energije u jajetu. Ovo direktno određuje koliko će rezerve odnosno žumanjčane vrećice biti prisutno pri izlijeganju, što pak utječe na mogućnost preživljavanja kornjačica.

Rezultati su dakle omogućili interesantan uvid u životni ciklus glavate želve, a predviđanja modela su se jako dobro poklapala s većinom podataka. Međutim, kako to često biva, onim predviđanjima modela koja se *nisu* idealno poklapala sa podacima je posvećeno više pažnje. Konkretno, iako je trajanje embrionalnog razvoja (inkubacije) predviđeno sa zadovoljavajućom točnošću, model je predviđao veću veličinu pri izlijeganju od zabilježene. Predviđena dob pri dostizanju spolne zrelosti (13 godina), iako unutar raspona izračunatog i zabilježenog za glavate želve, niža je od dobi određene u većini novijih studija (20 ili više godina). Znači li to da glavate želve počinju ulagati energiju u razmnožavanje puno ranije nego što se trenutno smatra? Ili sam svojom pretpostavkom da se glavate želve prilagođavaju uvjetima u okolišu toliko efikasno da manje varijacije u okolišnim uvjetima nemaju bitnog utjecaja na njihov rast i ostale fiziološke procese (što rezultira između ostalog von Bertalanffijevom krivuljom rasta), pretjerano pojednostavila uvjete u prirodi? Interesantan rezultat bio je i rast kornjačica, jer su kornjačice rasle brže nego što je model (uzimajući u obzir njihov općenito brži metabolizam) predviđao. Je li moguće da kornjačice rastu brže jer je njihov metabolizam brži od onoga što model predviđa? Ovakav obrazac rasta je već prepoznat kao "rastrošno brzanje" (eng. *"waste-to-hurry"*), i prisutan je kod vrsta koje moraju brzo rasti, čak i kada to znači rastrošno korištenje dostupnih resursa. Evolucijski, brži rast kornjačica (koje se izliježu tijekom ljeta kad su resursi dostupni u izobilju) bio bi povoljan, jer su kornjačice najosjetljivije dok su male i primamljive brojnim predatorima. Istovremeno, brži rast kornjačica, kao i druga zabilježena odstupanja od predviđanja modela, mogli bi biti posljedica specifičnosti podataka (primjerice različita kvaliteta hrane kojom su kornjačice hranjene u odnosu na hranu kojom se hrane odrasle jedinke, i slično). Zbog toga je bilo potrebno proučiti zaseban set podataka koji opisuje neku drugu populaciju, kako bih mogla potvrditi ili odbaciti svoje sumnje i hipoteze.

Proučavanje mediteranske populacije, koja je manja na nekoliko različitih načina (manji broj manjih jedinki koje žive na manjem području), bio je treći veliki korak mog istraživanja. Kako bih dobila prvi uvid u to koliko se zapravo veličinski razlikuju jedinke iz sjeverno-atlantske i mediteranske populacije, kao i moguće uzroke tih razlika, analizirala sam podatke o veličini (duljini i masi) želvi iz tih populacija, te ih usporedila za dva trenutka u životu glavate želve: izlijeganje iz jaja i polijeganje jaja. U obzir sam također uzela veličinu jaja, jer su prethodne studije pokazale da veličina jaja (relativno konstantna unutar pojedinih populacija) može objasniti veliki dio razlike u veličini izleglih kornjačica tih populacija. Rezultati ovog prvog (morfološkog) dijela analize pokazali su da, u usporedbi sa sjeverno-atlantskom populacijom, mediteranske kornjače manje i pri izlijeganju i pri gniježđenju. Iznenađujuće je bilo što se omjeri mase i duljine na treću potenciju (tj. indeksi kondicija) nisu razlikovali između populacija. Međutim, razlikovali su se između razvojnih stadija. U diskusiji vezanoj uz ovaj dio analize, raspravljam o nekoliko čimbenika koji su mogli uzrokovati razliku u veličini jedinki ovih dviju populacija: od uvjeta u gnijezdu tijekom inkubacije, do dostupnosti hrane u okolišu kasnije tijekom života kornjača. Nijedan od ovih čimbenika međutim nije mogao biti uzrokom tako velike razlike u prosječnoj veličini jedinki pri gniježđenju, a istovremeno podržavati stopu razmnožavanja koja je zabilježena u Mediteranu. Odgovor na ovu zagonetku otkriven je tek nakon određivanja parametara DEB modela za mediteranske glavate želve, i proučavajući implikacije modela. S obzirom na to da je u Mediteranu dostupno manje hrane, podaci o jedinkama koje sazrijevaju mlađe i pri manjoj veličini nisu bili intuitivni, jer je općenito u uvjetima s manje hrane jedinkama potrebno dulje vremena da dosegnu spolnu zrelost. Model je ukazao da je glavno objašnjenje niži stupanj zrelosti koje mediteranske jedinke moraju doseći kako bi postale spolno zrele, te da taj stupanj zrelosti proporcionalan njihovoj veličini. Implikacije ovog fenomena su dvojake: (i) mediteranske želve moraju uložiti ukupno manje energije kako bi postale spolno zrele, što znači da mogu doseći spolnu zrelost u ranijoj dobi i pri manjoj veličini nego sjeverno-atlantske želve, i (ii) mediteranske želve, nakon dostizanja spolne zrelosti, moraju dnevno ulagati manje energije za održavanje tog (maksimalnog) stupnja zrelosti, što znači da je dnevno više energije dostupno za razmnožavanje. Značajke koje je model predviđao (primjerice manja dob i veličina mediteranskih želvi pri dostizanju spolne zrelosti, ali stopa razmnožavanja jednaka onoj sjeverno-atlantskih želvi), bile su u skladu s izmjerenim podacima, a tehničko mehanističko objašnjenje bilo je u skladu s DEB teorijom. Model je predviđao veću veličinu kornjačica pri izlijeganju nego što je zabilježeno, te relativno nisku dob pri spolnom sazrijevanju, kao što je bio slučaj i za sjeverno-atlantske želve. Rast kornjačica, koji je unutar ovog poglavlja analiziran detaljnije i istovremeno za kornjačice iz obje populacije, potvrdio je da je metabolizam kornjačica tijekom analiziranog perioda uistinu veći od očekivanoga, rezultirajući bržim rastom. Ista analiza ukazala je i na moguće probleme u analizi izmjerenih brzina rasta, jer je podatak da mediteranske kornjačice rastu brže od sjeverno-atlantskih kornjačice iste starosti otkriven tek nakon što su brzine rasta standardizirane za istu referentnu temperaturu i količinu hrane (što je teško moguće napraviti koristeći klasične krivulje i podatke za rast). Dodatno, koristeći također DEB model simulirala sam značajnu promjenu u dostupnosti hrane tijekom života glavate želve, te gledala kakav utjecaj ta promjena ima na rast. Dobivena krivulja rasta odgovarala je dvofaznom rastu, što odgovara modelima rasta koje je predložila nekolicina autora, dok ih je većina koristila klasične (jednofazne) modele rasta. Dvofazni ili čak višefazni rast bi zapravo rezultirao i većom dobi pri dostizanju spolne zrelosti, te je model rasta koji bi svakako valjalo dodatno istražiti. Iako je ovako upečatljiva krivulja rasta značajan rezultat koji je u skladu sa podacima iz literature, nisam mogla sa sigurnošću tvrditi je li promjena u razini hrane jedina zaslužna za promjene u brzini rasta, ili je potrebno uzeti u obzir i temperaturu mora? I na koji točno način svaki od tih čimbenika utječe na cjelokupan energijski proračun i relevantne procese?

Posljednji dio mog putovanja (i posljednji dio disertacije) usmjeren je na proučavanje, prvo nezavisno a onda istovremeno, utjecaja hrane i temperature na energijski proračun.

Eksperimentalno je vrlo teško, ako ne i nemoguće, održavati uvjete nepromijenjenima tijekom cijelog života glavate želve (65 godina), a još je teže ovo postići za onoliko želvi koliko bi bilo potrebno za ispitati sve kombinacije hrane i temperature od interesa, nadajući se pri tome da su kornjače koje su u programu testiranja reprezentativne za svoju vrstu i/ili populaciju. Jedna od mnogih prednosti korištenja pristupa mehanističkog modeliranja je upravo mogućnost da se preispitaju ovakvi scenariji. Fokusirajući se opet na sjeverno-atlantsku populaciju, simulirala sam realistične raspone količine hrane i temperature kojima su izložene glavate želve. Utjecaj dostupnosti hrane odrazio se i na brzinu rasta, ali je bio najizraženiji na krajnju veličinu odraslih jedinki. Utjecaj temperature bio je najizraženiji za brzine rasta i sazrijevanja. Oba okolišna čimbenika značajno su utjecala na stopu razmnožavanja. Duljina oklopa pri spolnom sazrijevanju nije bila bitno različita niti za različitu dostupnost hrane, niti za različitu temperaturu, potvrđujući zaključke pojedinih autora da, usprkos tome što duljina pri dostizanju spolne zrelosti varira unutar određene populacije, u usporedbi s drugim značajkama predloženima za indikatore spolne zrelosti (dob, naglo usporavanje rasta), ovo je jedna od najmanje varijabilnih značajki. Rezultati su također potvrdili zaključke o unutarnjim (fiziološkim) razlikama koje omogućuju mediteranskim glavatim želvama spolno sazrijevanje pri manjim veličinama. Zatim sam, koristeći i model za mediteranske glavate želve, usporedila kako jedinke koje pripadaju različitim populacijama (i shodno tome se fiziološki razlikuju) reagiraju na iste okolišne uvjete. Ova simulacija je bitna jer su jedinke sjeverno-atlantske populacije često zabilježene u Mediteranu. Nedavno su brzine rasta i sazrijevanja jedinki sjeverno-atlantskog i mediteranskog podrijetla zasebno analizirane za Mediteran, što mi je omogućilo provjeru mojih rezultata. Rezultati dobiveni korištenjem DEB modela bili su u skladu s objavljenim rezultatima i zaključcima, uspješno reproducirajući brži rast i ranije sazrijevanje mediteranskih želvi. Dodatno, izuzetno niska reproduktivna stopa sjeverno-atlantskih želvi predviđena modelom dala je odgovor na pitanje zašto se sjeverno-atlantske želve ne gnijezde u Mediteranu.

Naposlijetku, na red je došao i onaj okolišni pritisak koji je i pokrenuo sve kotačiće - otpad porijeklom od ljudi, te utjecaj pojedene plastike na energijski proračun. Utjecaj na energijski proračun modeliran je u kontekstu sintetizirajućih jedinica, odnosno preciznije rečeno asimilacijskih jedinica (eng. *Assimilation Units*, AU) koje su općenito zadužene za pretvorbu pojedene hrane u rezervu i osiguravanje energije za sve potrebne procese (rast, sazrijevanje, održavanje sustava, i razmnožavanje). Pojednostavljeno, AU mogu biti ili zauzete obrađujući čestice hrane (ili neke inertne tvari) ili slobodne kako bi prihvatile novu česticu. Kada sve više čestica hrane postane zamijenjeno inertnim česticama plastike ili drugog otpada, tada je sve veći udio zauzetih AU zauzeto tim inertnim česticama , a bez ikakvog energijskog dobitka. Prvo sam pretpostavila da je vrijeme potrebno za obradu čestica plastike jednako onome potrebnome za obradu čestica hrane, i onda sam kvantificirala dugoročne posljedice one količine pojedene plastike koliko je bilo pronađeno u probavilu morskih kornjača. Podaci za volumni udio želuca glavatih želvi zauzet plastičnim otpadom kreću se od 0 do 25% (u prosjeku 3%), ali volumni udio čitavog probavnog sustava je vjerojatno veći obzirom da je udio plastičnog otpada u sadržaju crijeva veći od onoga u sadržaju želuca. Zatim, imajući na umu podatak kako je izmjereno vrijeme zadržavanja plastike u probavnom sustavu nekoliko puta veće od izmjerenog vremena zadržavanja hrane, simulirala sam scenarij gdje plastika koja zauzima 3% volumena probavnog sustava zahtjeva duže vrijeme obrade. Dakle, prvo sam simulirala raspon realnih količina pojedene plastike koja se u probavilu zadržava jednako dugo kao hrana, a zatim sam simulirala raspon različitih vremena zadržavanja plastike koja ukupno zauzima 3% volumena probavnog sustava. Utjecaj pojedene plastike pokazao se značajnim, izazivajući istovremeno u meni osjećaj znanstvenog ushita i moralne nevjerice. Pojedena plastika efektivno je imala jednake posljedica kao smanjen unos hrane, uzrokujući sporiji rast (odnosno povećanu opasnost od predatora), manju konačnu veličinu, i manju stopu razmnožavanja. Pod pretpostavkom jednakog vremena zadržavanja plastike i hrane u probavilu glavatih želvi, već je 14% volumena probavnog sustava ispunjeno plastikom prouzročilo tako nisku stopu reprodukcije da je realno pretpostaviti da se glavate želve ne bi uopće razmnožavale (jednako niska stopa razmnožavanja predviđena je za sjeverno-atlantske jedinke u Mediteranu, čije gniježđenje na mediteranskim plažama uistinu nije često zabilježena pojava). Ako plastika zauzima veći postotak volumnog udjela probavnog sustava, jedinke nemaju dovoljno raspoložive energije niti da bi dosegle spolnu zrelost. Kada je vrijeme zadržavanja plastike simulirano kao tri ili više puta duže od vremena zadržavanja hrane, jednaki učinak postignut je već pri nižem udjelu plastike (3%). U prirodi, udio pojedene plastike nije konstantan, niti sve pojedene čestice imaju jednako vrijeme zadržavanja. Podjednako realne mogućnosti su i da: (i) glavate želve mogu podnijeti kratku (akutnu) izloženost količini plastike koja zauzima više od 14% volumena probavnog sustava i oporaviti se, te (ii) želva koja pojede i manju količinu otpada će umrijeti od izgladnjivanja, jer je (s obzirom na to da je do tada jela hranu u većim količinama) narasla do veće veličine i potrebno joj je više energije za održavanje, koju sada više ne može pribaviti u dovoljnoj količini.

Diskusija i zaključak

Rezultati istraživanja ove disertacije omogućili su novu perspektivu i nove spoznaje o životnom ciklusu glavate želve. Na moje veliko zadovoljstvo, brojna pitanja su odgovorena na zadovoljavajući način. Istovremeno, oblikovalo se nekoliko novih zanimljivih pitanja, na koja bih rado nastavila tražiti odgovore. Primjerice, zašto model predviđa da će veličina kornjačica pri izlijeganju biti veća nego što je zabilježeno, i to dosljedno za obje proučavane populacije? Može li taktika “rastrošnog brzanja” objasniti brži rast kornjačica, i vrijedi li ovo objašnjenje za sve populacije glavatih želvi, ili čak za sve vrste morskih kornjača koje su izložene sličnim okolišnim pritiscima? Uzrokuje li kombinacija manje količine dostupne hrane i niže temperature u pelagijalnom okolišu otvorenog mora, te veće količine dostupne hrane i više temperature u neritičkom okolišu priobalnog mora, dvofaznu krivulju rasta kod većine spolno nezrelih jedinki? Mogu li ova dva obrasca, jedan kao rezultat metabolizma ("waste-to-hurry"), a drugi kao rezultat okoliša (promjene u količini hrane i temperaturi), istovremeno objasniti pretpostavku višefaznog rasta i neslaganje između novijih procjena (20-30 godina) i modelom procijenjene (13-15 godina) dobi pri dostizanju spolne zrelosti? Da li bi proučavanje utjecaja pojedene plastike, uzimajući u oblik takvu (višefaznu) krivulju rasta, rezultiralo predviđanjima za još višu dob pri dostizanju spolne zrelosti i još gorim scenarijima za budućnost populacija glavatih želvi? I, naposljetku, može li ovakvo produbljeno razumijevanje biologije i ekologije ove izvanredne vrste, te štetan utjecaj koji plastika ima na naš okoliš, objasniti zašto je brojnost nekih populacija glavate želve u opadanju usprkos zaštiti, te nas potaknuti da promijenimo svoje ponašanje?

Samenvatting

Mijn belangrijkste motivatie om deze reis te ondernemen, die resulteert heeft in een doctoraat, maar daar niet mee eindigt, was om uit te zoeken hoeveel schade wij berokkenen met plastic afval dat we in de zee kiepen. Het is algemeen bekend dat plastic er erg lang over doet om afgebroken te worden, precies een eigenschap waarom plastic is uitgevonden, en dien-ten-gevolge, overal aanwezig is. Waar blijft het plastic eigenlijk? Drijft het in de oceanen alsmaar ronddraaiend op de stromen? Komt het in de magen van zeedieren terecht? En wat zijn dan de gevolgen?

Rapporten vermelden dat minstens 300 verschillende soorten organismen iets met plastic doen. Ze raken erin verstrikt, eten het, gebruiken het als transport om nieuwe habitats te bereiken en daar soms zo succesvol zijn dat ze de oorspronkelijke soorten eruit drukken. Mag de aanhechting aan plastic om nieuwe habitats te bereiken en een nieuwe start maken nog hoopvol klinken, verstrikt raken in een oud vissersnet, of de hongerdood sterven door te veel plastic te eten lijkt aanzienlijk minder hoopvol. Dit tweede scenario komt, echter, veel vaker voor en is van toepassing op zeeschildpadden.

Zeeschildpadden zijn opmerkelijke beesten. Ze bestaan al meer dan 150 miljoen jaar zoals wij ze nu kennen - wat wil zeggen dat ze tijdgenoten waren van de grote dino's, maar ze zijn er nog steeds! Zij hebben vroege beschavingen gefascineerd, maar deze fascinatie heeft ze niet erg geholpen - alle 7 soorten zeeschildpadden komen nu op de IUCN lijst voor als bedreigde soorten, en de meest populaties krimpen, ondanks de bescherming. De meest kwetsbare dieren voor plastic zijn de lang-levende soorten, omdat hun vermogen om zich aan veranderende omgevingen aan te passen het meest beperkt is. Zeeschildpadden vallen in deze categorie.

Onechte karetschildpadden, zoals hun wat vreemde Nederlandse naam is, komen in alle gematigde oceanen voor over de hele wereld en hebben zich opgesplitst is vele populaties en sub-populaties. Ze worden minstens 65 jaar oud, en hun geslacht wordt door de temperatuur bepaald tijdens het laatste derde deel van hun 60 dagen durende embryonale ontwikkeling. Ze nemen tijdens hun leven 25 maal in lengte toe: vanaf 4 cm lange en 20 g 'zware' pasgeborenen die het nest verlaten tot volwassenen die meer dan 100 kg wegen en een schildlengte hebben van 100 tot 130 cm bij terugkomst op het strand waar ze geboren zijn. Deze twee momenten, geboren worden en een nest graven, zijn de enig momenten waarop ze met het land in aanraking komen. Het strand en de kust waren lange tijd ook de enige plaatsen waar je onechte karetschildpadden kon zien. De tussen-geleden periode, die 5 of 10 jaar of tientallen jaren kan duren, staat in de vakliteratuur bekend als 'lost period', een term die Archie Carr in 1986 gebruikte, en wat waarin gebeurde is voor lange tijd een mysterie gebleven.

Vooruitgang in de wetenschap heeft het mogelijk gemaakt zeeschildpadden te bestuderen en vele raadsels te ontrafelen. De levenscyclus van de onechte karetschildpad kan ruwweg in drie stadia verdeeld worden, embryo, juveniel en volwassen, en het is nu mogelijk waarnemingen te verzamelen over hun ecologie en levensstadia beter te definiëren. De juveniele periode kan opgesplitst worden in
(i) een pasgeborene, een individu dat juist is uitgekomen en over het strand rent,
(ii) een kleuter, een iets ouder individu tot 15 cm schildlengte en die dicht bij de kust blijft,
(iii) een oceaan-juveniel met een schild tussen 15 en 30 cm, die de oceaan op gaat en van plankton en andere pelagische organismen leeft en
(iv) een kust-juveniel met een schild tussen 30 en 50 cm, die langs de kust van bodem organismen leeft. De overgang tussen het oceaan- en kust-juveniel zijn is meestal vrij abrupt en wordt soms de kust-rekrutering genoemd; het gebeurt wanneer de dieren een bepaalde grootte of ontwikkelingsstadium bereiken, maar kan ook langer duren, of helemaal niet optreden met gevolg dat volwassenen op de open oceaan foerageren.

Er zijn vele studies gedaan, en er is veel over geschreven, maar deze studies beperkten zich meestal tot een bepaalde eigenschap of stadium. Dank zij (i) verschillende habitats, (ii) verschillende bemonstering (het meten van veranderingen in lengte op schillende manieren en dan formules gebruiken om ze in elkaar om te rekenen of groeisnelheden berekenen uit vang-en-terugvang data of uit groeiringen in beenderen) en (iii) verschillende analytische technieken (verandering in lengte, of in massa, en daar verschillen groeimodellen op fitten) ontbreekt het totaal beeld en zijn resultaten nogal eens tegenstrijdig. Het vaakst komen die voor met betrekking tot groei-snelheden en groeimodellen die worden toegepast op verschillende populaties en levensstadia, gebrek aan eensgezindheid of nu de minimum of gemiddelde lengte van nestelende vrouwtjes gebruikt moet als het gaat om de puberteit vast te stellen; de schattingen over de puberteits-leeftijd lopen uiteen van 6 tot 38 jaar. Diverse schrijvers wijzen er bovendien op dat er grote verschillen tussen populaties bestaan, zoals erg duidelijk is bij de grootte van Mediterrane adulten, vergeleken met anderen populaties, maar ook binnen populaties met betrekking tot groei-snelheden en verschillende formules om de ene naar de andere schildlengte om te rekenen (wel of niet rekening houdend met de kromming van de schilden).

Om het mysterie van het 'verloren plastic' op te lossen en het effect op onechte karetschildpadden vast te stellen, moest ik eerst veel meer weten over hun biologie en ecologie: Hoe lang doen ze erover om volwassen te worden? Waarom groeit de één harder dan de ander? Waarom zijn de schilpadden in de noordelijke Atlantische Oceaan groter dan die in de Middellandse Zee? Reproduceren grote individuen meer dan kleine? In welke mate zijn grootte en reproductie-snelheid afhankelijk van omgevings- dan wel fysiologische karakteristieken? Hoe reageren ze op wereldwijde veranderingen in de omgeving? Of, meer in het bijzonder, hoe beïnvloeden voedselbeschikbaarheid, zoutgehalte en temperatuur processen als groei, ontwikkeling, onderhoud en reproductie? En - als we de effecten van gegeten plastic op de energie allocatie naar deze relevante processen willen weten - hoeveel energie hebben schildpadden dagelijks nodig voor deze processen, en hoe komen ze eraan?

Het vaststellen en volgen van het energie budget van een schildpad is de meest logische stap om deze vragen te beantwoorden omdat enig effect van gegeten plastic zichtbaar moet worden als verandering in dit budget en/of levensduur. Voor dit doel heb ik gekozen voor de Dynamische Energie Budget theorie als pad naar mijn 'Heilige Graal': het DEB model van de onechte karetschildpad.

Het heeft alles wat ik nodig heb: het respecteert de wetten van de thermodynamica, maakt gebruik van verschillende typen homeostase dat elk systeem, van cellen tot ecosystemen, nastreeft, houd rekening met effecten van voedsel en temperatuur op het energie budget, en met interacties tussen verschillend deelprocessen zoals groei, onderhoud, ontwikkeling en reproductie. Bovendien was en is het de meest consistente theorie die er op dit moment beschikbaar is.

In het algemeen is massa meer relevant dan lengte als het aankomt op energetica, maar omdat dezelfde curve de relatie tussen lengte en massa goed beschrijft over de hele levenscyclus van het individu en voor verschillende populaties (Wabnitz and Pauly, 2008), heb ik vooral lengten gebruikt. Ik ben begonnen met de relatie tussen verschillen lengtematen voor schilden, omdat verschillende formules voor de verschillende populaties in omloop zijn, hetgeen verschillen in vorm suggereren gedurende de ontwikkeling en tussen populaties. Deze verschillen kunnen belangrijke consequenties hebben voor het energie budget model aangezien vorm-homeostase één van de aannamen is van het standaard DEB model. Veranderingen in vorm (d.w.z. afwijkingen van isomorfie) kunnen eenvoudig in model gebracht worden via de vorm coëfficient ($\delta_{\mathcal{M}}$), maar eerst moet het belang daarvan worden vastgesteld. Ik heb eerst voor de Noord-Atlantische populatie verschillen in geografie (noord, zuid) en levensstadia (kleuters, oceaan- en kustjuvenielen en adulten) onderzocht, omdat de literatuur verschillende conversies hiervoor rapporteert. Erg bleken echter geen belangrijke verschillen te bestaan, maar je moet wel voorzichtig zijn om resultaten van erg kleine individuen te vertalen naar die voor erg grote, en vice versa. Ik zag geen reden om met extra vorm-coëfficienten te werken, aangezien de fouten-marge slechts 5% was, en ik met het meest eenvoudige model kon werken: het standaard DEB model.

Het ontwikkelen en formaliseren van de complete levenscyclus van de onechte karetschildpad was de volgen stap. Het standaard DEB model beschrijft het individu met behulp van veranderingen in een drietal grootheden: structuur, reserve, maturatie (complexiteit) of (na puberteit) de reproductie buffer. De eerste twee (structuur en reserve) kunnen indirect gemeten worden via lengte en/of massa van een individu, terwijl de derde (ontwikkeling) formeel wordt gekwantificeerd door de gecumuleerde investering in ontwikkeling. Deze processen hebben een unieke dynamica die volledig wordt vastgelegd door de parameters, die alle tegelijkertijd uit data worden geschat, en omgevingsvariabelen. Het uitgangspunt was dat verschillen in populaties (Noord-Atlantisch of Mediterraan) en effecten van plastic eten of het energie budget zichtbaar worden als verschillen in parameter waarden en daarmee als verschillen in voorspellingen. Deze waarden moeten dus eerst worden vastgesteld. De parameterschattings-methode gebruikt alle levenscyclus data tegelijkertijd (zoals lengte en leeftijd bij geboorte en puberteit), en andere data (zoals groei en reproductie-curven) om de beste en meest realistische schattingen te vinden. Vanwege de grote variatie binnen één populatie was het niet doenlijk om de parameters van alle populaties tegelijkertijd te schatten. Ik heb alle primaire parameters kunnen schatten voor de Noord-Atlantische populatie, de grootste en best bestudeerde populatie in de wereld. Het model fitte de data erg goed, variëerend van incubatie tijd, groeisnelheden van lengten en gewichten, lengte bij puberteit, en reproductie. Zo kon ik het dagelijks energie budget van een individu bestuderen. De resultaten laten zien dat een kleuter de meeste energie besteedt aan groei en ontwikkeling terwijl een uitgegroeide adult driekwart van zijn budget gebruikt voor onderhoud. Ik kon bovendien nu bekijken wat het effect is van de voedingstoestand van de moeder op de ontwikkeling van het embryo: als de moeder goed doorvoed is, heeft het embryo nog minstens de helft van zijn reserve over bij het uitkomen, maar als de voedingstoestand van de moeder met 20% daalt, heeft het embryo minder dan de helft van zijn reserve over. Dit kan de overlevingskans van de kleuter sterk beïnvloeden.

Zoals wel vaker voorkomt zijn het juist de voorspellingen in niet goed uitpakken de meest interessante. Terwijl de incubatie-tijden behoorlijk goed voorspeld werden, was de grootte bij uitkomen overschat. De voorspelde leeftijd bij puberteit was aan de ondergrens van de waargenomen range (rond 13 jaar), terwijl de meer recente studies juist aan de bovengrens zitten (20 jaar en meer). Betekent dit dat de schildpadden eerder beginnen energie aan reproductie te besteden dan meestal wordt aangenomen? Of was de aanname van een min of meer constante omgeving te eenvoudig? Een interessant resultaat was ook dat kleuters sneller lijken te groeien dan voorspeld. Komt dit omdat hun metabolisme sneller werkt dan voorspeld door het standaard model? Dit patroon van versnelde groei komt vaker voor en staat bekend als 'waste to hurry', waarbij een verhoogde voedsel-inname en onderhoud de oorzaken zijn van een versnelde groei. De versnelde groei van kleuters (tijdens de zomer) zou evolutionair logisch zijn om zodoende sneller aan predatoren te ontsnappen. Snellere groei kan echter ook het gevolg zijn van een verhoogde voedselbeschikbaarheid voor de kleuters, ten opzichte van adulten.

De derde stap in mijn werk was de studie van de Mediterrane populatie, die in een aantal opzichten kleiner is, minder en kleinere individuen die in een kleinere omgeving leven. Om inzicht te krijgen in de grootte en de oorzaak van de verschillen, werd deze vergeleken met de Atlantische populatie voor pasgeborenen en nestelende individuen. Verschillen in ei-grootte werden ook meegenomen omdat eerder werd gevonden dat dit de oorzaak is van verschillen in grootte van pasgeborenen. De grootte tussen de populaties verschillen inderdaad sterk voor pasgeborenen en nestelende adulten. Merkwaardig genoeg verschilden de verhouding tussen gewichten en lengten-tot-de-macht-drie (de conditie-index) echter niet. Deze conditie-index verschilde echter wel tussen de verschillende levensstadia. Ik bespreek de mogelijke redenen voor de grootte verschillen, lopend van de omgeving tijdens incubatie naar voedselbeschikbaarheid voor juvenielen en adulten. Geen van deze factoren kon echter de combinatie verklaren van verschillen in grootte en reproductie van nestelende adulten. Het antwoord op deze puzzel werd mij pas duidelijk door de verschillen in parameter waarden tussen beide populaties te bekijken, en de gevolgen voor voorspelling van grootte en reproductie. Hoewel vroegere puberteit bij een kleinere grootte in een omgeving met minder voedsel niet voor de hand ligt, het bleek toch het belangrijkste verschil tussen de Mediterrane en Atlantische populatie te zijn als gevolgen van een lagere drempelwaarde voor de maturatie bij puberteit. Dit houdt twee dingen in: (i) de Mediterrane schildpadden hebben minder energie nodig om tot puberteit te komen, wat ze dan eerder doen bij een kleinere grootte, en (ii) ze hebben vervolgens minder energie nodig om dit lagere niveau van ontwikkeling te handhaven, zodat er relatief meer voor reproductie overblijft. De voorspelde eigenschappen (eerdere puberteit bij kleinere grootte maar geen effect op reproductie) van de Mediterrane populatie ten opzichte van de Atlantische is geheel consistent met de waarneming en nu mechanistisch verklaart door DEB theorie. De grootte van de pasgeborenen is lichtelijk overschat, en de voorspelde leeftijd bij puberteit was aan de ondergrens van de range zoals in de literatuur wordt vermeldt, voor beide populaties. De groei van de kleuters kon nu meer in detail bestudeerd worden voor beide populaties gezamenlijk; de metabole versnelling trad in beide populaties op. Mijn analyse lied zien waarom het zo moeilijk is om groeisnelheden te analyseren. Dat de Mediterrane kleuters sneller groeien dan de Atlantische wordt pas duidelijk na correctie van verschillen in temperatuur en voedselbeschikbaarheid. Met hulp van het DEB model kon ik ook vaststellen dat de voedselbeschikbaarheid gedurende het leven van de schildpadden behoorlijk verandert, met gevolgen voor de groei. De resulterende groeikromme suggereert twee fasen, zoals ook door diverse auteuren werd voorgesteld die verschillende standaard groeimodellen voor deze fasen gebruikten. Deze twee of meer fasen resulteren inderdaad in een hogere leeftijd bij puberteit, overeenkomend met de bovengrens van de gerapporteerde range; een patroon dat de moeite waard om verder uit te zoeken. Het was interessant om op deze groeipatronen uit te komen, maar het is niet duidelijk of alleen verschillen in voedselbeschikbaarheid hiervoor verantwoordelijk zijn of ook verschillen in temperatuur. En wat zijn precies de effecten van beide op het hele energie budget en de onderliggende processen?

Het meest recente deel van mijn reis (het laatste deel van mijn proefschrift) exploreert, eerst onafhankelijk en dan gezamenlijk, de effecten van voedsel en temperatuur op het energie budget. Het is experimenteel erg moeilijk, zo niet onmogelijk, om deze condities constant te houden over de hele levenscyclus van 65 jaar en het is zelfs nog moeilijker om dit te doen voor alle mogelijke combinaties die nodig zijn, in de hoop dat individuen waarmee we dit zouden proberen goede representanten van de soort zouden zijn. Dit is precies één van de sterke punten van mechanistisch modellen die het mogelijk maken verschillende scenario's door te rekenen. Weer opnieuw beginnend met de Atlantische populatie simuleerde ik realistische ranges van voedselbeschikbaarheid en temperatuur. Effecten van voedselbeschikbaarheid werden duidelijk op de groeisnelheid, maar vooral ook op de uiteindelijke grootte. Effecten van temperatuur betreffen vooral groei en ontwikkelings-snelheden. Beide factoren beïnvloeden de reproductie sterk. Lengte bij puberteit werd nauwelijks beïnvloed onder de geteste scenario's, hoewel variaties in deze lengte optraden, waren deze klein ten opzicht van variaties in leeftijd en groeisnelheid. De resultaten bevestigden de conclusie dat intrinsieke fysiologische verschillen met de Atlantische populatie, de Mediterrane populatie in staat stellen om puberteit eerder en bij een kleiner grootte te bereiken. Vervolgens heb ik dit ook gedaan voor Mediterrane populatie en het verschil met de Atlantische populatie gezien, als ze in een zelfde omgeving zouden leven. Deze vergelijking is realistisch omdat Atlantische dieren vaak ook de Middellandse Zee bezoeken. Hun groei en ontwikkelings-patroon werd recent gerapporteerd en vormen een goede validatie van mijn simulatie studies. De resultaten van de DEB modellen zijn in goede overeenstemming met gepubliceerde data en conclusies, vooral m.b.t. snellere groei en vroegere maturatie van Mediterrane onechte karetschildpadden. Mijn resultaten laten bovendien zien waarom Atlantische dieren niet nestelen in op Middellandse Zee-kusten; hun reproductie is zeer laag onder deze omstandigheden.

Tenslotte bestudeerde ik waar het allemaal om te doen was: de druk op de omgeving in de vorm van effecten van afval dat door menselijk toedoen wereldwijd aanwezig is. Effecten op het energie budget werd gemodelleerd in de context van 'Synthesizing Units', of beter 'Assimilatie Units' (AUs) die normaliter gebruikt worden om gegeten voedsel in reserve om te zetten om zodoende de energie voor het metabolisme te leveren voor

groei, onderhoud, ontwikkeling of reproductie. Simpelweg is een AU of bezig met de afhandeling van een voedseldeeltje (er energie uit te halen) of wachtend om een nieuw deeltje te ontvangen. Wanneer nu een stijgend deel van de voedseldeeltjes vervangen wordt door plastic (of ander inert afval), verdoen steeds meer AUs hun tijd met nutteloos werk dat niets oplevert. Ik doordacht eerst het scenario dat de afhandelingstijd per plastic deeltje hetzelfde zou zijn als voedsel deeltjes en kwantificeerde de lange termijn effecten van eten van plastic tot besteding van reserve. Het gerapporteerde deel van het maagvolume dat door plastic wordt ingenomen is gemiddeld 3%, uiteenlopend van 0 tot 25%, maar is waarschijnlijk meer als je het hele spijsverteringsstelsel erbij betrekt omdat het plastic gehalte van de darm groter is dan dat in de maag. Met het oog op het feit dat de verblijftijd van plastic in de darm een veelvoud is van dat van voedsel, simuleerde ik effecten van 3% plastic in gegeten materiaal. Eerst simuleerde ik geen effect op de verblijftijd in darm, daarna een langere verblijftijd voor plastic dat 3% volume in de darm inneemt. Het effect van gegeten plastic bleek, tot mijn niet geringe wetenschappelijke opwinding en grote morele teleurstelling, zeer substantieel te zijn. Gegeten plastic heeft effectief hetzelfde effect als een daling van de voedselinname, resulterend in lagere groei (dus groter predatie risico), kleinere uiteindelijke grootte en een lagere reproductie. In afwezigheid van effect op de darmverblijftijd is 14% van het volume van het spijsverteringsstelsel dat door plastic wordt ingenomen al voldoende om nagenoeg de hele reproductie onmogelijk te maken (vergelijkbaar met die Atlantische dieren die de Middellandse Zee bezoeken en daar niet blijken te reproduceren). Zou het plastic nog meer volume innemen, dan zou zelfs puberteit niet meer kunnen worden bereikt. Als ik aannam dat plastic een drie maal langere verblijftijd in de darm heeft dan voedsel, dan werd dit effect al bereikt bij 3% plastic in het spijsverteringsvolume. Het aandeel van plastic in gegeten materiaal is niet constant in de natuur, en de verschillende deeltjes hebben verschillende darmverblijftijden. Realistische scenario's zijn
(i) onechte karetschildpadden kunnen kortdurend percentages van 14% of hoger overleven en hiervan herstellen, en
(ii) opnamen van zelfs lagere hoeveelheden kunnen tot de hongerdood leiden, omdat inmiddels grote dieren (opgegroeid in een schoon milieu) meer onderhoud nodig hebben en dus een hogere onderhoudsbehoefte hebben.

Dit werk heeft, als onderdeel van mijn proefschrift, verschillende waardevolle inzichten naar voren gebracht, maar er blijven nog veel vragen over - waar ik in de toekomst verder aan wil werken. Waarom, bijvoorbeeld, wordt de grootte van pasgeborenen systematisch overschat? Kan het 'waste to hurry' patroon de snellere groei van de kleuters verklaren en geldt dit voor alle populaties onechte karetschildpadden, of zelfs voor alle zeeschildpadden die bloot zijn gesteld aan vergelijkbare omgevings-drukken? Is het combineerde effect van een pelagisch milieu met lagere voedselbeschikbaarheid en temperatuur, en een kust milieu met hogere voedselbeschikbaarheid en temperatuur de oorzaak van de twee fasen in de groeikromme van de meeste juvenielen? Kunnen deze twee patronen, één gedreven door het metabolisme ('waste to hurry') en door het milieu (voedseldichtheid en temperatuur) tezamen de meer-fase groei verklaren als wel het verschil tussen gerapporteerde (20-30 jaar) en voorspelde (13-15 jaar) leeftijd bij puberteit? Zou het effect van plastic op meer-fasige groeikommen een zelfs hogere leeftijd bij puberteit voorspellen met nog zwaarmoedigere scenario's voor de toekomst van zeeschildpadden? En, tenslotte, zou dit diepere inzicht in de biologie en ecologie van deze prachtige soort verklaren waarom sommige populaties onechte karetschildpadden nog steeds krimpen ondanks de bescherming die ze krijgen, en ons motiveren om ons gedrag te veranderen?

Appendix C

List of publications

2016

- Marko Jusup, Tania Sousa, Tiago Domingos, Velimir Labinac, Nina Marn, Zhen Wang, Tin Klanjšček. Physics of metabolic organization // submitted to Phys. Life Rev.
- Ivana Maguire, Nina Marn, Goran Klobučar. Morphological evidence for hidden diversity in the threatened crayfish species *Austropotamobius torrentium* // submitted to Zoomorphology

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Appendix D

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Time for new questions, research, and adventures!

image from latortugaverde.com

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The image of the plastic items featured on the front cover is based on an actual photograph of plastic debris found in a gut contents of a single turtle (original photograph by Victoria Gonzalez Carman from Seaturtle.org). The background image is by Arseniy Gutov, and the sea turtle was drawn lovingly by Dora Marn, images are used with permission.

The specific aim of my research was to answer a simple question "What are the effects of plastic ingestion on loggerhead turtle's biology and ecology?". The overall aim was to provide new insights into biology and ecology of this protected migratory species.

I focused on the processes important for the life cycle and ecology (growth, maturation, and reproduction). My methods included using the collected experimental and literature data, developing a life cycle model based on the Dynamic Energy Budget (DEB) theory, and using the model to compare North Atlantic and Mediterranean populations, and to study the the environmental (food density and temperature) and anthropogenic (plastic pollution) pressures on loggerhead turtles.

By far the most shocking result was the effect of plastic ingestion on the energy budget of loggerhead turtles, however several other results deserve attention as they provide valuable insight into the biology and ecology of loggerhead turtles. The analyses of the three life stages and two neighbouring North Atlantic subpopulations resulted in recommended carapace conversion formulae (for specific life stages as well as the whole size span) and highlighted in which cases the discovered morphological differences between life stages might play an important role. Comparison of individuals from Mediterranean and North Atlantic populations explored the effect of the environment and the effect of population-specific physiological traits (reflected as population-specific parameter values) on various life history traits, growth, maturation, and reproduction of loggerhead turtles. Analysis of the estimated (population-specific) DEB parameters and their implied properties, as well as analysis of the cases when model predictions deviate from the measured data, suggest posthatchlings might maximize their growth during the first several weeks, individuals might allocate energy to reproduction earlier than thought, and the ontogenetic habitat shift might be resulting in a bi-phasic growth curve. Finally, plastic ingestion affects the individuals similar to food dilution, resulting in small ultimate size and no reproduction when it occupies over 14% of the digestive system volume. When a several times longer residence time of ingested plastic than that of food is assumed, the effect is similar (small ultimate size and no reproduction) already when plastic occupies 3% of the digestive system volume.

Keywords: DEB, loggerhead turtle, mechanistic model, environmental changes, plastic ingestion, Mediterranean, North Atlantic