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Corso di Laurea magistrale in Marine Biology

**TESI DI LAUREA**

# Assessment of reproductive maturity of loggerhead sea turtles (*Caretta caretta*) found stranded along Veneto coastline, Northern Adriatic Sea

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**Abstract**

Knowledge on species' reproductive biology is essential to understand population dynamics and manage conservation efforts. The present study analyses the reproductive maturity of loggerhead sea turtles (*Caretta caretta*) stranded along the Veneto coastline, northern Adriatic Sea, in the period January 2023 to February 2025. Focus was set on exploring the level of gonad maturity in stranded individuals using gonad gross morphology and histology, and investigating the relationship with size. Moreover, the aim was to understand any spatiotemporal trends in the stranding data with regard to the individuals' size, sex, and maturity level. Lastly, the main sexual dimorphism in adult sea turtles, the difference in tail length, was analysed. Results found an evident seasonality with higher stranding frequency in the summer months, as well as high occurrence in stranding zones 7 and 6, which correspond to coastal areas close to inlets to the Venice lagoon. Based on gonad gross morphology, most individuals were assessed to be prepubescent (41.2%), followed by pubescent (25%) and mature individuals (20.6%). Mature individuals were either in condition preparing to breed, in regression after nesting season, or in quiescence. Histology of six individuals confirmed the macroscopic assessment of their maturity stage, although the analysis was compromised by tissue decomposition. Mature individuals were significantly larger than individuals with prepubescent and pubescent gonads, however the curved carapace length (CCL) of individuals with different gonad development showed a large overlap, demonstrating variability in size at maturity. Using generalized linear models (GLMs) and generalized additive models (GAMs), it was found that male loggerhead sea turtles begin to develop a longer tail than females between 50.2 and 56.2 cm, depending on which tail measurement was used. Overall, this study presents the first assessment of gonad development and reproductive maturity in loggerhead sea turtles in the northern Adriatic Sea and underlines the importance of this habitat for individuals in different phases of their life.

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## List of abbreviations

| Abbreviation | Definition |
|---|---|
| BCA | Department of Comparative Biomedicine and Food Science |
| CaTT | Carapace – tip of the tail |
| CMR | Capture-mark-recapture |
| CCL | Curved carapace length |
| CERT | Cetacean strandings Emergency Response Team |
| CTL | Cloaca – tip of the tail |
| CTLV | Coordinamento Tartarughe marine del Litorale Veneto |
| GAM | Generalized additive model |
| GLM | Generalized linear model |
| IUCN | International Union for Conservation of Nature |
| PMI | Post-mortem investigation |
| PT | Plastron – tip of the tail |
| RMU | Regional Management Unit |
| SCL | Straight carapace length |
| SSM | Size at sexual maturity |
| SST | Sea surface temperature |
| TL | Tail length |
| TSD | Temperature-dependent sex determination |
| UNIPD | University of Padova |

# 1. Introduction

## 1.1. The loggerhead sea turtle in the Mediterranean and northern Adriatic Sea

The loggerhead sea turtle (*Caretta caretta*) is the most abundant sea turtle species in the Mediterranean Sea (Margaritoulis et al., 2003; Casale et al., 2018). The most important nesting sites are concentrated in the eastern basin, and predominantly in western Greece, Turkey, Libya and Cyprus (Casale et al., 2018). However, in recent years a beginning shift in nesting behaviour has been observed, with increasing nest occurrences in the western Mediterranean Sea (Hochscheid et al., 2022). The northern Adriatic Sea in the central Mediterranean Sea is a key foraging and overwintering habitat for this species (Casale et al., 2004; Casale et al., 2012). While the Mediterranean Sea is inhabited by loggerhead sea turtles originating from different populations, genetic studies have shown that the Adriatic Sea is almost exclusively frequented by loggerhead sea turtles from the Mediterranean Regional Management Unit (RMU) (Tolve et al., 2018), and particularly, a connectivity to the Greek nesting rookeries has been confirmed (Baldi et al., 2023a; Lazar et al., 2004; Tolve et al., 2018; Zbinden et al., 2008).

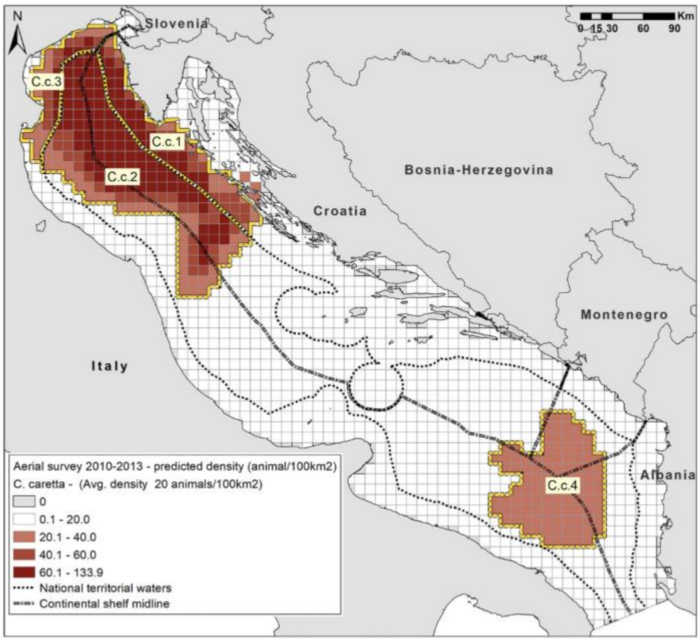



***Figure 1**: Predicted density of sea turtles in the Adriatic Sea based on 2010 and 2013 aerial surveys (darker red scale = higher relative density) (Fortuna et al., 2018).*

Aerial surveys in the summers 2010 and 2013 demonstrated that the density of loggerhead sea turtles in the northern Adriatic Sea is about twice the density that can be found in the whole Adriatic Sea (0.405 turtles/km$^2$ vs. 0.203 turtles/km$^2$) (Fortuna et al., 2018, Fig. 1). Although migration to southern parts of the Adriatic Sea during colder months has been observed, individuals stay in the northern Adriatic Sea also during winter months, which makes this area an important habitat year-round (Baldi et al., 2023a; Casale et al., 2004; Casale et al., 2012). While the beaches in the northern Adriatic Sea are generally not considered as a nesting area for loggerhead sea turtles, in 2021 two nests were recorded for the first time in the Veneto Region, which candidates this coastline as a diffuse nesting area (Pietroluongo et al., 2023).

#### 1.1.1. Threats

Loggerhead sea turtles in the northern Adriatic Sea are exposed to a variety of anthropogenic impacts. The most relevant are related to the high fishing effort in the region (Agabiti et al., 2024; Casale et al., 2010; Marisaldi et al., 2023). Due to their neritic foraging behaviour and specialization on benthic prey (Mariani et al., 2023; Palmer et al., 2021), loggerhead sea turtles are particularly susceptible to be bycaught in trawling fishery, and high incidences of loggerhead bycatch by Italian trawlers have been recorded (Casale et al., 2004; Lucchetti et al., 2017). However, unless there is physical evidence of e.g. ingested fishing gear, mortality caused by fishery impact is often difficult to determine (Marisaldi et al., 2023). Other anthropogenic threats include the entanglement and ingestion of marine litter (Hama et al., 2020; Lazar and Gračan, 2011), and the collision with vessels (Casale et al., 2010; Hama et al., 2020; Mihaljević et al., 2024). Moreover, cold-stunning as a consequence of sudden temperature drop in winter has been determined as a relevant cause of death in stranded juvenile loggerhead turtles along the Croatian Adriatic coast (Mihaljević et al., 2024).

#### 1.1.2. Conservation

While the conservation status of loggerhead sea turtles globally was last assessed as “Vulnerable (VU)” (Casale and Tucker, 2017), the Mediterranean subpopulation has been classified as "Least Concern (LC)" by the International Union for Conservation of Nature (IUCN) Red List (Casale, 2015). However, knowledge gaps on reproductive parameters as well as variation in monitoring effort and methodologies leave uncertainties in the assessment (Casale, 2015). Since the recovery of the population in recent years is the result of continuous conservation efforts, Casale (2015) urges the population's status to be considered as "conservation dependent".

In the Mediterranean, loggerhead sea turtles are protected under the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention), the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention, Annex II), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Appendix I), the Convention on the Conservation of Migratory Species of Wild Animals (CMS/ Bonn Convention, Appendix I and II), and the EU Habitats Directive (Annex II and IV) (Casale and Margaritoulis, 2010; European Commission, 1992).

## 1.2. Reproductive biology in loggerhead sea turtles

### 1.2.1. Reproductive cycle

Sea turtles are migratory animals known to travel far distances between their foraging and breeding sites (Miller, 1996). After hatching, young loggerhead sea turtles experience an oceanic phase drifting at the sea surface and distributed with currents due to their limited swimming and diving capacity (Casale et al., 2018). With continuous growth, juveniles recruit to habitats closer to the coast and switch their diet towards benthic prey. In Mediterranean loggerheads this ontogenetic shift seems to occur already at an early juvenile stage (Casale et al., 2008). The gonadal tissue of sea turtles differentiates into male and female organs already during embryonic development (Sari and Kaska, 2016; Yntema and Mrosovsky, 1980), and continues to grow during prepuberty (Miller and Limpus, 2003). Puberty marks the transition to adulthood, where ovaries and oviducts, testes and epididymides undergo maturation processes, and the changes are observable with macroscopic gonad assessment (Hamann et al., 2003; Limpus, 1990; Pérez-Bermúdez et al., 2012).

Sea turtles are an iteroparous species, which means that they reproduce several times throughout their lifetime, and the gonads of mature individuals undergo cyclical morphological changes (Miller, 1996; Miller and Limpus, 2003). Before the start of the nesting season, sea turtles build up energy reserves in their foraging areas (Hamann et al., 2003). While spermatogenesis is assumed to be completed before the courtship period (Hamann et al., 2003; Wibbels et al., 1990), follicle maturation continues throughout the nesting season in several sea turtle species, including loggerheads and green sea turtles (*Chelonia mydas*) (Bruno et al., 2025; Miller and Limpus., 2003). Mature females depart from their foraging sites to the nesting area with a complete set of large vitellogenic follicles, as well as numerous follicles of smaller size that will finish their maturation during the nesting season (Bruno et al., 2025; Miller and Limpus, 2003). As other chelonian species, loggerhead sea turtles are known to exhibit follicular hierarchy, which means that follicles in different stages co-occur in the ovaries, of which not all will be ovulated (Bruno et al., 2025; Hamann et al., 2003; Miller and Limpus, 2003). Instead, atretic follicles will stop developing after a certain time and serve as energy deposit for the female (Bruno et al., 2025; Hamann et al., 2003). Mating takes place offshore the nesting area and triggers the successive ovulation in the female (Manire et al., 2008). Over the course of the nesting season, females lay several clutches (~100 eggs) and the number of follicles in the ovaries decreases, while *corpora lutea* as remnants of the ovulated follicles degenerate to *corpora albicantia*, which will remain visible as white ovarian scars after the end of the nesting season (Hamann et al., 2003; Miller and Limpus, 2003). Non-ovulated follicles will be reabsorbed and become atretic (Miller and Limpus, 2003). After the nesting season, the ovaries therefor bear few larger follicles, as well as ovarian scars and atretic follicles (Miller and Limpus, 2003). Due to the high energetic expenditure of the nesting process, female turtles normally do not nest annually, and Mediterranean loggerhead females have an average remigration interval of 2 to 3.5 years (Casale et al., 2018). In this intermediate period of quiescence, the females return to their feeding ground, and their ovaries show no larger follicles, but ovarian scars and atretic follicles

(Miller and Limpus, 2003). The reproductive process is less exhaustive for males, which may return annually to their breeding site to mate (Hamann et al., 2003; Hays et al., 2010), and Mediterranean male loggerhead turtles have an average remigration interval of 1 to 1.8 years (Casale et al., 2018). Their quiescent period may therefore be much shorter, and during this time the testes have a flaccid appearance due to the absence of spermatogenesis (Hamann et al., 2003).

### 1.2.2. Sex ratios and sex determination

Sex ratios are an important parameter to understand population demography and dynamics (Maffucci et al., 2013). Sea turtles have temperature-dependent sex determination (TSD), with warmer incubation temperatures leading to predominantly female offspring (Harry and Williams, 1991; Yntema and Mrosovsky, 1980). In the Mediterranean, incubation temperatures vary widely among loggerhead turtle nesting sites, however an overall female-biased hatchling production is assumed (Casale et al., 2018). Nevertheless, sex ratio seems to be less biased in later life stages (Casale et al., 2006; Casale et al., 2014; Maffucci et al., 2013). Male and female loggerhead turtles do not seem to differ in size (Casale et al., 2018), and the main sexual dimorphism is the tail length in mature individuals, which grows longer in males (Casale et al., 2005). This distinct feature however only starts to develop at a curved carapace length (CCL) of about 70 cm, which is why a precise sex determination for turtles ≤ 75 cm CCL can only be obtained by examination of the gonads during *post-mortem* analysis (Casale et al., 2005). Gonad gross morphology is a common sex determination technique during necropsies (e.g. da Silva Fabrício et al., 2019; Craven et al., 2019, Mihaljević et al., 2024). However, this method is only reliable for loggerheads with CCL > 30 cm, and it is suggested to use histology for sex determination of smaller individuals (Lazar et al., 2008).

### 1.2.3. Size at maturity

The general distinction between different turtle age classes is commonly based on carapace length. However, size and age at maturity vary widely between individuals (Avens et al., 2015; Miller, 1996; Tiwari and Bjorndal, 2000), and classification into different age classes is not standardized in studies on Mediterranean loggerhead sea turtles (Casale et al., 2018). It has been suggested that on average, female sea turtles start reproducing at a size slightly below the average breeding size of their population (Limpus, 1990). In the Mediterranean, the average size of nesting females (weighted for rookery contribution) is considered to be 79.1 cm CCL, and ranges between 66.5 cm and 84.7 cm CCL (Casale et al., 2018). Based on the size at which male tail elongation begins, Casale et al. (2005) assume that male Mediterranean loggerheads reach maturity between 75 and 80 cm CCL. Studies on kemp's ridley sea turtles (*Lepidochelys kempii*) in Texas (USA), green sea turtles (*Chelonia mydas*) in Brazil, and hawksbill sea turtles (*Eretmochelys imbricata*) in Cuba have shown that carapace length is not always a reliable indicator for maturity (Craven et al., 2019; da Silva Fabrício et al., 2019; Pérez et al., 2010), and that juvenile and adult turtles may overlap in carapace length (Craven et al., 2019; Pérez et al., 2010). Validation of maturity based on gonad examination of dead stranded or bycaught individuals is therefore a valuable tool to understand the reproductive biology of a population (da Silva Fabrício et al., 2019; Craven et al., 2019; Ishihara and Kamezaki, 2011).

### 1.3. Informative value of sea turtle strandings

Dead stranded sea turtles can provide valuable information about the population at sea (e.g. Casale et al., 2010; Guarino et al., 2020; Tomás et al., 2008). Depending on their decomposition, they may give an insight into the population structure (da Silva Fabrício et al., 2019; Guarino et al., 2020), foraging ecology (Palmer et al., 2021; Mariani et al., 2023), cause of mortality and anthropogenic impact (Hamas et al., 2020; Mihaljević et al., 2024), in particular related to fisheries (Agabiti et al., 2024; Casale et al., 2010; Marisaldi et al., 2023). However, when and where carcasses are washed ashore depends on a variety of physical and biological factors (Agabiti et al., 2024; Santos et al., 2018), and their detection depends on monitoring effort, for which stranding data must be interpretated with caution (Casale et al., 2010; Mihaljević et al., 2024).

The macroscopic investigation of gonads obtained from dead stranded or bycaught sea turtles to understand their state of maturity has been performed on loggerhead sea turtles from Japan (Ishihara and Kamezaki, 2011), kemp's ridley sea turtles from

Texas (USA) (Craven et al., 2019), green sea turtles from Brazil (da Silva Fabrício et al., 2019) and green and hawksbill turtles from Cuba (Pérez et al., 2010). Lazar et al. (2008) used gonad gross morphology to determine the sex of juvenile loggerhead sea turtles from the Adriatic Sea and central Mediterranean Sea and compared these results with histology. Histological analysis of gonadal tissue allows the most precise analysis of maturity stages and has been used in the mentioned kemp's ridley sea turtles from Texas (USA) (Craven et al., 2019), green sea turtles from Brazil (da Silva Fabrício et al., 2019; Failla et al., 2018), hawksbill turtles from Cuba (Pérez-Bermúdez et al., 2012), and loggerhead sea turtles from Campania (south-western Italy) (Guarino et al., 2020).

### 1.4. Aim of this study

The comparison of different sexing techniques by Lazar et al. (2008) presents the only publication analysing loggerhead sea turtles' gonad tissue in the northern Adriatic Sea, and so far, no study has investigated the gonad development of the species in this area. However, details on the population's demography and reproductive maturity in the northern Adriatic Sea are of high interest to implement effective conservation measures, especially with regard to the recent detection of nests in the Veneto region (Pietroluongo et al., 2023).

Respecting the limitations that data collected from stranded sea turtles inhere, this study aims to:

1) Explore the general demography of loggerhead sea turtles in the northern Adriatic Sea and investigate potential spatiotemporal stranding trends;
2) Analyse the reproductive maturity of stranded individuals based on gonad gross morphology and histology, and explore the relationship with CCL;
3) Explore the sexual dimorphism between male and female turtles regarding the tail elongation.

# 2. Materials and methods

## 2.1. Study area

The Adriatic Sea is an elongated basin in the central Mediterranean Sea (Fig. 2), which is commonly divided into three sub-basins based on distinct oceanographic characteristics: northern, central, and southern Adriatic Sea (Artegiani et al., 1997). The northern Adriatic Sea is the shallowest part, with an average depth of 35 m (Artegiani et al., 1997), and is recognized as important wintering and foraging habitat for adult and juvenile loggerhead sea turtles from the Mediterranean RMU (Casale et al., 2004). Sea water temperature varies strongly between the seasons, and throughout the whole water column (Artegiani et al., 1997).

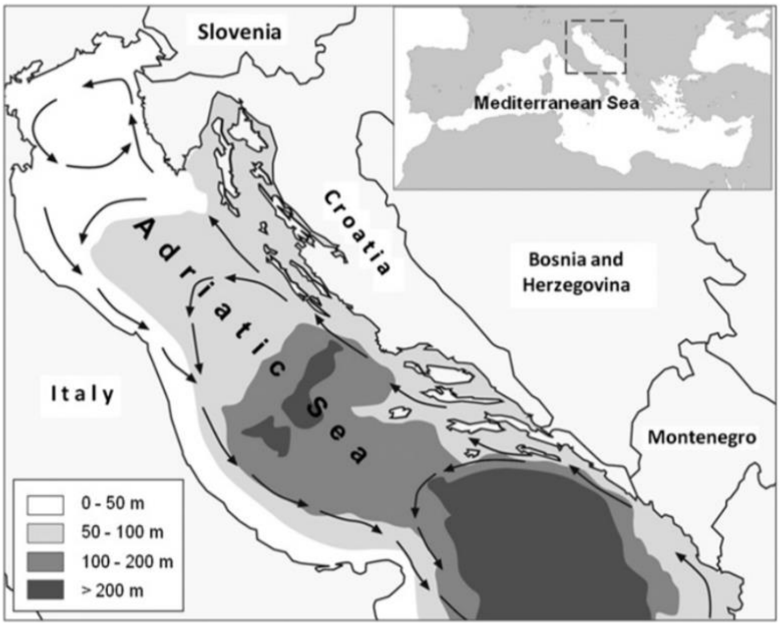



***Figure 2**: Adriatic Sea with bathymetry and directions of prevailing sea currents (Lazar and Gračan, 2011).*

The northern Adriatic Sea is bordered by Croatia and Slovenia on the eastern part, while the north and western part is covered by Italian coastline. The Veneto region constitutes a substantial part of the Italian north-western Adriatic coastline, stretching over a longitude of about 160 km (Ruol et al., 2018).

## 2.2. Sampling and post-mortem investigations

2.2.1. Sampling

Between January 2023 and February 2025, the carcasses of 247 stranded loggerhead sea turtles were reported along the Veneto coastline. The sampling area was divided into 14 zones (Fig. 3). Two carcasses recovered from the Emilia-Romagna region were included, as they were found just behind the border of zone 14. Dead stranded sea turtles were reported to the Italian Coast Guard emergency number 1530 and collected by the Cetacean strandings Emergency Response Team (CERT) of the Department of Comparative Biomedicine and Food Science (BCA), University of Padova (UNIPD), which is part of the regional stranding network *Coordinamento Tartarughe marine del Litorale Veneto* (CTLV).

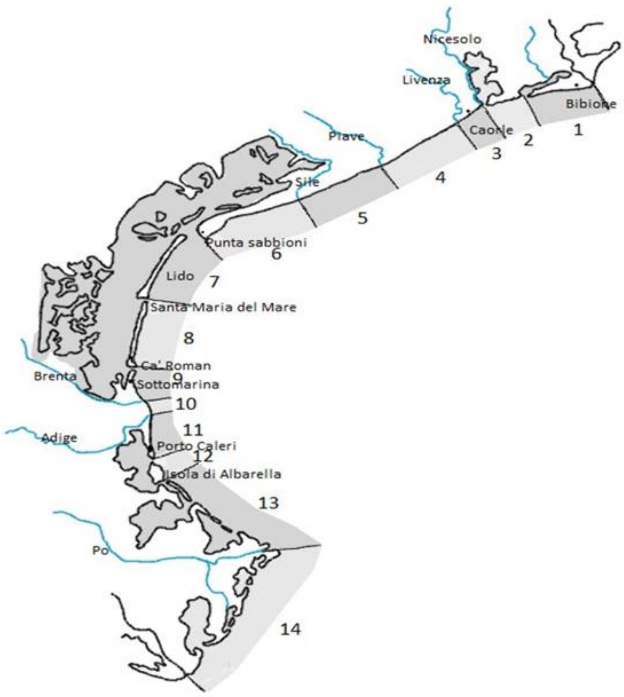



***Figure 3***: *Map of the Veneto coastline divided into stranding zones.*

2.2.2. Post-mortem investigations

Post-mortem investigations (PMIs) were performed at the facilities of the Agripolis campus in Legnaro (BCA-UNIPD). If PMIs were not performed immediately, the carcasses were stored at +5°C or frozen at -20°C, depending on the time scheduled for the analysis. Each individual was identified with a Turtle ID (e.g. T01/23 for the first individual stranded in 2023). Necropsies were performed by trained veterinary pathologists of the BCA with the assistance of trained volunteers and interns, according to the NETCET Standard Protocol for Post-Mortem Examination on Sea Turtles (Poppi and Marchiori, 2013). All data were recorded on a standardized necropsy form (Scheda Necroscopia Tartarughe Marine, CERT-BCA-UNIPD, Appendix A).

Each individual analysed was given a carcass decomposition code (DCC) which determined which kind of analyses could be performed (Table 1).

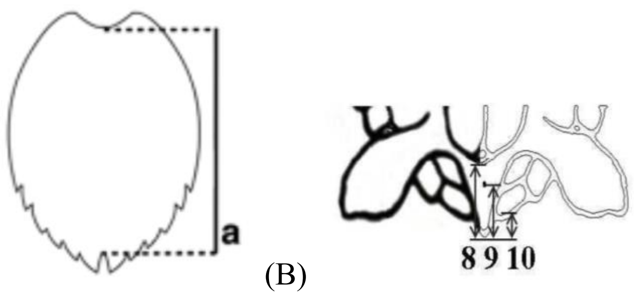
***Table 1**: Overview of DCCs.*

| DCC | Characteristics |
|---|---|
| **1** | fresh carcass |
| **2** | minimal decomposition |
| **3** | moderate decomposition |
| **4** | advanced decomposition |
| **5** | mummified carcass or skeleton remains |

*2.2.2.1. Morphometric measurements*

Morphometric measurements were taken for carcasses with DCC 1 - 5 to the closest 0.5 cm with a flexible tape measure according to Bolten (1999) and Poppi and Marchiori (2013) (Fig. 4):

- CCLmin: Curved carapace length from notch to notch (cm)
- SCLmin: Straight carapace length from notch to notch (cm)
- PT: end of the plastron to the tip of the tail (cm)
- CT: middle of the cloaca to the tip of the tail (cm)
- CaTT: notch between the supracaudal scutes to the tip of the tail (cm)




(A) (B)

***Figure 4**: Morphometric measurements recorded during necropsy. **A**: CCLmin/SCLmin: curved/straight carapace length notch to notch (Bolten, 1999); **B**: PT: plastron-tip of the tail (8); CT: cloaca-tip of the tail (9); CaTT: and carapace-tip of the tail (10) measurements (image from CERT necropsy form).*

The measurement CCLmin (hereafter CCL) describing the turtle size, is different to CCLn-t (curved carapace length from notch to tip), which is used in other studies (e.g. Casale et al., 2005; Baldi et al., 2023a, Baldi et al., 2023b; Marisaldi et al., 2023). However, other studies do not mention which of the two measurements were used, and as the difference between the two measurements is small, they are treated as identical in the present study. Based on the CCL, individuals were classified into three age classes representing different life stages, adapted from Casale et al. (2005), Margaritoulis et al. (2003) and Lai et al. (2015):

- Juvenile: ≤ 40 cm
- Sub-adult: 41 - 70 cm
- Adult: > 70 cm

*2.2.2.2. Analysis of reproductive organs*

Depending on the state of decomposition, the gonads were observed to assess individuals' sex and level of gonad development. Sex was determined based on the presence of testes and epididymides for male turtles, or ovaries and oviducts for female turtles. For the present study, the level of gonad maturity was assessed for individuals collected in 2024 and 2025. Follicle diameter and testes length and width were measured with a ruler. A first macroscopic assessment of gonad maturity was performed during the necropsy, classifying individuals as prepubescent, pubescent or mature, according to Miller and Limpus, 2003 (see paragraph 2.3.1.). Gonads were photographed together with a size reference for subsequent more detailed analysis. Based on the DCC (1 - 4), gonads were sampled for histology by taking 1 $cm^3$ of the gonadal tissue. Samples were preserved in formaldehyde (corresponding to 10% formalin solution in phosphate buffered solution) in plastic containers (see Paragraph 2.3.2).

### 2.3. Assessment of gonad maturity

2.3.1. Gonad gross morphology (macroscopic assessment)

If not recorded during the necropsy, follicle and testes size were measured based on the available gonad pictures with size reference using the software ImageJ (Version 1.53). Using the available pictures, the macroscopic maturity assessment recorded during necropsy was re-evaluated following Miller and Limpus (2003), Hamann et al., (2003) and Pérez-Bermúdez et al. (2012), according to the characteristics listed in Table 2 for females and Table 3 for males. Based on the number of large follicles and the presence of ovarian scars, mature females were further classified as "preparing to breed", "in regression" after nesting season and "in quiescence" (Miller and Limpus, 2003). Since the succession between regression and quiescence in males is assumed to be more rapid due to the potential for annual breeding, and the macroscopic differences seem to be minor, mature males were only classified as "preparing to breed" and "in quiescence" (Miller and Limpus, 2003). Besides the macroscopic gonad appearance also the stranding month was taken into consideration, based on the known timings of the reproductive cycle in Mediterranean loggerhead sea turtles (peak mating period: April – May, peak nesting period: June – July; Casale et al., 2018).

***Table 2**: Characteristics of different maturity stages in female loggerhead sea turtles, adapted from Miller and Limpus (2003) and Pérez-Bermúdez et al. (2012).*

| **Maturity stage** | **Characteristics** |
|---|---|
| **Post-hatchling/ Prepubescent** | ovary: compact stroma with uniform previtellogenic follicles (< 0.1 mm)<br>oviduct: white, straight, < 2 mm flattened diameter |
| **Pubescent** | ovary: enlarged stroma, increased distance between previtellogenic follicles (mostly uniform size, up to ca. 2 mm); atretic follicles may be present<br>oviduct: partly convoluted, < 15 mm flattened diameter |
| **Mature** | ovary: stroma strongly expanded, appearing like a curtain with follicles of different sizes, including previtellogenic follicles (1 - 3 mm), vitellogenic follicles (4 - 25 mm) and mature follicles (> 25 mm), presence of atretic follicles, *corpora lutea* or *corpora albicantia*<br>oviduct: pink, strongly convoluted, > 15 mm flattened diameter |
| **Mature, in quiescence** | ovary: absence of large, yolk-filled follicles; presence of *corpora albicantia* and scattered atretic follicles<br>oviduct: flaccid, very convoluted |

*Table 3: Characteristics of different maturity stages in male loggerhead sea turtles, adapted from Miller and Limpus (2003) and Hamann et al. (2003)*

| Maturity stage | Characteristics |
|---|---|
| **Post-hatchling/ Prepubescent** | testis: solid, smooth structure with small tubules (visible with 10 x magnification); pale salmon-coloured<br>epididymis: attached to the body wall next to the testes; appears as thickened tissue mass; less visible in very young animals |
| **Pubescent** | testis: ellipsoidal shape; increase in size<br>epididymis: raised from the body wall forming a distinct ridge |
| **Mature, preparing to breed** | testis: increased size; cylindrical shape; evident and distended seminiferous tubules;<br>epididymis: pendulous and distinct from the body wall; white coils turgid and enlarged |
| **Mature, in quiescence** | testis: cylindrical shape, but flaccid appearance; seminiferous tubules pale white to salmon<br>epididymis: pendulous from the body wall; not turgid; pale white colour |

The detection of atretic follicles and ovarian scars in female ovaries, as well as the color of the gonadal structures were strongly subject to the carcass decomposition. An additional indicator considered for female maturity was the presence of flipper tags that are applied at nesting beaches and provide information on the individuals nesting history (Craven et al., 2019).

### 2.3.2. Histology (microscopic assessment)

The gonad samples (ovaries and testes) were processed following standard procedures for histology. Samples were fixed in 4% formaldehyde (corresponding to 10% formalin solution in phosphate buffered solution) to stop autolytic processes. Following, the sampled were trimmed under an aspiration hood (trimming path 2200, BIOPTICA s.p.a.) to obtain a suitable size for the histological cassettes. Trimmed sections were dehydrated using an automatic tissue processer

(Histo-pro200 Tissue Processor, HISTO LINE LABORATORIES S.r.l.) and embedded in paraffin. Paraffined samples were cut into 4 µm sections with a retractable rotary microtome (RM2145, LEICA), treated with cold water and at 45 to 48°C to reduce potential folds, and placed onto glass slides, which were first left to dry at room temperature and following in the oven at 60°C for 30 minutes. Paraffin was removed before staining by embedding the samples successively into xylene, alcohol and distilled water using an Austostainer from the brand LEICA. Stains used were haematoxylin and eosin and mason's trichome, which were applied using the same Autostainer.

The prepared slides were observed under an electron microscope. Classification of follicular stages for females followed Pérez-Bermúdez et al. (2012), who described the ovarian follicular development in hawksbill turtles, and which has been used in the histological assessment on green sea turtles (da Silva Fabrício et al., 2012), as well as loggerhead sea turtles (Guarino et al., 2020). Histologically, the differentiation between prepubescent, pubescent and adult ovarian tissue is mainly characterized by the augmenting oocyte diameter, the development of the surrounding follicular cells, theca and zona pellucida, the position and appearance of the nucleus, and the presence and magnitude of yolk platelets and lipids in the ooplasm (Pérez-Bermúdez et al., 2012). A detailed list of the criteria used as described by Pérez-Bermúdez et al. (2012) can be found in Table 4.

***Table 4:*** *Stages of ovarian follicular development, adapted from Pérez-Bermúdez et al., 2012.*

| Maturity level | Follicular stage | Characteristics |
|---|---|---|
| **prepubescent, pubescent, adult** | **I** | **Folliculogenesis**: oocyte partly surrounded by squamous follicular cells inside the germinal bed; nucleus spherical and in the cell center with one nucleolus; ooplasm with smooth and homogenous surface; oocytes of relatively homogenous size |
| | **II** | **Beginning of previtellogenesis**: oocyte still located in germinal bed and with increased |

| | | |
|---|---|---|
| | | diameter, completely surrounded by follicular cells (granulosa); nucleus grows according to the increase in oocyte diameter, spherical shape and located in the cell center, containing nucleoli of variable size in its periphery, as well as lampbrush chromosomes; ooplasm granulated and translucent; diameter of oocytes more variable |
| | **III** | **Previtellogenesis**: oocyte diameter increases; oocyte outside the germinal bed, surrounded by follicular cells and a theca out of fibroblasts; formation of a thin zona pellucida the periphery; nucleus spherical and eccentric, with an increased number of nucleoli at its periphery, lampbrush chromosomes still visible but less evident; ooplasm granulated, containing lipid vacuoles that form a lipid band and underlying lipid region in the follicle periphery and around the nucleus |
| **pubescent, adult** | **IV** | **End of previtellogenesis**: oocyte increases further in diameter, surrounded by cuboidal to flattened follicular cells and a differentiated theca (internal theca rich in fibroblasts and external theca more fibrous); zona pellucida of increased size and differentiated into a zona radiata and a hyaline band; position and appearance of nucleus unchanged, but no longer presence of lampbrush chromosomes; ooplasm is occupied by larger lipid vacuoles and smaller lipid droplets |
| **adult (breeding)** | **V** | **Beginning of vitellogenesis**: oocyte of increasing diameter; surrounding zona pellucida, granulosa and theca show similar appearance to the previous stage; nucleus spherical and located at the oocytes' animal pole; ooplasm contains yolk platelets of various sizes, some with visible |

| | | |
|---|---|---|
| | | granulated and pigmented material, as well as yolk platelets and lipid droplets |
| | **VI** | **Vitellogenesis**: oocyte of increasing diameter; theca increases in dimension; nucleus of similar morphology and position, but with reduced number and size homogeneity of peripheral nucleoli; ooplasm with higher density of spherical yolk platelets of different size |
| | **VII** | **Vitellogenesis**: oocyte of increasing diameter; granulosa and theca remain similar in appearance; zona pellucida less evident, position and shape of the nucleus and number of nucleoli persist, spherical yolk platelets and lipid droplets increase strongly in diameter and are located close to the zona pellucida, reduced prevalence of smaller platelets |
| | **VIII** | **End of vitellogenesis:** preovulatory condition, characteristics of nucleus, yolk platelets, lipid droplets, granulosa, zona pellucida, and theca remain similar, while the diameter increases |

The assessment of male gonad development stages was based on the appearance and dimension of the seminiferous tubules and interstitial tissue, and the stage of spermatogenesis. For the present study, assessment followed the criteria developed by da Silva Fabrício et al., (2012) (Table 5).

*Table 5: Testes characteristics in different development stages according to da Silva Fabrício et al., 2012.*

| Maturity level | Characteristics |
|---|---|
| **prepubescent** | seminiferous tubules with small lumen, germinal epithelium with Sertoli cells and a single row of spermatogonia, difficult differentiation from surrounding, well-developed interstitial tissue |
| **pubescent** | seminiferous tubules with increased diameter and luminal space, containing spermatogonia and spermatocytes, reduction of interstitial tissue |
| **adult** **(active spermatogenesis)** | seminiferous tubules with increased diameter, germinal epithelium with several cell layers, spermatozoa abundant in the lumen |

### 2.4. Data analysis

All relevant information from the necropsy forms, as well as the results from the histological analysis were entered into an Excel database. All analyses were performed using the software R (version 4.3.1) within the RStudio environment (version 2023.09.0+463) (R Core Team, 2023).

Since in 2025 only the months January and February were sampled, any analyses regarding temporal aspects only included the individuals stranded in 2023 and 2024. Information on gonad development was only available for the years 2024 and 2025, for which all analyses of this aspect only included data collected between January 2024 and February 2025. Consequently, temporal trends in gonad development were only analyzed for individuals stranded in 2024.

Descriptive analysis and data visualization were performed using the packages "DescTools" (Signorell et al., 2025), "ggplot2" (Wickham et al., 2025), "RColorBrewer" (Neuwirth, 2023) and "leaflet" (Cheng et al., 2025). For data wrangling, the packages "dplyr" (Wickham et al., 2023) and "tidyr" (Wickham et al., 2024) were used. Chi-squared tests were used to investigate the dependency of categorical variables using the "car" package (Fox and Weisenberg, 2019). In case of significance, the significant groups were identified by comparing their standardized residuals to the critical value, which was calculated with the qnorm() function in R based on a Bonferroni-adjusted alpha level to account for multiple comparisons (alpha_adj = alpha/no. of comparisons). A Shapiro-Wilk test was used to test variables for normality, and homogeneity of variance was tested with a Levene's test ("car" package, Fox and Weisenberg, 2019). A one-way ANOVA was performed to examine differences in CCL among the stranding seasons and zones, and in case of significance a post-hoc Tukey's HSD test was performed to identify significant groups. The comparison of CCL across individuals with different stages of gonad development was done using a Kruskal-Wallis test and post-hoc analysis was performed with a Dunn's test to identify significant groups. Comparisons of male and female CCL were analysed with a Students t-test.

The relationship between CCL and Sex and the tail measurements PT, CTL and CaTT was investigated using Generalized Linear Models (GLMs) and Generalized Additive Models (GAMs) with a "gamma" family and a "log" link function. GAMs were generated using the "mgcv" package in R (Wood, 2017). In the GLMs, the explanatory variables CCL and Sex were tested for interactions, which were removed if they were not significant. In that case a reduced model with only main effects of CCL and Sex was computed. The response variable CaTT had a continuous, right-skewed distribution including negative values. To allow the usage of the "gamma" family with "log" link function, the response variable was shifted by +5 and the models were calculated with this shifted value called "CaTT_shift". For the model predictions and interpretation, this value was shifted back to the original scale. In the GAMs, the linearity of smooth terms was evaluated based on the effective degrees of freedom (EDF). The goodness of fit criteria used for GLMs were the AIC and the percentage of deviance explained by the model ((total deviance – remaining deviance after model fit) / total deviance) * 100). GAM fit was assessed by the adjusted $R^2$, the percentage of deviance explained by the model, and the AIC.

Based on the GAM models, the CCL at which males and females begin to show significantly different tail measurements was calculated. For this purpose, the difference between male and female predicted values was calculated, as well as their upper and lower 95% confidence interval. The point at which males show a significantly higher tail measurement than females was defined as the first value at which the lower confidence interval was larger than 0.

The significance level for all statistical analyses was defined as $\alpha = 0.05$.

# 3. Results

## 3.1. General stranding information

Between January 2023 and February 2025, a total of 247 loggerhead sea turtles were found stranded along the Veneto coastline. One individual in 2023 was not recovered, which led to 246 analyzed carcasses (133 in 2023, 111 in 2024 and 2 in 2025).

### 3.1.1. Spatiotemporal stranding distribution

In 2023 and 2024, the highest number of strandings were recorded in the summer months (64.8%, n = 158/244, followed by autumn (19.7%, n = 48/244), spring (12.3%, n = 30/244) and winter (3.3%, n = 8/244) (Fig. 5). The seasonal distribution of stranding events was different between the years, with stranding frequency being significantly higher in winter and spring in 2024 than in 2023 (Chi-squared test, $x^2 = 19.54$, df = 3, $p < 0.001$).

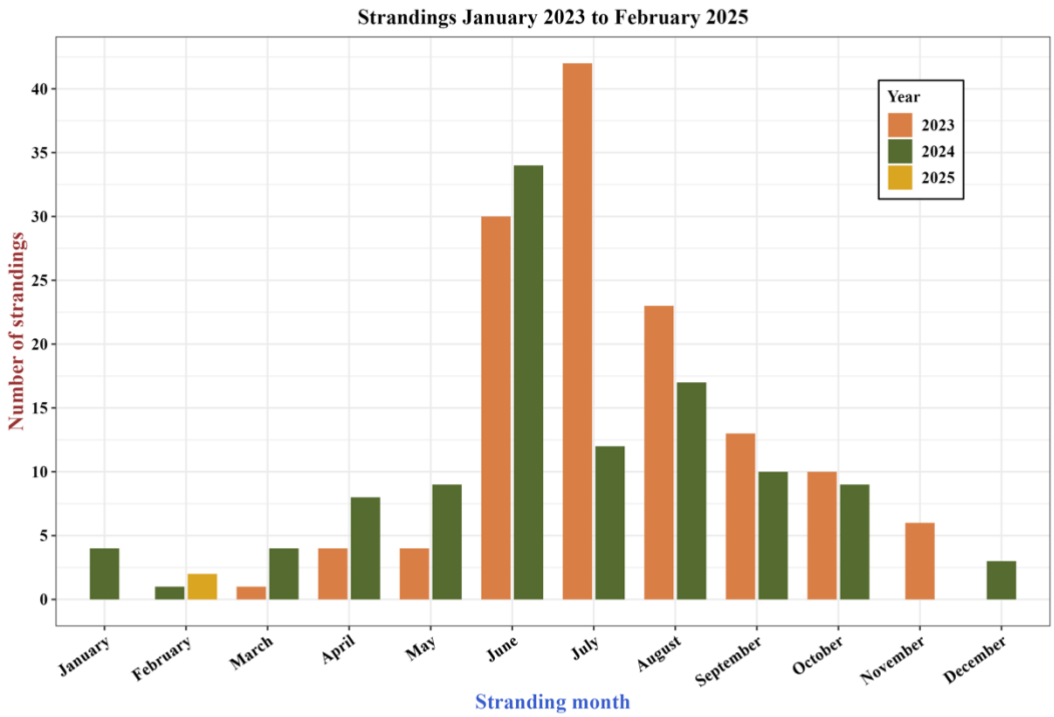



***Figure 5**: Monthly frequency of C. caretta strandings along the Veneto coastline in the sampling period January 2023 to February 2025.*

In the period January 2023 to February 2025 the highest number of strandings was recorded in zone 7 (21.5%, n = 53/246), followed by zone 6 (12.2%, n = 30/246), 5 and 1 (both 9.8%, n = 24/246) (Fig. 6). Fewest stranding events occurred in zone

10 (0.8%, n = 2/246), 12 (1.2%, n = 3/246) and 2 (2.0%, n = 5/246). There was no significant difference in the distribution of strandings along the Veneto coastline between 2023 and 2024 (Chi-squared test, $x^2 = 15.531$, df = 13, p = 0.2754).

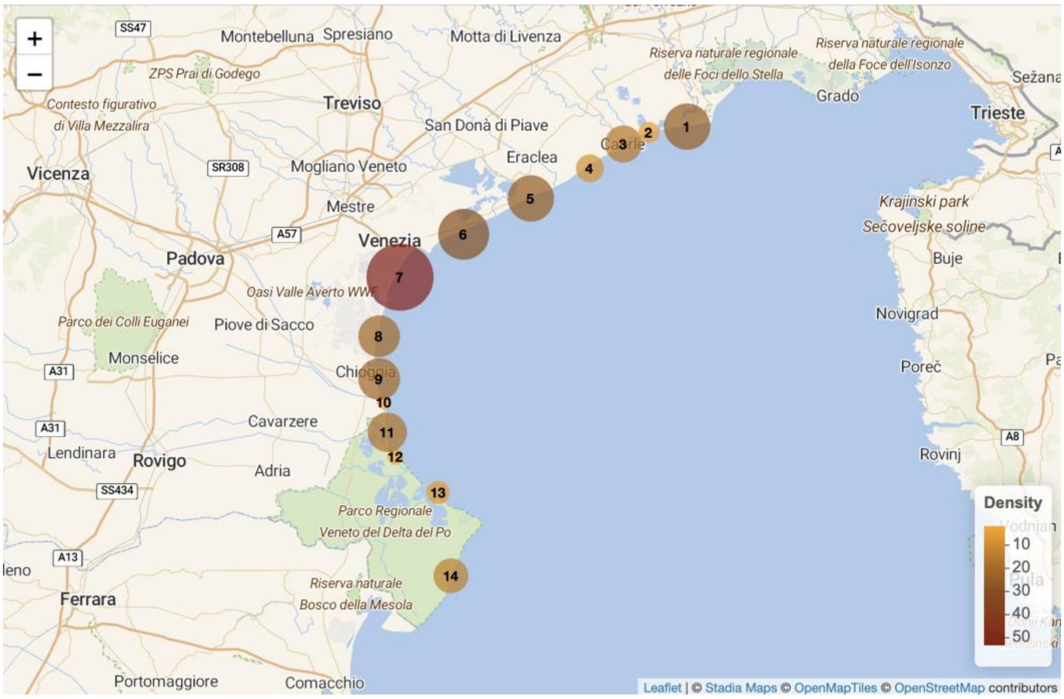



***Figure 6**: Spatial distribution of C. caretta along the Veneto coastline in the sampling period January 2023 to February 2025.*

3.1.2. Size and sex

CCL of stranded individuals ranged between 21 cm to 92 cm, with a mean value of 58.15 ± 15.49 cm (Fig. 7). Most individuals were classified as sub-adults (61%, n = 150/246), followed by adults (22%, n = 54/246), while smaller juvenile turtles ≤ 40 cm were less frequent (13.4%, n = 33/246). For 9 individuals (3.7%) the age class could not be determined due to broken or incomplete carapace. The most frequent CCL measured was 70 cm (6.9%, n = 17/246).

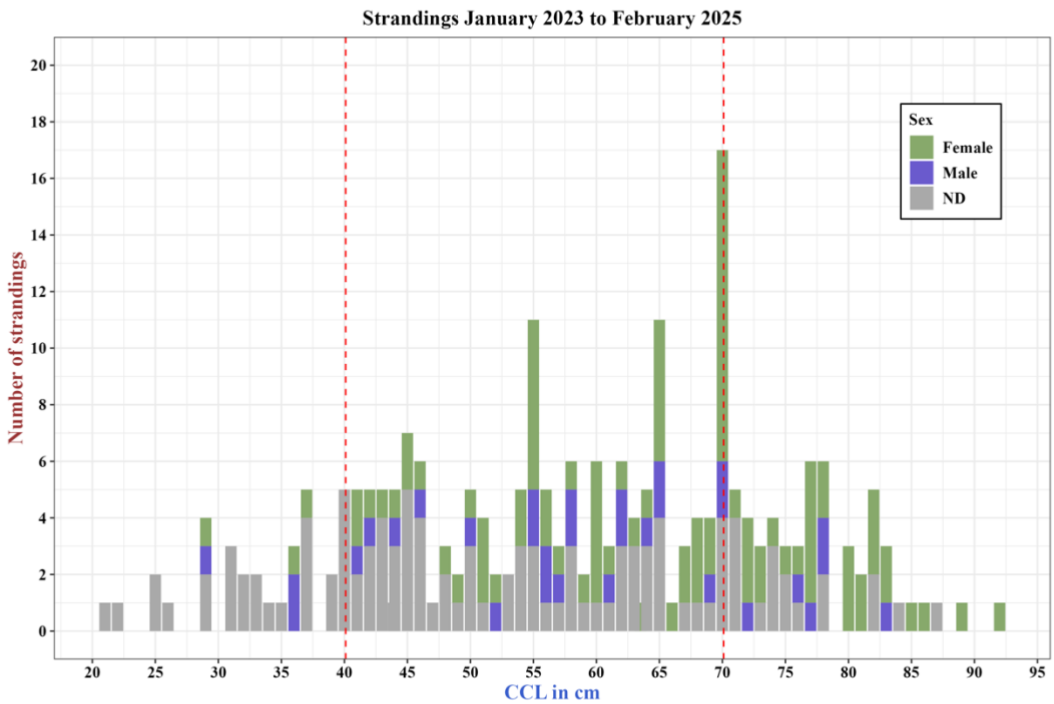



***Figure 7:*** *Curved carapace length of stranded C. caretta January 2023 to February 2025 per sex. Dashed lines represent thresholds for age classes at ≤ 40 cm and > 70 cm CCL.*

Sex was determined in 51.2% animals (n = 126/246), of which 75.4% were females (n = 95/126) and 24.6% were males (n = 31/126), leading to a sex ratio of 3:1 (F:M) (Fig. 7). This sex ratio was significantly different from a 1:1 sex ratio (Chi-squared test, $x^2 = 32.508$, df = 1, $p < 0.001$). Females had a significantly larger CCL than males (Two Sample t-test; t = 2.113, df = 124, p-value = 0.0366), with a mean of 64.64 ± 13.2 cm in contrast to 58.81 ± 13.77 cm in males. There was no dependency between the stranding zone and the sex of the individuals (Chi-squared test, $x^2 =$ 12.926, df = 12, p = 0.3744), and the sex of the turtles was also independent from the season for turtles stranded in 2023 and 2024 (Chi-squared test, $x^2 = 0.75346$, df = 3, p-value = 0.8606). There was no significant difference in individuals' CCL between the stranding zones (ANOVA, F = 0.968, p = 0.484). However, in 2023 and 2024, there was a significant difference in CCL among the seasons (ANOVA, F = 4.003, $p < 0.01$), and a Tukey's HSD test revealed that this difference was significant between summer, where on average the highest CCL values were measured (60.3 ± 13.9 cm), and spring, which was the season with the smallest stranded turtles (50.2 ± 20.1 cm) (p = 0.005, 95% CI [2.34, 17.96]; Fig. 8).

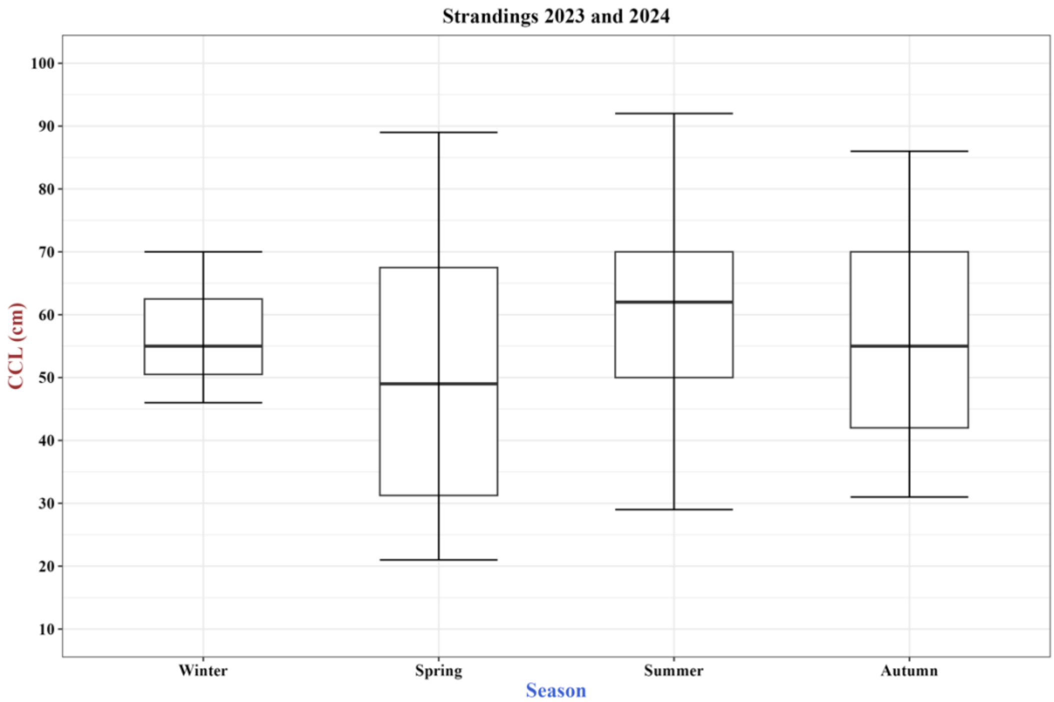



***Figure 8****: Distribution of CCL over stranding seasons in 2023 and 2024.*

3.1.3. Condition of the carcasses

The majority of the carcasses was in a state of advanced decomposition (DCC 4; 60.6%, n = 149/246) or mummified or skeletal remains (DCC 5; 25.6%, n = 63). 11.4% were moderately decomposed (DCC 3; n = 28/246), 1.2% only showed minimal decomposition (DCC 2; n = 3/246), and 0.8% of the carcasses were fresh at the time of necropsy (DCC 1; n = 2/246).

**3.2. Assessment of gonad maturity**

The assessment of gonad maturity was performed for individuals stranded between January 2024 and February 2025, if the DCC was allowing it. Out of 113 individuals stranded in this period, sex was determined for 68 (60.2%). Of those, 72.1% were females (n = 49/68), and 27.9% were males (n = 19/68), leading to a sex ratio of 2.6:1. This sex ratio was slightly less female-biased than the complete sample including all animals stranded from January 2023 to February 2025, but was nevertheless significantly different from a 1:1 sex ratio (Chi-squared test, $x^2$ = 13.235, df = 1, $p < 0.001$).

3.2.1. Gonad gross morphology (macroscopic assessment)

The level of gonad development was determined macroscopically for 59 out of 113 individuals (52.21%; 58 in 2024 and 1 in 2025). Gonads of 27 individuals had been photographed during necropsy, which allowed a more detailed analysis.

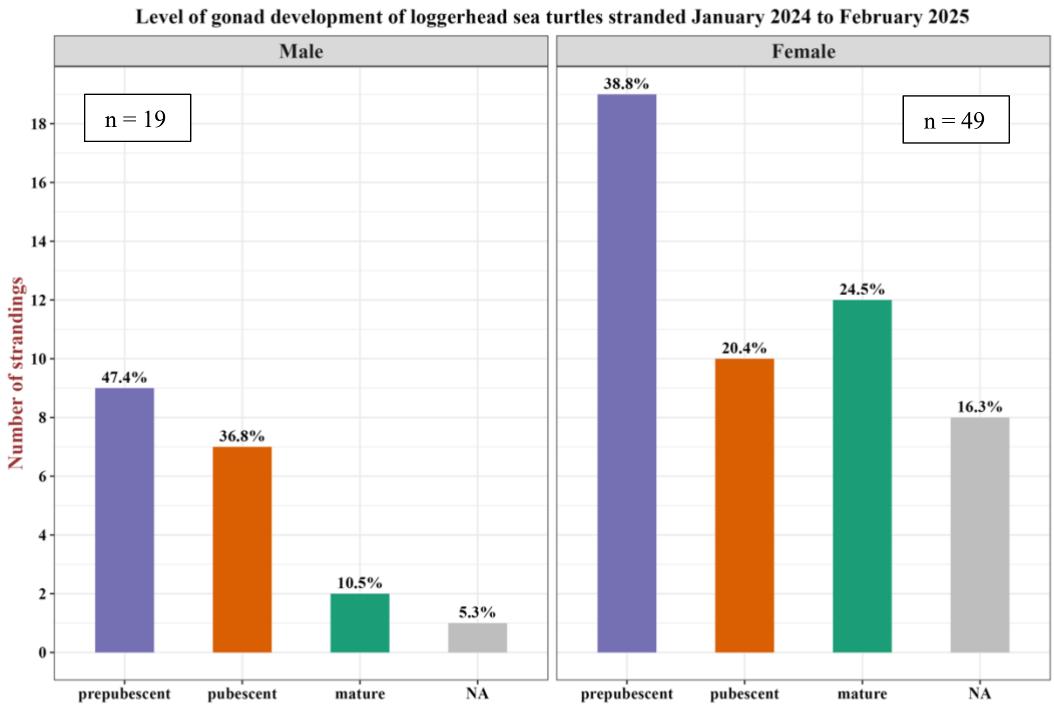



***Figure 9:*** *Level of gonad development in 68 individuals sampled between January 2024 and February 2025.*

Out of 19 turtles identified as male, 9 showed immature gonads in prepubescent development stage (47.4%), 7 had immature gonads in pubescent development stage (36.8%), and two individuals were assessed as mature (10.5%) (Fig. 9). In the 49 females, 19 individuals had prepubescent gonads (38.8%), 10 had pubescent gonads (20.4%), and 12 individuals showed mature gonads (24.5%). For 9 out of 68 individuals with determined sex (13.2%), the gonad development could not be assessed. Males and females did not show a significant difference in gonad development level (Chi-squared test, $x^2$ =2.6857, df = 2, p-value = 0.2611).

*3.2.1.1. Females*

In prepubescent females, the ovaries were loosely attached to the body wall, showed a pale-pink color in animals with a fresh DCC, and contained uncounted previtellogenic follicles of < 1 mm diameter (Fig. 10). The oviduct was straight and pale-pink to white and connected to the ovaries on both sides by a sparsely vascularized mesotubarium.

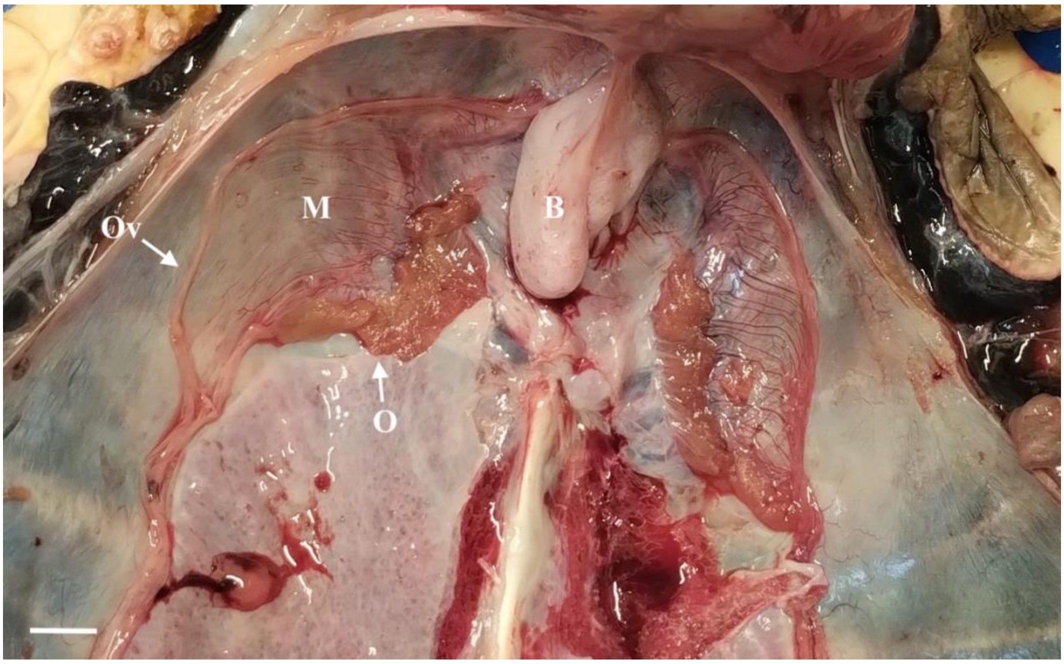



***Figure 10:*** *Ovaries (O) and oviducts (Ov) of prepubescent female T01/25. The oviducts are connected to the ovaries by the mesotubarium (M). B = urinary bladder. Size bar = 1cm.*

Pubescent females showed an expanded ovarian stroma of dark red color. Previtellogenic follicles were measured in 6 individuals and ranged between 1 to 3 mm, however their visibility was highly compromised due to DCC (Fig. 11). Atretic follicles were also not detectable in this development stage due to DCC.

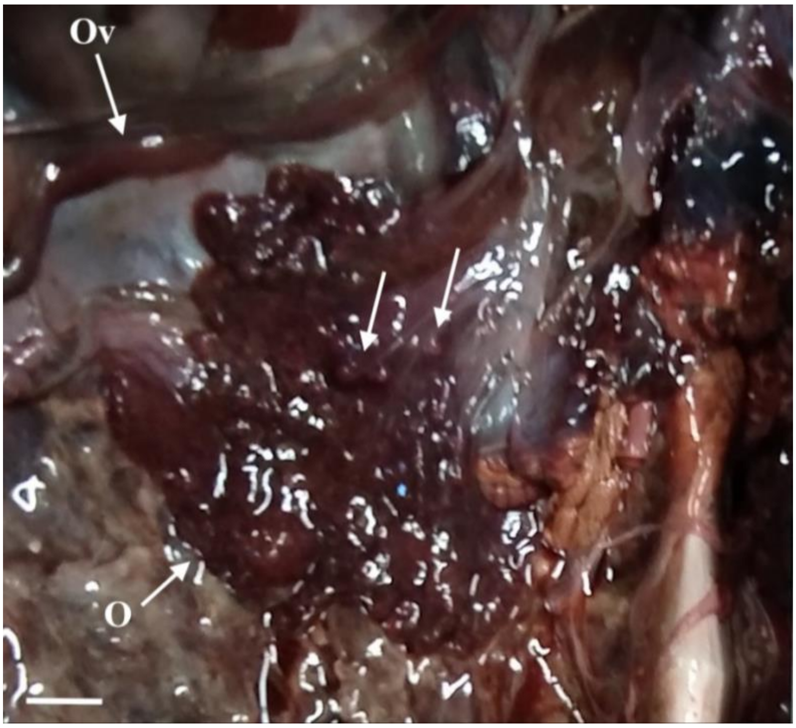



***Figure 11****: Ovary (O) and oviduct (Ov) of pubescent female T16/24. Small arrows = previtellogenic follicles. Size bar = 1cm.*

Mature females showed ovaries with strongly expanded stromata that were hanging free in the coelomic cavity and bore follicles in different developmental stages. The oviducts were long, well developed and muscular, and either convoluted or straightened and flattened (Fig. 12).


***Figure 12:*** *Ovaries and oviduct of mature female T17/24 stranded in April 2024, preparing to breed. O=ovary; Ov=oviduct; M=mesotubarium; V=vitellogenic follicle. Size bar = 5cm.*

The gonads of mature females were found in three different reproductive states: preparing to breed, regressing from previous nesting period, or quiescent (Tab. 6).

Three mature females which stranded in January, April and May 2024 (individuals T03/24, T17/24, T20/24), showed ovaries with follicles of variable size, including numerous larger vitellogenic follicles with a maximum diameter of 1.5 cm, 3 cm and 1.6 cm. Ovarian scars and atretic follicles were not detected. The oviducts were well-developed, muscular and convoluted, and both oviducts and ovaries were well-vascularized (Fig. 12). These individuals were assessed to be preparing to breed in the upcoming nesting season.

Five females with gonads regressing after nesting season were found between May and October 2024. The ovaries contained fewer large follicles than seen in individuals preparing to breed, numerous ovarian scars and atretic follicles. In the case of individual T104/24 an external metal tag on the front flipper revealed that this female had nested in July 2024 in Kyparissia Bay, Greece, where it was first tagged (ARCHELON, personal communication 2024). This information confirmed the regressing character of the individuals' gonads, which showed few follicles in different developmental stages, ovarian scars and atretic follicles (Fig. 13).

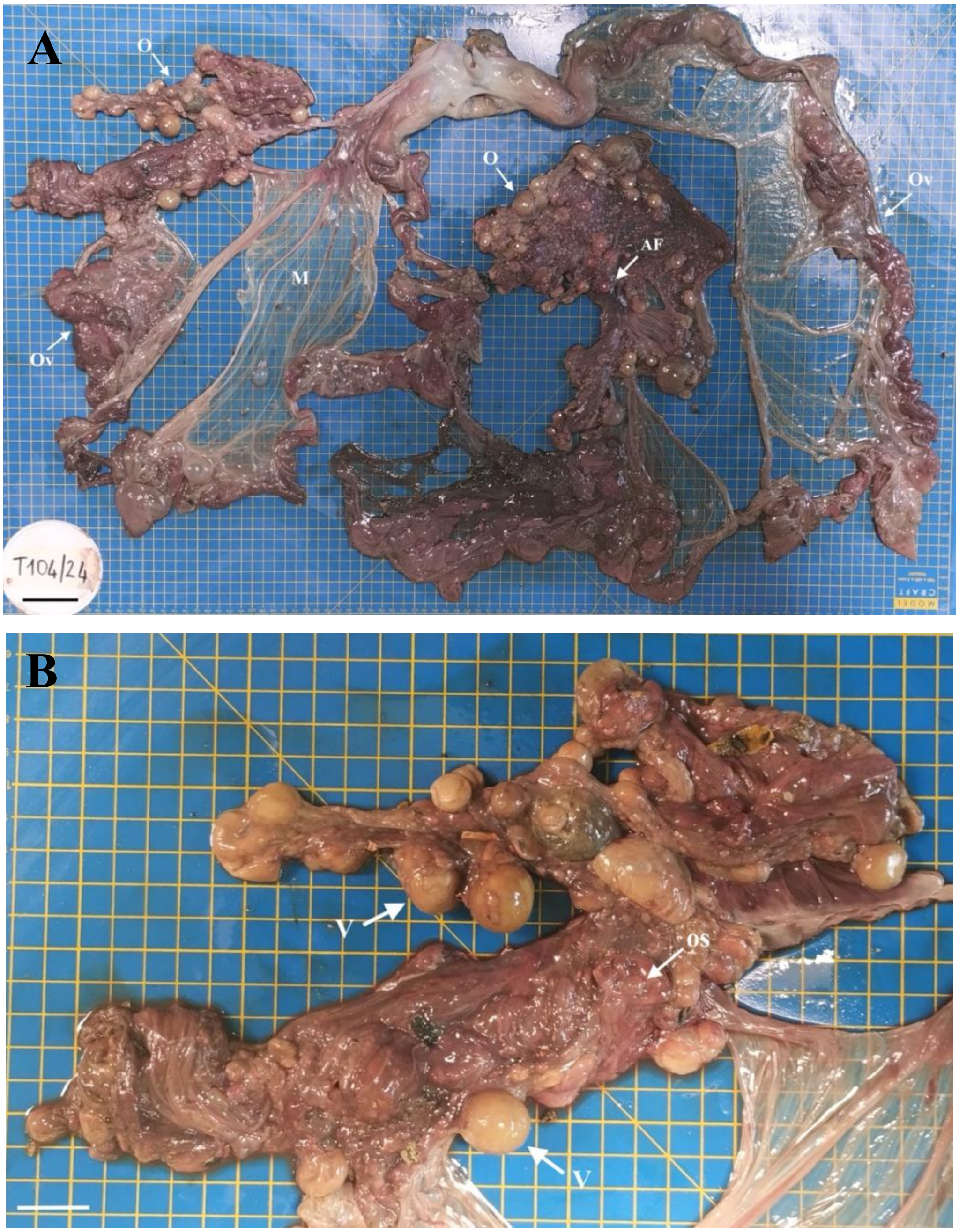



***Figure 13: A****: Ovaries and oviducts of mature female T104/24, in regression after nesting season. O=ovary; Ov=oviduct; AF = atretic follicle; M=mesotubarium. Size bar = 5mm.* ***B****: ovary close-up with vitellogenic follicles (V) and ovarian scars (os). Size bar = 2cm.*

One female found stranded in October 2024 was assessed to be "mature, in quiescence" (T93/24). This individual had an expanded ovarian stroma with few small follicles of ca. 0.5 cm diameter, as well as numerous ovarian scars. The oviducts were long and muscular, but flattened (Fig. 14).

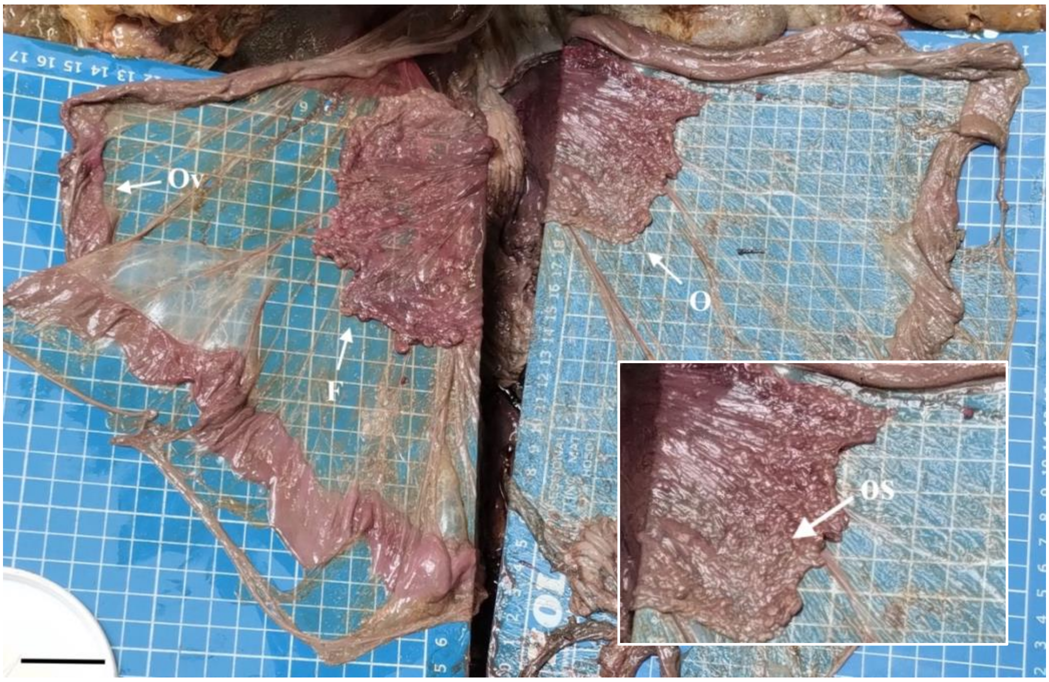



***Figure 14:*** *Ovaries (O) with small follicles (F) and oviducts (Ov) of mature female T93/24 in quiescence. Inlet: close-up of the ovary displaying ovarian scars (os). Size bar = 3cm.*

***Table 6:*** *Summary of the reproductive history of mature females.*

| Turtle ID | Sex | Stranding month | Gonad characteristics | Interpretation |
|---|---|---|---|---|
| **T03/24** | F | January | oviduct thick and strongly convoluted, follicles in different developmental stages, follicle diameter ca. 0.9 - 1.5 cm | mature, preparing to breed |
| **T17/24** | F | April | oviduct thick and strongly convoluted, follicles in different developmental stages, follicle diameter ca. 1 – 3 cm | mature, preparing to breed |
| **T20/24** | F | May | oviduct thick and strongly convoluted, follicles in different developmental stages, follicle diameter ca. 0.3 - 1.6 cm | mature, preparing to breed |
| **T23/24** | F | May | 12 larger follicles (diameter ca. 1.7 - 2 cm), numerous ovarian scars, oviduct fully developed but flaccid | mature, in regression |
| **T59/24** | F | June | 20 larger follicles (diameter ca. 1.8 - 2 cm) and several smaller follicles, including atretic follicles, numerous ovarian scars | mature, in regression |

| | | | | |
|---|---|---|---|---|
| **T84/24** | F | August | several follicles of different sizes, atretic follicles and ovarian scars not visible, oviduct fully developed but flattened | mature, in regression |
| **T93/24** | F | September | oviduct well-developed, but flaccid and little convoluted, expanded ovarian stroma with 12 small follicles (diameter ca. 0.5 cm), | mature, in quiescence |
| **T103/24** | F | October | 16 larger follicles (diameter ca. 1.2 – 1.6 cm) of which some atretic, presence of ovarian scars compromised by DCC | mature, in regression |
| **T104/24** | F | October | presence of follicles of different sizes (diameter ca. 1 - 2 cm), numerous ovarian scars and atretic follicles, oviduct little convoluted | mature, in regression |

*3.2.1.2. Males*

In prepubescent males, the testes were thin structures attached to the dorsal body wall, with a characteristic pink or orange color and a flat and even surface (Fig. 15). The epididymides were not evident at this early stage of development.

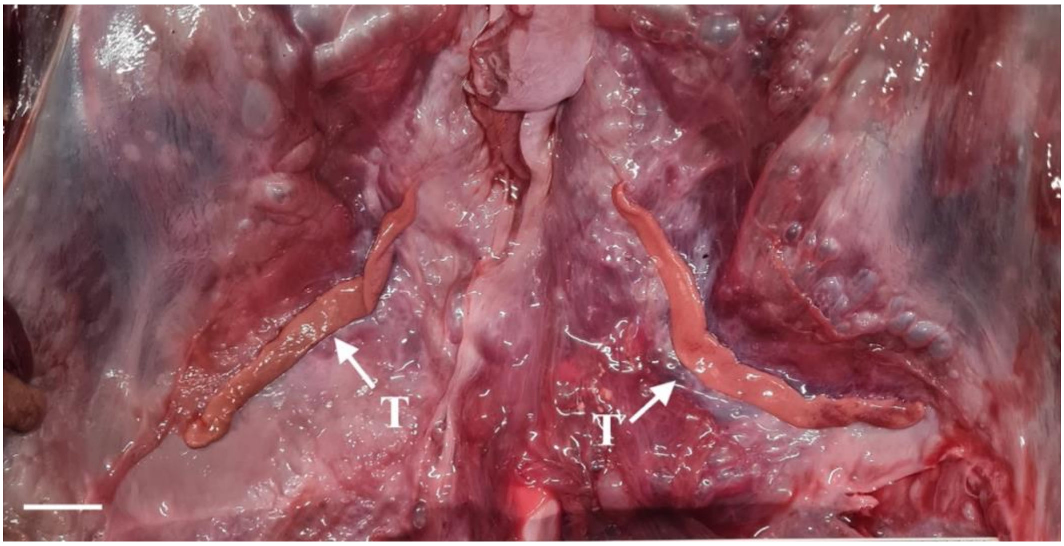



***Figure 15:*** *Testes (T) of prepubescent male T13/24. Size bar = 1cm.*

With advanced development, the testes in pubescent males elongated, thickened and showed a dark color, still attached to the dorsal body wall. In one pubescent male also the epididymides were distinguishable as a dark, thickened mass next to the testes (Fig. 16).


***Figure 16:*** *Testes (T) and epididymides (E) of pubescent male T26/24. Ruler in cm (left side).*

In adult males, the differentiation of the male reproductive organs into testes and epididymides was well visible (Fig. 17). The testes were thickened structures of a long, but irregular shape, with the rounded tips pointing outwards the body cavity. They had a dark or pale-pink color, and seminiferous tubules were not macroscopically visible. The epididymides were attached dorsolaterally to the testes, leading to the *vas deferens*, and showed a of dark (T46/24) or whiteish color (T41/24).

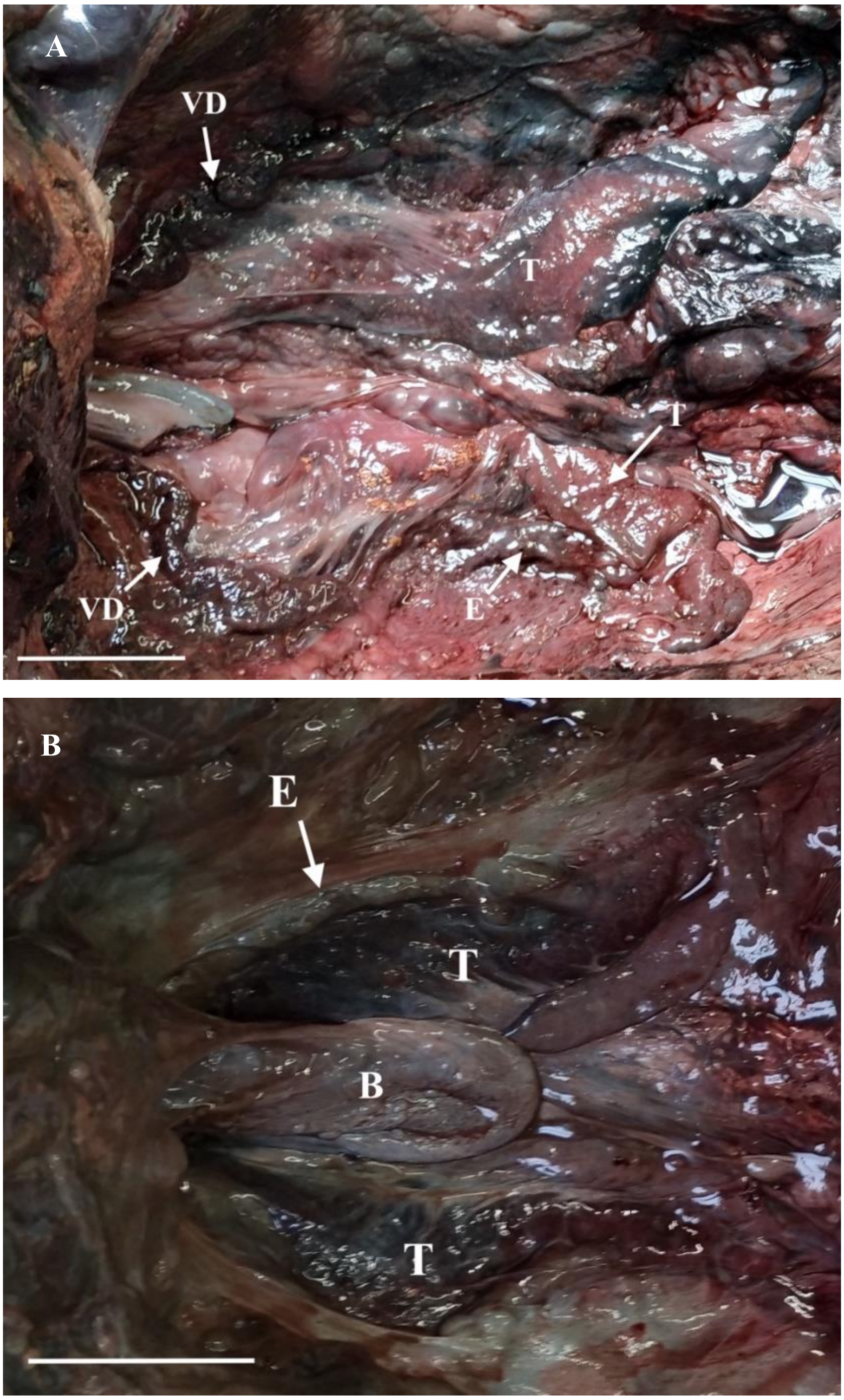



***Figure 17:*** *Gonads of two mature males stranded in June.* ***A****: Testis (T) of T46/24, with dorsolateral epididymis (E), leading to the vas deferens (VD). Size bar = 5 cm.* ***B****: Testes (T) and epididymis (E) of T41/24. B= urinary bladder. Size bar = 5 cm.*

Considering the appearance of the testes and epididymides and the stranding month (June), the two mature males were assessed to be in quiescence (Tab. 7). While the gonads showed mature development, the testes and epididymides in both males were not turgid and had a slightly flaccid appearance, indicating absence of active spermatogenesis.

*Table 7: Summary of the reproductive history of mature males.*

| Turtle ID | Sex | Stranding month | Gonad characteristics | Interpretation |
|---|---|---|---|---|
| **T41/24** | M | June | long and well-developed testes (ca. 14 x 3 cm) of dark color, appears flaccid; epididymis of white color | mature, in quiescence |
| **T46/24** | M | June | long and well-developed testes (ca. 23.5 x 3 cm) of dark to pale-pink color, thickened but not turgid; epididymis of dark color | mature, in quiescence |

### 3.2.2. Histology (microscopic assessment)

The testes and ovaries of six individuals (five females and one male) were sampled for histological confirmation of their reproductive status.

***Prepubescent female***

The prepubescent individual T01/25 presented numerous small oocytes in early stages of follicular development (Fig. 18). The oocytes were of variable diameter and densely aggregated with limited stromal space between them. Early previtellogenic oocytes and previtellogenic oocytes were surrounded by thin layers of follicular cells. Nuclei were spherical and either central (beginning of previtellogenesis) or eccentric (previtellogenesis), with several peripheral nucleoli. The ooplasm was granulated to translucent, with no sign of yolk platelets. Oocytes were of variable diameter and small dimension.

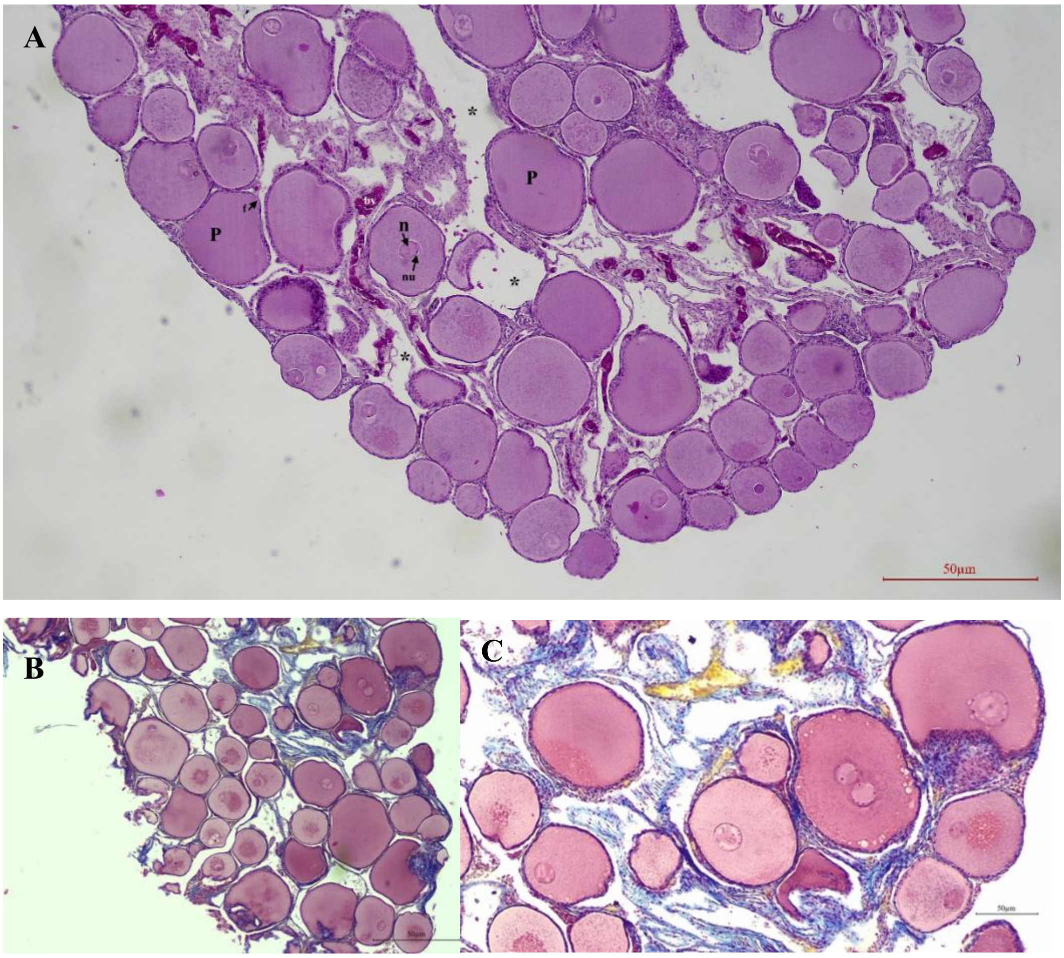



***Figure 18:*** *Previtellogenic follicles of prepubescent female T01/25.* ***A****: Previtellogenic follicles (P) surrounded by follicular cells (f), some showing nuclei (n) with several nucleoli (nu); bv=blood vessel; * = technical artefact; Hematoxylin-eosin, size bar=50µm.* ***B*** *and* ***C****: Masson's trichrome, size bar=50µm.*

### *Pubescent female*

The histological sections of the pubescent individual T16/24 were strongly affected by autolysis (Fig. 19). Oocyte diameter could not be accurately measured, however an increase compared to the prepubescent individual can be seen. Moreover, in some cell peripheries a beginning differentiation into a zona pellucida could be recognized, indicating and advanced stage of previtellogenesis. These cells were also surrounded by a more developed theca out of fibroblasts compared to early previtellogenic follicles seen in the prepubescent female.

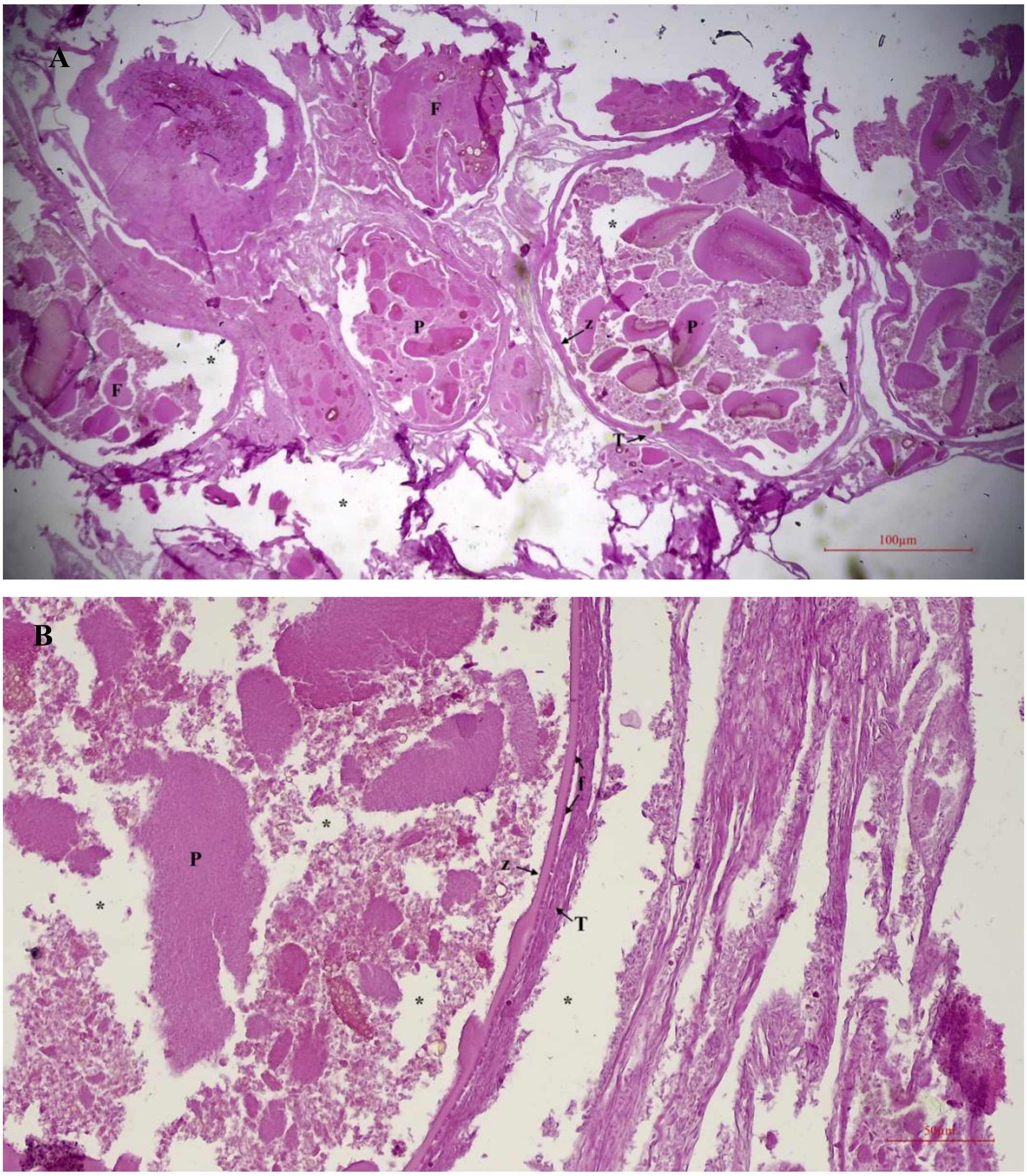



*__Figure 19:__ Ovarian section of pubescent T16/24, strongly affected by autolysis. __A__: showing remnants of follicles (F) of unknown developmental stage and previtellogenic follicles (P) surrounded by a thin zona pellucida (z) and theca (T). Hematoxylin-eosin, size bar=100µm. __B__: close-up of autolytic previtellogenic follicle (P) with surrounding zona pellucida (z), follicular cells (f) and theca (T) . * = technical artefact. Hematoxylin-eosin, size bar=50µm.*

### *Mature females*

The histological sections of the mature female T03/24 were strongly degraded by autolytic processes and allowed only limited analysis (Fig. 20). One large follicle was identified, with increased diameter compared to the prepubescent and pubescent ovarian sections. Its ooplasm was occupied by yolk platelets, indicating vitellogenesis, and surrounded by remnants of a zona pellucida.

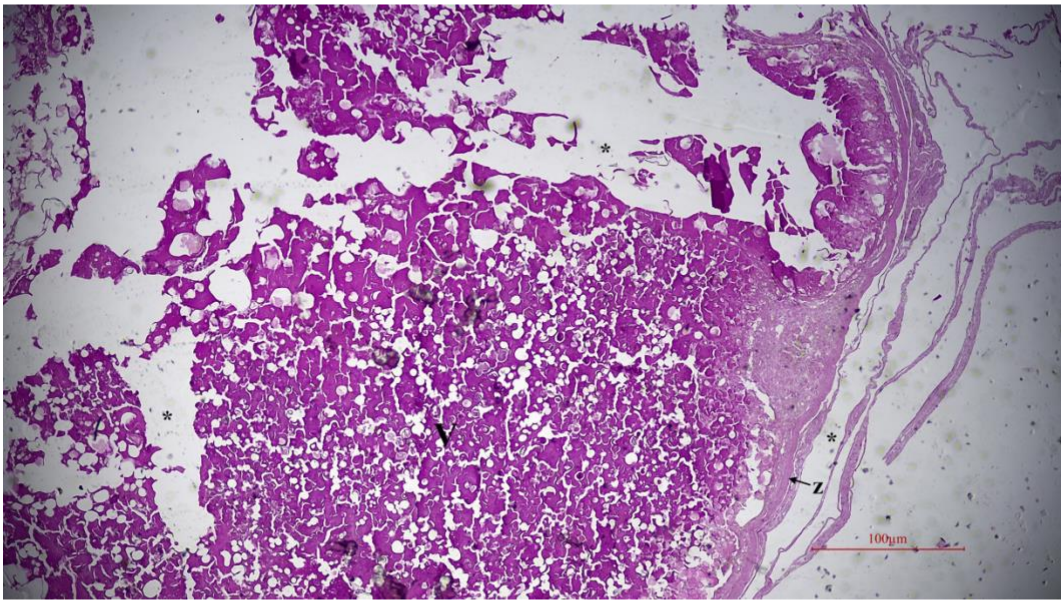



***Figure 20:*** *Large vitellogenic follicle (V) of T03/24 strongly affected by autolysis. The ooplasm is occupied by yolk platelets in degradation, surrounded by remnants of a zona pellucida (z). * = technical artifact. Hematoxylin-eosin, size bar = 100 µm.*

The female T17/24 presented oocytes of different diameter and development stages, confirming the mature status of the gonads. In late previtellogenic follicles the oocytes were surrounded by a broad zona pellucida, which was surrounded by a theca out of fibroblasts (Fig. 21). Larger vitellogenic follicles were strongly affected by autolysis and identified by their increased diameter.

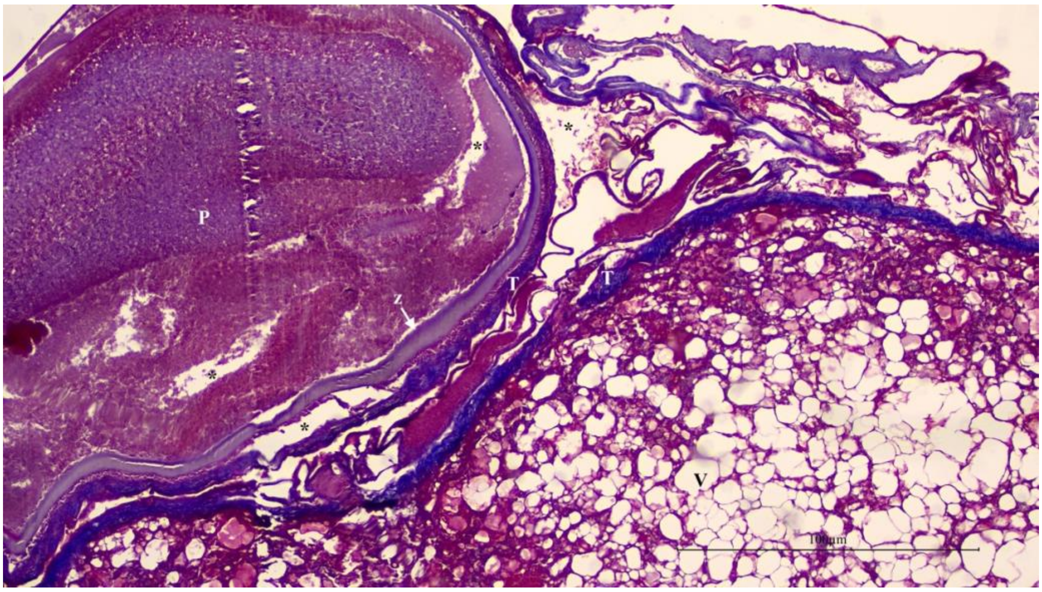



***Figure 21:*** *Ovarian section of adult T17/24. Late previtellogenic follicle (P) with well-developed zona pellucida (z), surrounded by a theca (T) out of fibroblasts, next to a larger vitellogenic follicle (V) in advanced autolysis. * = technical artefact. Masson's trichome, size bar = 100µm.*

The histological sections of T104/24 confirmed the presence of follicles of different sizes, however a more detailed analysis was not possible due to the tissue decomposition (Fig. 22).

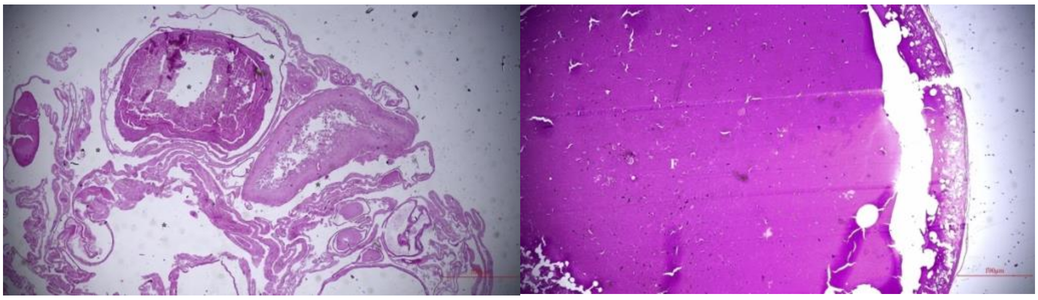



***Figure 22:*** *Ovarian sections of mature female T104/24 displaying follicles (F) of different sizes. Hematoxylin-eosin, size bar = 100 µm.*

***Prepubescent male***

Histological sections of T64/24 testes confirmed the immature gonad development stage of this individual. Seminiferous tubules were inconspicuous and showed little differentiation from the surrounding interstitial tissue, which was highly vascularized (Fig. 23). Their lumen was small and showed single rows of homogenous germinal cells with no signs of spermatogenetic activity. No spermatocytes, spermatids or spermatozoa were present.

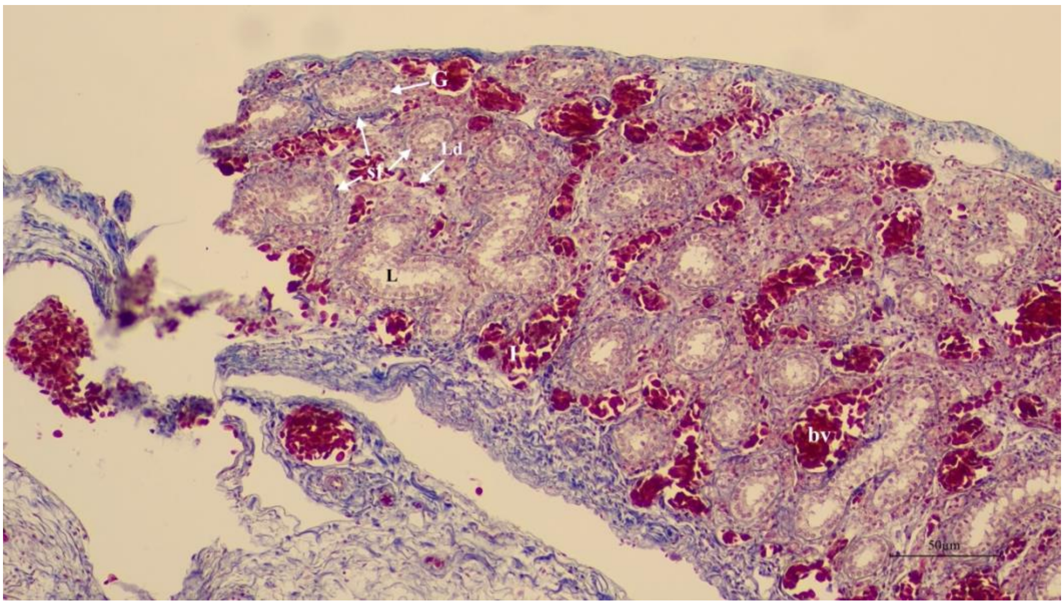



***Figure 23:*** *Section of the testes of prepubescent T64/24. Small seminiferous tubules (sf) with germinal cells (G) and narrow lumen (L), surrounded by interstitial tissue (I) containing blood vessels (bv) and Leydig cells (Ld). * = technical artifact. Masson's trichome; size bar = 50 µm.*

3.3. Gonad development and CCL

In the sub-sample of individuals for which the gonad maturity could be determined (n = 59), females had a significantly larger CCL than males (Two sample t-test, t = 2.2773, df = 58, p-value = 0.04387), having a mean CCL of 67.1 ± 13.48 cm in contrast to 58.17 ± 15.56 cm in males. Prepubescent, pubescent and mature individuals showed an overlapping range of CCL (Fig. 24).

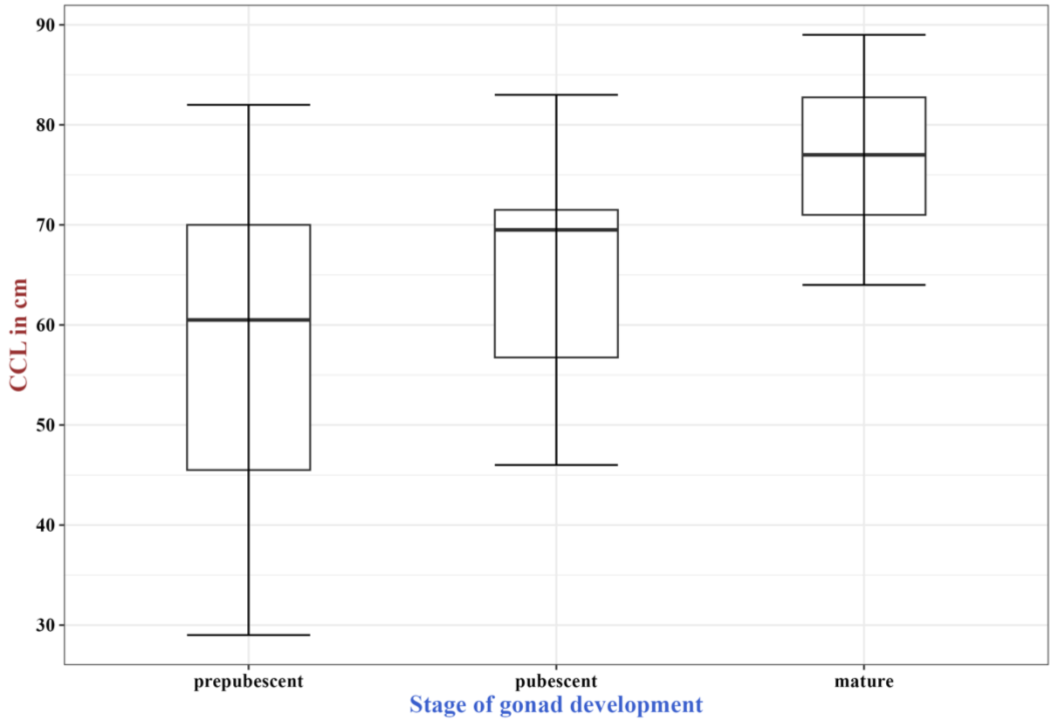



***Figure 24:*** *CCL of prepubescent (n=28), pubescent (n=17) and mature individuals (n=14).*

The size of prepubescent animals ranged from 29 to 82 cm (mean 57.29 ± 14.85 cm), pubescent individuals were found between 46 and 83 cm (mean 65.35 ± 11.03 cm), and mature turtles ranged between 64 and 89 cm CCL (mean 77.36 ± 7.45 cm) (Tab. 8). A Kruskal-Wallis test revealed significant differences in CCL among individuals with different stages of gonad development (Kruskal-Wallis $x^2 = 19.19$, df = 2, $p < 0.001$). Post-hoc pairwise comparisons using Dunn's test indicated that mature individuals had a significantly higher CCL than prepubescent ($p < 0.001$) and pubescent ($p = 0.0265$) individuals, while no significant difference between prepubescent and pubescent turtles' CCL was found ($p = 0.292$).

*Table 8:* *CCL ranges, mean and median of prepubescent, pubescent and mature individuals.*

| Stage of gonadal maturation | Number | CCL min (cm) | CCL max (cm) | CCL mean (cm) | CCL median (cm) |
|---|---|---|---|---|---|
| Prepubescent | 28 | 29 | 82 | 57.29 ± 14.85 | 60.5 |
| Pubescent | 17 | 46 | 83 | 65.35 ± 11.03 | 69.0 |
| Mature | 14 | 64 | 89 | 77.36 ± 7.45 cm | 77 |

Due to the low sample size of male turtles per group, statistical comparisons between males and females with different gonad development stages were not adequate. Nevertheless, it can be observed that the CCL ranges in prepubescent, pubescent and mature turtles overlap in both sexes (Tab. 9). The smallest mature turtle was a male of 64 cm CCL, while the smallest mature female had a CCL of 68 cm.

*Table 9:* *CCL ranges, mean and median of prepubescent, pubescent and mature males and females. * Note that the group of mature males only included two individuals.*

| Stage of gonadal maturation | Sex | Number | CCL min (cm) | CCL max (cm) | CCL mean (cm) | CCL median (cm) |
|---|---|---|---|---|---|---|
| Prepubescent | F | 19 | 29 | 82 | 61.26 ± 14.1 | 67 |
| | M | 9 | 29 | 70 | 48.89 ± 13.49 | 52 |
| Pubescent | F | 10 | 48 | 82 | 64.5 ± 10.05 | 66.5 |
| | M | 7 | 46 | 83 | 66.57 ± 13.04 | 70 |
| Mature | F | 12 | 68 | 89 | 78.5 ± 6.92 | 79 |
| | M* | 2 | 64 | 77 | 70.5 ± 9.19 | 70.5 |

### *3.3.1. Gonad development and age classes*

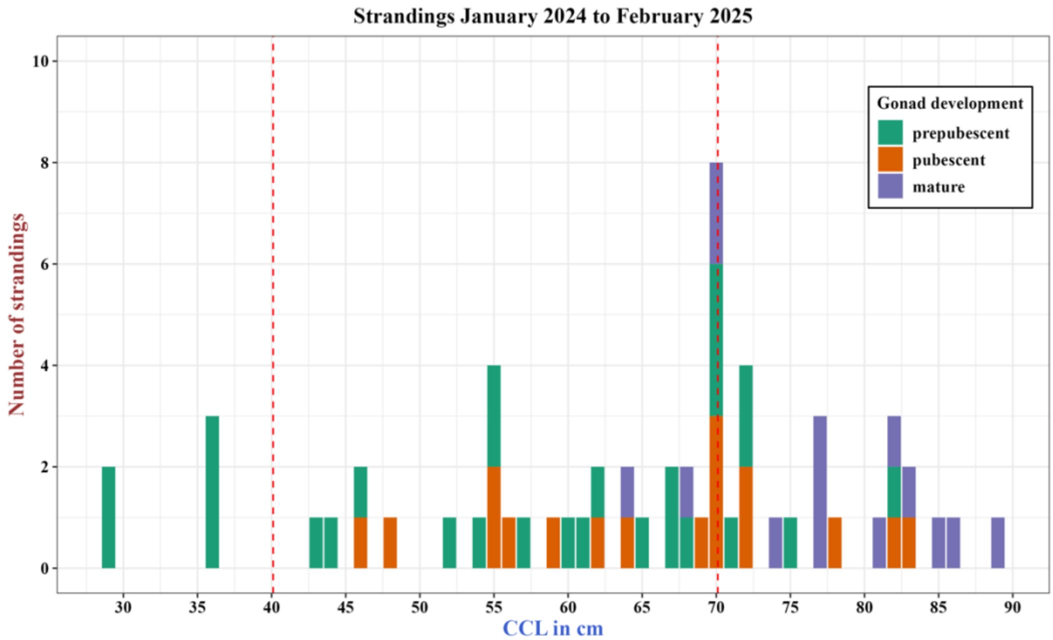



***Figure 25:*** *CCL range of individuals stranded between January 2024 to February 2025 for which a gonad assessment was conducted (n=59). Dashed lines indicate the CCL thresholds from literature for juveniles (≤ 40 cm) and sub-adults (≤ 70 cm).*

The level of gonad development did not correspond to the age classes found in the literature (Fig. 25). While 100% of the juvenile turtles showed prepubescent gonads (n = 5/5), the sub-adult and adult age class were composed of individuals with variable gonad development. 52.9% of sub-adult individuals had prepubescent gonads (n = 18/34), 35.3% had pubescent gonads (n = 12/34) and 11.8% showed gonads with mature characteristics (n = 4/34). Among the turtles classified as "adult" based on CCL, 50% were assessed as mature (n = 10/20), while turtles with prepubescent and pubescent gonads made up 25% of this age class each (n = 5/20).

## 3.4. Spatiotemporal trends in maturity

Association between season and gonad development was only analyzed for individuals stranded in 2024, since the sampling period in 2025 did not encompass the whole year. Pubescent and mature turtles were found throughout the year and their occurrence was not significantly dependent on the season, while prepubescent turtles were found in significantly higher frequencies in the summer months (Chi-squared test, $x^2$ = 16.286, df = 6, p-value = 0.0123) (Fig. 26). In 2024, no prepubescent turtles were found in the winter months, however in 2025 a prepubescent turtle stranded in February, indicating that prepubescent turtles are not entirely absent in winter.

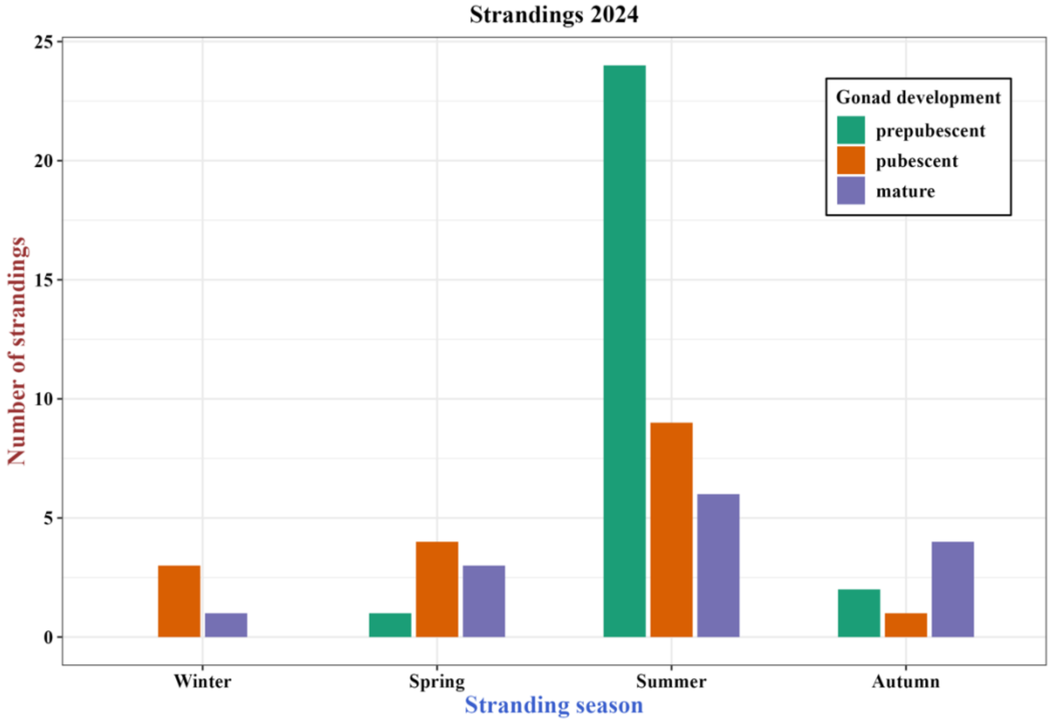



***Figure 26:*** *Seasonal stranding frequency for turtles with available gonad assessment in 2024 (n = 89).*

There was no association between the gonad development of individuals and their distribution along the Veneto coastline (Chi-squared test, $x^2 = 21.898$, df = 22, p-value = 0.466; Fig. 27).

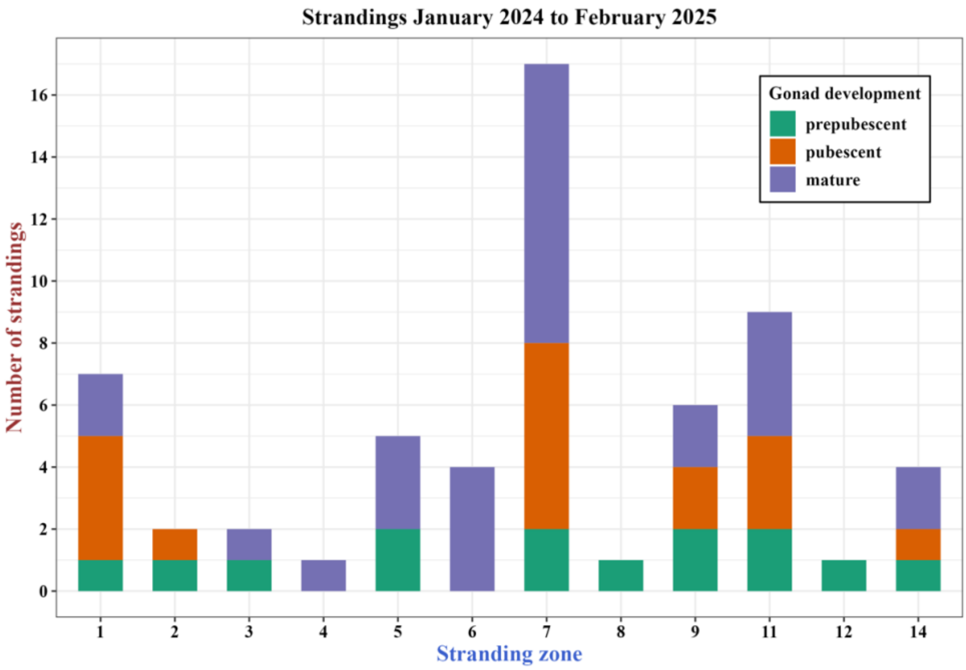



***Figure 27:*** *Frequency of strandings in the 14 stranding zones for individuals with available gonad assessment (n = 59), stranded between January 2024 to February 2025.*

### 3.5. Beginning of tail elongation

Three different tail measurements were taken for all individuals stranded between January 2023 and February 2025: the distance between the plastron and the tip of the tail (PT), the distance between the cloaca and the tip of the tail (CTL) and the distance between the carapace and the tip of the tail (CaTT). The effects of CCL and Sex on all three measurements were separately investigated with generalized linear models (GLMs) and generalized additive models (GAMs).

3.5.1. Plastron – tip of the tail vs. CCL and Sex

The GLM "PT ~ CCL * Sex" showed a significant effect of CCL on PT, which was stronger in male than in female turtles ($p < 0.001$), indicating an overall stronger increase in PT length with increasing CCL in male turtles (Tab. 10). The model explained 80.23% of the deviance in the data, indicating a good fit to the observations (Fig. 28).

***Table 10:*** *Summary of parameter estimates for the GLM model PT ~ CCL * Sex.*

| Parameter | Estimate | SE | t-value | p-value | Dev. exp. | AIC |
|---|---|---|---|---|---|---|
| (Intercept) | 1.210402 | 0.089507 | 13.523 | **< 0.001** | 80.23% | 582.46 |
| CCL | 0.022597 | 0.001364 | 16.568 | **< 0.001** | | |
| SexM | -0.599189 | 0.164362 | -3.646 | **< 0.001** | | |
| CCL:SexM | 0.013848 | 0.002661 | 5.205 | **< 0.001** | | |

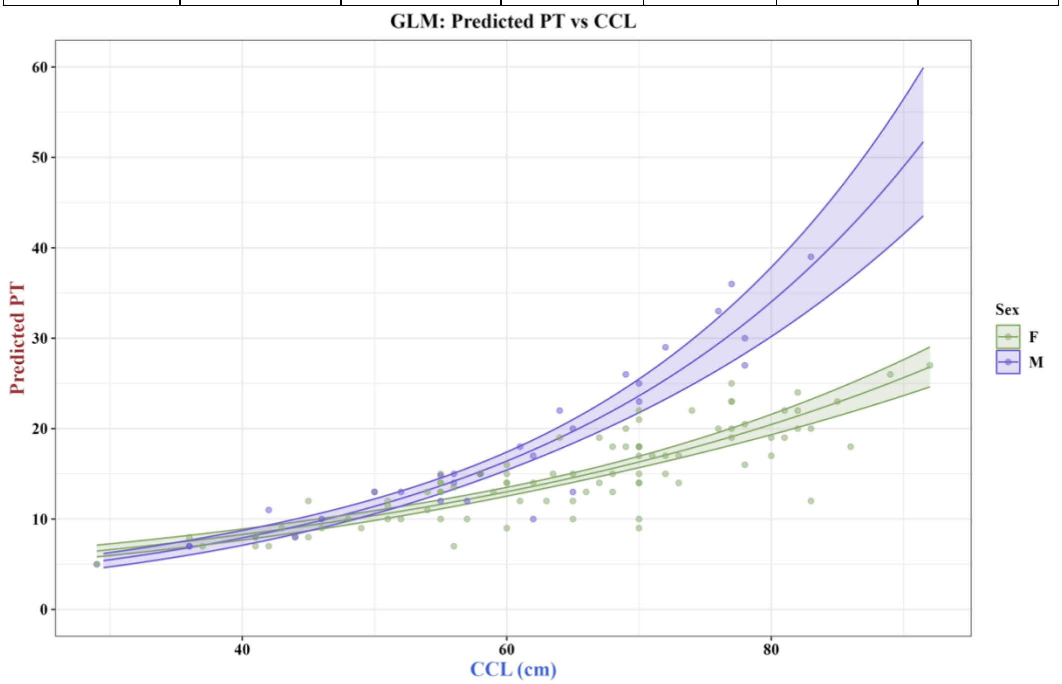



***Figure 28:*** *Predicted PT curve vs. CCL (cm) based on GLM PT ~CCL * Sex. Dots = observations, shaded area = 95% confidence intervals.*

To compare with a more flexible approach, a GAM was applied to the same data, with a smooth function for CCL separately for males and females. The GAM confirmed that the relationship between CCL and PT was significantly different between males and females, with males exhibiting higher PT values and a stronger increase with increasing CCL ($p < 0.001$, Tab. 11). The smooth terms for both sexes were strongly significant, indicating that PT changes with increasing CCL in both males and females (Tab. 12). While the effect for females was slightly nonlinear (EDF = 1.785), the CCL smooth term for males followed a clear linear trend (EDF = 1) (Fig. 29). The GAM had an adjusted $R^2$ of 0.809 and explained 80.7% of the deviance in the observations, indicating a good model fit that is very similar to the previously shown GLM. The AIC of 581.57 is slightly lower than the AIC of the GLM (582.46). The distance however is very small ($\Delta AIC < 2$), which indicates a similar model performance.

***Table 11:*** *Summary of parameter estimates for the GAM model PT ~ s(CCL, by = Sex) + Sex.*

| **Parameter** | **Estimates** | **SE** | **t-value** | **p-value** | **adj. $R^2$** | **Dev. explained** | **AIC** |
|---|---|---|---|---|---|---|---|
| Intercept | 2.63 | 0.02 | 145.53 | **< 0.001** | 0.809 | 80.7% | 581.57 |
| SexM | 0.27 | 0.04 | 7.41 | **< 0.001** | | | |

***Table 12:*** *CCL smooth terms separated for males (SexM) and females (SexF) in the GAM model PT ~ s(CCL, by = Sex) + Sex.*

| **s(CCL)** | **edf** | **Ref. df** | **F value** | **p-value** |
|---|---|---|---|---|
| SexF | 1.785 | 2.26 | 125.2 | **< 0.001** |
| SexM | 1.000 | 1 | 260.0 | **< 0.001** |

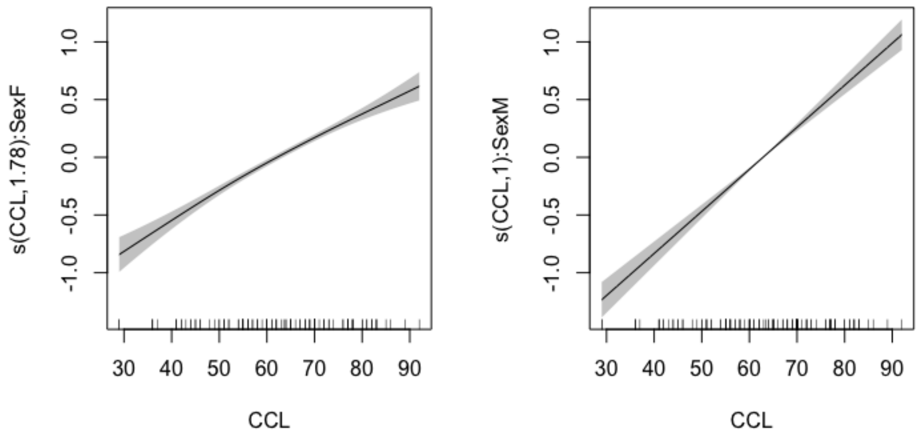



***Figure 29:*** *Smooth curves for females (left) and males (right) for the GAM model "PT ~ s(CCL, by = Sex) + Sex".*

Based on the differences between the values for males and females predicted by the GAM, the CCL at which males begin to develop significantly longer tails than females was estimated as 50.2 cm (Fig. 30).

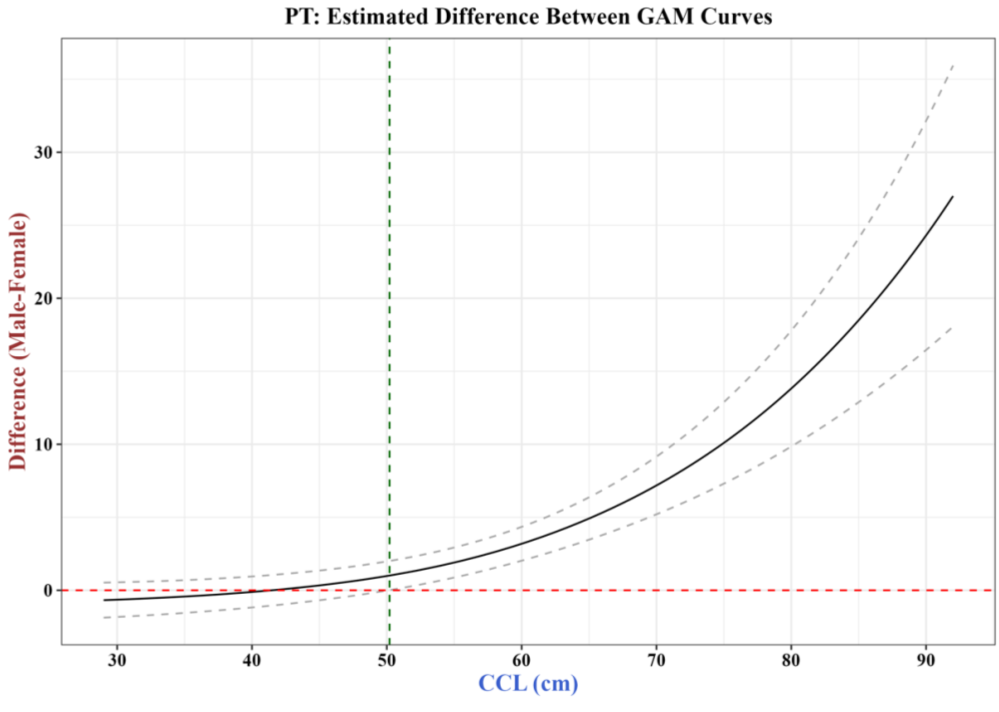



***Figure 30:*** *Estimated difference between the male and female GAM curves for the GAM "PT ~ s(CCL, by = Sex) + Sex". Black line = difference between curves; grey dashed lines = 95% confidence intervals; dark green dashed line = estimated point of significant divergence of male and female CCL curves.*

3.5.2. Cloaca – tip of the tail vs. CCL and Sex

The GLM predicting the distance between the cloaca and the tip of the tail (CTL) by CCL and Sex found that the change in CTL with increasing CCL was not significantly different between males and females ($p = 0.201$; Tab. 13). In a reduced model only considering the main effects of CCL and Sex on CTL separately, both factors were found to have significant effects ($p < 0.001$ and $p < 0.01$, respectively). Both models had a very similar AIC (503.55 and 503.73) and explained 28.17% and 26.9% of the deviance in the data. The predicted CTL curve based on the model with only main effects of CCL and Sex is shown in Fig. 31.

***Table 13:*** *Summary of parameter estimates for the two CTL GLMs. "*" indicates that the model includes interaction effects between the factors, while "+" indicates that only main effects of the two factors are included.*

| **Model** | **Parameter** | **Estimate** | **SE** | **t-value** | **p-value** | **Dev. exp.** | **AIC** |
|---|---|---|---|---|---|---|---|
| CTL ~ CCL * Sex | (Intercept) | 0.222939 | 0.285140 | 0.782 | 0.436 | 28.17% | 503.55 |
| | CCL | 0.018068 | 0.004354 | 4.150 | **< 0.001** | | |
| | SexM | -0.340737 | 0.522485 | -0.652 | 0.516 | | |
| | CCL:SexM | 0.010877 | 0.008461 | 1.286 | 0.201 | | |
| CTL ~ CCL + Sex | (Intercept) | 0.03373 | 0.23687 | 0.142 | 0.88700 | 26.9% | 503.73 |
| | CCL | 0.02103 | 0.00359 | 5.858 | **< 0.001** | | |
| | SexM | 0.32008 | 0.11141 | 2.873 | **< 0.01** | | |

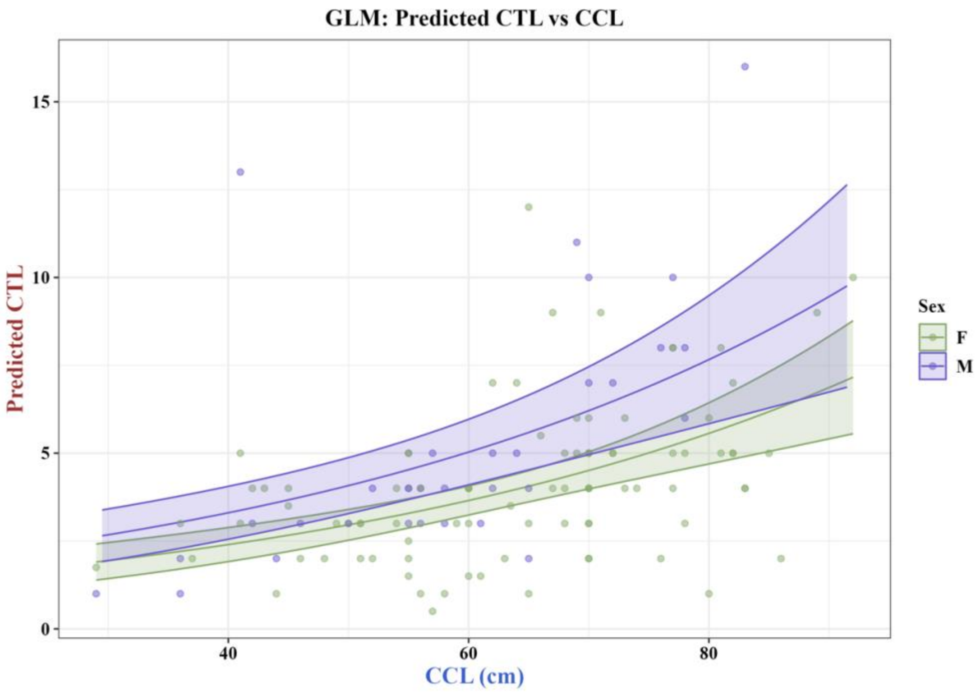



***Figure 31:*** *Predicted CTL curve according to GLM "CTL ~ CCL + Sex". Dots = observations, shaded area = 95% confidence intervals.*

Applying a GAM with separate smooth terms for males and females, the relationship between CTL and PT differed significantly between the sexes, with males showing higher CTL measurements and a steeper increase with increasing CCL (< 0.01, Tab. 14). The smooth CCL terms for both sexes were significant (< 0.001), indicating that in both male and female turtles a significant relationship between CCL and CTL exists (Tab. 15). While the female CCL smooth term was linear (EDF = 1), the male CCL smooth term showed a high degree of nonlinearity (EDF = 4.038) (Fig. 32). The GAM had a slightly lower AIC than the two linear models (499.83), and explained a slightly higher percentage of the deviance in the observations (33.9%). However, in both GLMs and the GAM the percentage of explained deviance was relatively low, which suggests that additional variables may influence CTL beyond CCL and Sex.

***Table 14:*** *Summary of parameter estimates for the GAM model "CTL ~ s(CCL, by=Sex) + Sex".*

| **Parameter** | **Estimates** | **SE** | **t-value** | **p-value** | **adj. $R^2$** | **Dev. explained** | **AIC** |
|---|---|---|---|---|---|---|---|
| Intercept | 1.35665 | 0.05287 | 25.660 | **< 0.001** | 0.371 | 33.9% | 499.83 |
| SexM | 0.34341 | 0.10999 | 3.122 | **< 0.01** | | | |

***Table 15:*** *CCL smooth terms separated for males (SexM) and females (SexF) in the GAM model "CTL ~ s(CCL, by=Sex) + Sex".*

| **s(CCL)** | **edf** | **Ref. df** | **F value** | **p-value** |
|---|---|---|---|---|
| SexF | 1.000 | 1.000 | 20.952 | **< 0.001** |
| SexM | 4.038 | 4.986 | 5.917 | **< 0.001** |

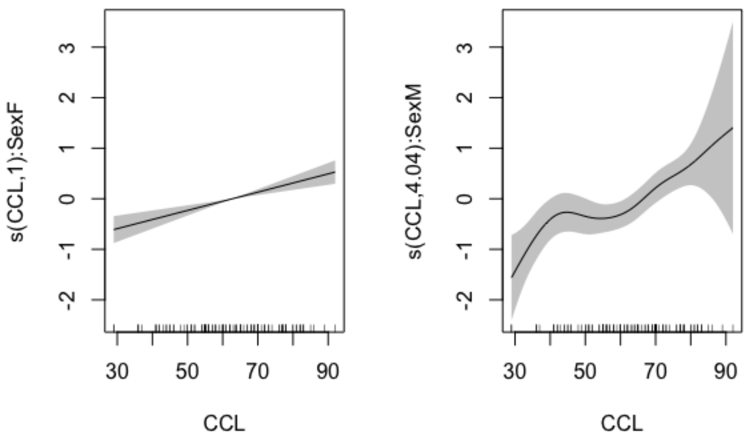



***Figure 32:*** *GAM smooth curves for females (left) and males (right) for the GAM model "CTL ~ s(CCL, by=Sex) + Sex".*

Based on the GAM, the CCL at which male and female turtles show a significantly different distance between the cloaca and the tip of the tail has been calculated at 70.3 cm (Fig. 33).

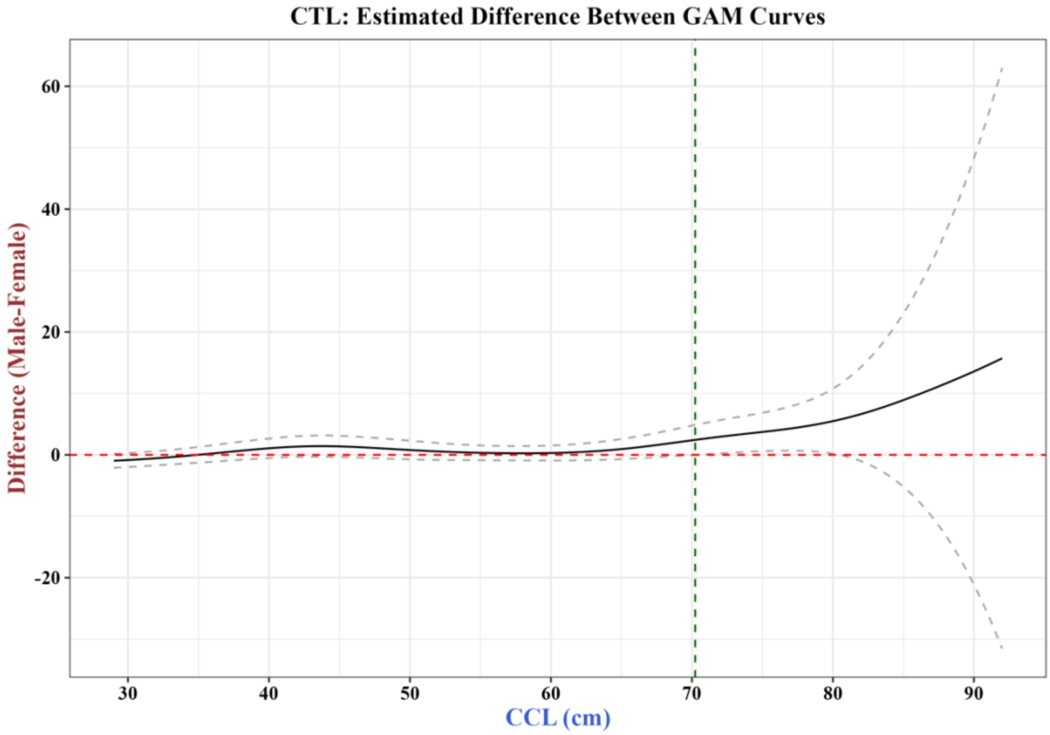



***Figure 33:*** *Estimated difference between the male and female GAM curves for the GAM "CTL ~ s(CCL, by=Sex) + Sex". Black line = difference between curves; grey dashed lines = 95% confidence intervals; dark green dashed line = estimated point of significant divergence of male and female CCL curves.*

3.5.3. Carapace – tip of the tail vs. CCL and Sex

To meet the assumptions of the gamma distribution with a log link function, all CaTT values were shifted by +5 to ensure positivity.

The GLM showed a significant effect of CCL on CaTT, with a steeper slope in males than in females ($p < 0.001$; Tab. 16). The model explained 43.62% of the deviance in the data. Predicted CaTT values were transformed back to the original scale for comparison with the observations (Fig. 34).

*Table 16: Parameter estimation from GLM model CaTT_shift ~ CCL * Sex using a shift value (+5) for CaTT.*

| Parameter | Estimate | SE | t-value | p-value | Dev. exp. | AIC |
|---|---|---|---|---|---|---|
| (Intercept) | 1.601277 | 0.186445 | 8.588 | **< 0.001** | 43.62% | 515.82 |
| CCL | 0.001906 | 0.002850 | 0.669 | 0.504979 | | |
| SexM | -1.187569 | 0.335656 | -3.538 | **< 0.001** | | |
| CCL:SexM | 0.026925 | 0.005433 | 4.956 | **< 0.001** | | |

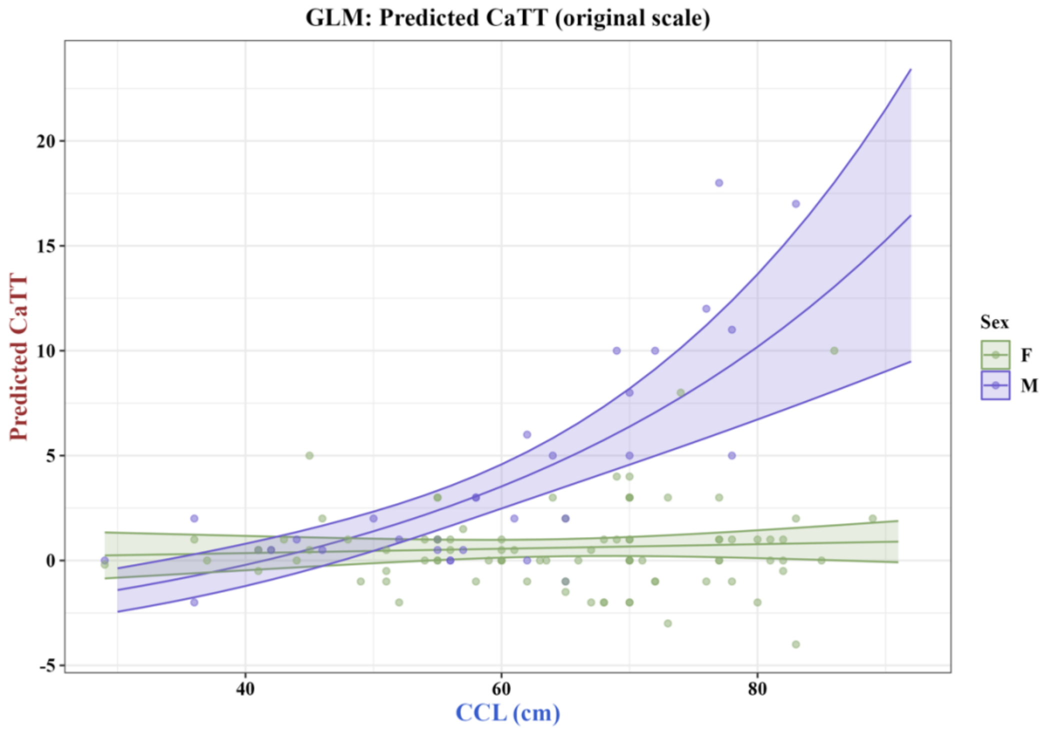



*Figure 34: Predicted CaTT curve corrected for the shift transformation based on the GLM model CaTT ~ CCL * Sex. Dots = observations, shaded area = 95% confidence intervals.*

Similarly to the GAMs analyzing PT and CTL, the GAM revealed a significantly different relationship between CaTT and CCL for male and female turtles, with males presenting higher CaTT values and a stronger increase with increasing CCL ($p < 0.001$, Tab. 17). The CCL smooth term for females was not significant ($p = 0.493$) and linear (EDF = 1), suggesting that CaTT increases only slightly with body size in females (Tab. 18). The CCL smooth term for males was significant ($p < 0.001$) and strongly nonlinear (EDF = 2.082), confirming the significant effect of increasing body size on CaTT length in male turtles (Fig. 35). The GAM explained the deviation between model and observations slightly better than the GLM (46.9%) and had an adjusted $R^2$ of 0.593. Also, the AIC was slightly lower than in the GLM (510.81 vs. 515.82).

***Table 17:*** *Summary of parameter estimates for the GAM model CaTT_shift ~ s(CCL, by=Sex) + Sex using a shift value (+5) for CaTT.*

| **Parameter** | **Estimates** | **SE** | **t-value** | **p-value** | **adj. $R^2$** | **Dev. explained** | **AIC** |
|---|---|---|---|---|---|---|---|
| Intercept | 1.72083 | 0.03636 | 47.323 | **< 0.001** | 0.593 | 46.9% | 510.81 |
| SexM | 0.50212 | 0.07344 | 6.837 | **< 0.001** | | | |

***Table 18:*** *CCL smooth terms for males (SexM) and females (SexF) in the GAM model CaTT_shift ~ s(CCL, by=Sex) + Sex using a shift value (+5) for CaTT.*

| **s(CCL)** | **edf** | **Ref. df** | **F value** | **p-value** |
|---|---|---|---|---|
| SexF | 1.000 | 1.001 | 0.473 | 0.493 |
| SexM | 2.082 | 2.606 | 17.956 | **< 0.001** |

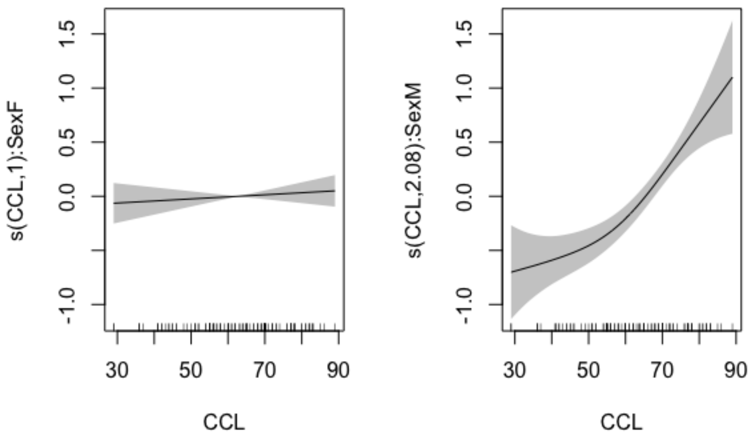



***Figure 35:*** *GAM smooth CCL curves for females (left) and males (right) for the GAM model "CaTT_shift ~ s(CCL, by=Sex) + Sex" using a shift value (+5) for CaTT.*

The CCL at which the CaTT value started to differ significantly between male and female turtles was calculated as 56.2 cm (Fig. 36).

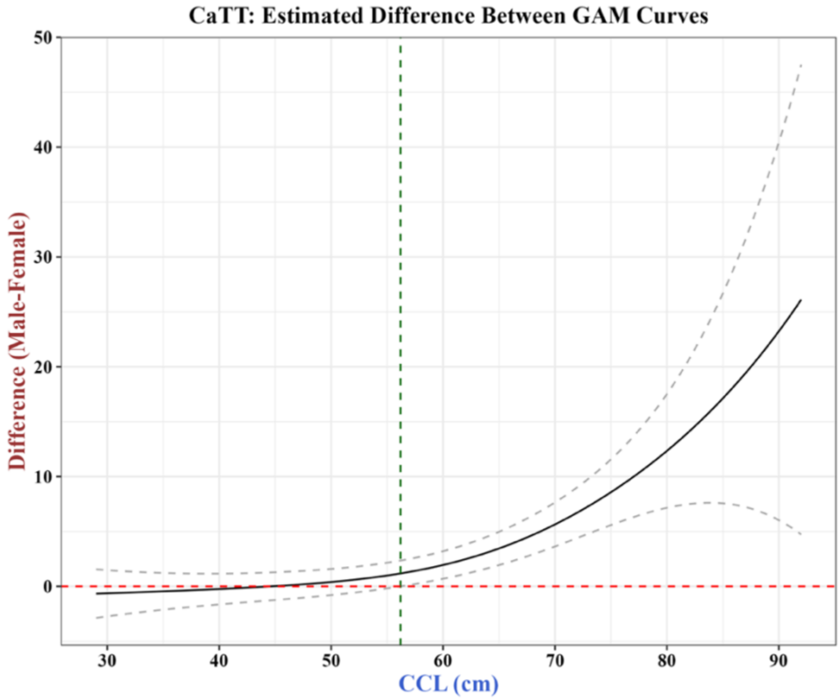



***Figure 36:*** *Estimated difference between the male and female GAM curves for the GAM "CaTT_shift ~ s(CCL, by=Sex) + Sex" using a shift value (+5) for CaTT. Black line = difference between curves; grey dashed lines = 95% confidence intervals; dark green dashed line = estimated point of significant divergence of male and female CCL curves.*

# 4. Discussion

### 4.1. Population demography and spatiotemporal stranding distribution

Loggerhead sea turtles stranded along the Veneto coastline throughout the years 2023 and 2024, which confirms the importance of the northern Adriatic Sea for loggerhead sea turtles demonstrated in previous studies (e.g. Agabiti et al., 2024; Casale et al., 2004; Fortuna et al., 2018; Marisaldi et al., 2023). The stranding records in those years showed a clear seasonality of strandings, with most loggerhead turtles (64.8%, n = 158/244) stranded in the summer months. The seasonality was significantly more evident in 2023, where strandings were completely absent in winter, and only few records were found in spring. Similar trends were reported by Marisaldi et al. (2023), analyzing turtle strandings between 2019 and 2022 in the coastal area south of the Po delta (Emilia-Romagna, Italy), which is directly adjacent to the most southern stranding zone 14 in this study. Also along the Croatian northern Adriatic coastline strandings peaked in summer (Mihaljević et al., 2024). These results reflect the high loggerhead sea turtle density in the northern Adriatic Sea in summer months as demonstrated by aerial surveys in 2010 and 2013 (Fortuna et al., 2018). Lower stranding reports in winter as seen in this study have also been reported by Marisaldi et al. (2023) for Emilia-Romagna and Casale et al. (2010) for the whole northern Adriatic Sea in a study encompassing the years 1980 to 2008. Higher stranding records in the central and southern Adriatic Sea in winter months suggest that individuals migrate southwards when the sea water temperature decreases (Casale et al., 2010; Mihaljević et al., 2024). However, over the whole sampling period, strandings were not absent in winter, which confirms that the northern Adriatic Sea serves as a wintering habitat for some individuals, as previously shown by satellite tracking and CMR studies (Baldi et al., 2023a; Casale et al., 2012).

Aside from a higher occurrence of individuals, a higher mortality in a given season may lead to higher stranding numbers (Mihaljević et al., 2024). Fishing activity is one of the major threats for sea turtles in the Mediterranean (Casale, 2011; Casale et al., 2018; Lewison et al., 2014), and vulnerability and mortality rate depends on the type of fishery (Casale, 2011). In the northern Adriatic Sea, the trawling industry reports the highest incidental sea turtle captures (Casale et al., 2004; Casale et al., 2010). This type of fishery is particularly dangerous for benthic feeders such as loggerhead sea turtles, who are known to recruit to neritic foraging grounds at a small size in the Mediterranean (Casale et al., 2008; Lazar et al., 2011; Mariani et al., 2023). Nevertheless, the actual mortality from this fishing type is unclear, since the survival rate of individuals returned to the sea after capture is unknown (Casale et al., 2004; Casale, 2011). In EU waters, trawling activity is completely prohibited within the 3 nm zone or 50 m isobath all year (Council of the European Union, 2006), and in Italy, a seasonal ban of bottom and pelagic trawling activity within the 6 nm zone in late summer is defined every year. The so-called "ferma pesca" was between 29.07. – 09.09. in 2023 (MASAF, 2023) and 31.07. – 13.09. in 2024 (MASAF, 2024), therefore encompassing the whole month of August in both years. Russo et al. (2020) found that trawling activity was lowest in summer in the years 2015 and 2016, and if this trend is representative for following years, this would indicate that trawling effort does not explain the higher stranding frequencies recorded in 2023 and 2024. However, it should be noted that the activity of artisanal and small-scale fisheries cannot be monitored in contrast the commercial ones, due to the lack of mandatory tracking systems onboard, and whether these comply with the fishing bans can therefore not be assessed (Casale, 2011). This is important to consider, as this type of fisheries is possibly responsible for as many captures as commercial fisheries (Casale, 2011), and this data gap must be addressed to properly evaluate the impact of fisheries on sea turtles in the northern Adriatic Sea.

Individuals with prepubescent gonads were found in significantly higher frequencies in summer. However, since the size range of prepubescent individuals was very large (29 cm to 82 cm) it is difficult to associate this finding to environmental factors such as higher sea surface temperature (SST) during summer, or the distribution by currents, since loggerhead turtles > 40 cm are able to swim independently from currents (Revelles et al., 2007). A possible misidentification of prepubescent individuals will be discussed in chapter 4.2.1., which could explain the confounding results and would stress the importance of further studies with standardized gonad maturity assessment. There was no dependency between the stranding month and individuals' sex or age class, however it was found that in summer, the mean CCL was significantly higher than in spring (60.3 vs. 50.2 cm CCL). This may be explained by the fact that during spring, a greater proportion of mature adult turtles, which are more likely to be of larger size, aggregate at their breeding sites to mate (Casale et al., 2018), leaving only smaller individuals at the foraging sites, while until the end of summer, most adult turtles that had reproduced, return to their foraging areas. However, this contradict with the high frequency of prepubescent turtles found in summer, for which further studies are needed to understand temporal stranding trends. Moreover, Marisaldi et al. (2023) demonstrated variability in seasonal CCL distribution among sampling years, and emphasized that long-term studies encompassing several years are needed to explore and ascertain temporal trends.

On the spatial scale, zones 7 and 6 were the areas with the highest numbers of turtle strandings (21.5%, n = 53/246 and 12.2%, n = 30/246). These zones represent the northern and southern coastal strips bordering the channel that opens to the islands of Venice. Zone 7 corresponds to the Lido di Venezia area, which lies between the inlets of the Malamocco and the channel opening to the islands of Venice. These passages are highly frequented by commercial and touristic ships entering the Venice lagoon (Scarpa et al., 2007). Both marine traffic and touristic pressure are considered threats for marine wildlife. These factors might contribute to increased sea turtle mortality in the zones 6 and 7 and reduce the habitat quality for marine turtles and other animals. The absence of a spatial pattern of individuals of different CCL, sex and gonad maturity stages indicates that these characteristics had no significant effect on the stranding location.

The occurrence of strandings in a particular area or season depends on the number of animals present, mortality risk, and environmental and physical parameters that determine the drift of the carcass (Mihaljević et al., 2024; Santos et al., 2018). Moreover, the detection of strandings depends on observer effort (Casale et al., 2010; Mihaljević et al., 2024). The higher human presence on the beaches during certain seasons, and the better accessibility of certain coastline areas may lead to increased observer effort and a higher likelihood to detect stranded turtles. The high frequency of strandings recorded during the summer can be associated to more tourists visiting the beaches along the Veneto coastline during this season, especially as zones 6 and 7 correspond to areas that are popular for beach tourism.

4.1.1. Size and sex

Individuals stranded along the Veneto coastline between January 2023 and February 2025 showed a wide range of CCL, which confirms that the northern Adriatic Sea is an important habitat for loggerhead turtles of different sizes and age classes (Casale et al., 2012; Zbinden et al., 2008). The majority of stranded turtles belonged to the sub-adult age class (61%; n = 150/246), which encompasses individuals with a CCL between 41 cm to 70 cm. It should be noted that 70 cm was the most frequently measured CCL (n = 17/246; 6.9%), which was the threshold between the sub-adult and adult age class used in this study. Small juvenile turtles ≤ 40 cm were less frequent (13.4%; n = 33/246), which is similar to findings from Emilia-Romagna, where most turtles stranded turtles were categorized as large juveniles and sub-adults, while juveniles ≤ 30 cm CCL were rare (Marisaldi et al., 2023). The average CCL of turtles stranded in Emilia-Romagna was 57.2 cm (Marisaldi et al., 2023) and therefor well comparable to the average size reported in the present study for Veneto, which was 58.15 cm. Combined, this indicates that loggerhead sea turtles stranded on the Italian side of the northern Adriatic Sea are larger than those stranded in the Croatian part, where small juvenile turtles ≤ 40 cm were the most frequent size class, and turtles > 65 cm CCL were less abundant (Mihaljević et al., 2023).

In the present study female turtles were significantly larger than males in the total sample (January 2023 to February 2025; 64.64 cm vs. 58.81 cm), as well as in the sub-sample (January 2024 to February 2025; 67.1 cm vs. 58.17 cm). This contrasts with the stranding reports from Emilia-Romagna, where adult male turtles had a significantly larger CCL than adult female turtles (81.8 cm vs. 77 cm) (Marisaldi et al., 2023). Since there is no clear evidence for a sexual size dimorphism for Mediterranean loggerhead turtles (Casale et al., 2018) this suggests a habitat preference of larger males for the area south of Veneto. However, it should be noted that Marisaldi et al. (2023) determined sex only in turtles larger than 70 cm based on tail length, for which they could not determine sex-specific CCL differences in smaller individuals. The sex ratios in the present study were female-biased in the total sample (January 2023 to February 2025), as well as in the sub-sample that only included the years in which gonad development was assessed (January 2024 to February 2025), with sex ratios of 3:1 and 2.6:1 F:M respectively. This goes in line with the assumption that the hatchling production on Mediterranean loggerhead sea turtle nesting beaches is largely female-biased (Casale et al., 2018) and would suggest that the sex ratio has a similar order in later life stages. Necropsies of stranded loggerhead turtles along the Croatian Adriatic coast and in a dietary study on loggerhead sea turtles in the central Adriatic Sea also revealed a higher prevalence of female turtles (Mariani et al., 2023; Mihaljević et al., 2023). More females than males were also recorded in Emilia-Romagna, however, as mentioned before, the authors determined the sex based on tail length and therefor only considered individuals larger than 70 cm CCL (Marisaldi et al., 2023). This is however in contrast to other studies on juvenile and adult Mediterranean loggerhead sea turtles, where the sex ratio was more balanced, and a possibly different habitat use of male and female turtles which would influence the sex ratio must be considered (Casale et al., 2006; Casale et al., 2014; Maffucci et al., 2013). For example, Schofield et al. (2013) demonstrated with satellite tracking that 24 % of the observed male turtles reproducing in Zakynthos (Greece) stayed close to the breeding area, in contrast to only 3% of the tracked females, which suggests that adult male turtles are less likely to be found in more distant foraging areas. It should be noted that in the present study, the sex could only be determined in 51.2% individuals (n = 126/246) in the total sample, and 60.2% (n = 68/113) in the sub-sample, which leaves uncertainty on the sex of 58.8% and 39.8% of the individuals.

Temperature-dependent sex determination (TSD) usually favors the occurrence of one sex, which has been suggested to be an evolutionary advantage in sea turtles, as a higher number of females in a population increases the number of nests that can be laid in one season (Hays et al., 2017; Santidrián Tomillo and Spotila, 2020). A female-biased primary sex ratio (PSR) in hatchlings may translate into a less biased operational sex ratio (OSR), which considers only the adult individuals breeding in a particular season (Santidrián Tomillo and Spotila, 2020). This is because male turtles can breed more frequently than females and can fertilize several females in one season, so that less males are needed to keep the population viable (Hays et al., 2017). However, concerns have been raised that while TSD in sea turtles has been an advantage in the past, due to anthropogenic climate change nest incubation temperatures increase at a much higher scale, which can lead to high in-nest mortalities and complete feminization of sea turtle populations in the long-term (Santidrián Tomillo et al., 2015). Continuous monitoring of sex ratios is therefore crucial to understand their survival probabilities in response to climate change and to adapt conservation strategies.

### 4.2. Reproductive maturity of loggerhead sea turtles stranded along the Veneto coastline

The analysis shows that the northern Adriatic Sea is inhabited by loggerhead sea turtles in different stages of reproductive maturity. While 84.2% (n = 16) of male and 59.2% (n = 29) of female individuals were immature and showed prepubescent and pubescent gonads, there was a considerable number of mature female turtles (n = 12/49, 24.5%). Only two out of 19 male turtles were found to be mature adults (10.5%).

Mature females were found in all seasons; however, they were in different phases of their reproductive cycle. Three females with ovaries preparing to breed were found in January, April and May (T03/24, T17/24 and T20/24). Female Mediterranean loggerheads migrate to their breeding sites between April and May (Casale et al., 2018), and it can be assumed that these females would have departed to their breeding sites if they had not died. Their ovaries contained developing follicles of variable dimensions, including large, vitellogenic follicles, indicating advanced follicular development as described by Miller and Limpus (2003) and Pérez-Bermúdez et al. (2012). Follicular size hierarchy has been described in different reptile species, such as the tortoise *Chelonoidis denticulatus* (Mayor et al., 2023), as well as green sea turtles and loggerhead turtles (Bruno et al., 2025; Miller and Limpus, 2003). Bruno et al. (2025) recently demonstrated that vitellogenesis continues throughout the nesting season in green sea turtles in Tortuguero (Costa Rica), which allows the females to efficiently use the limited space in the coelomic cavity, but also to use the energy from reabsorbed follicles that were not ovulated in a clutch for the vitellogenesis in successive clutches. Since both green and loggerhead sea turtles exhibit follicular size hierarchy, it is possible that loggerhead turtles show a similar strategy. However, further data from necropsies of breeding individuals are needed to confirm this hypothesis.

Five females with gonads regressing after nesting season were found between June and October 2024 (T23/24, T59/24, T84/24, T103/24, T104/24). Peak loggerhead nesting season in the Mediterranean occurs between June and July, and females return to their foraging sites between July and August (Casale et al., 2018; Zbinden et al., 2008). Two mature females whose ovaries showed characteristics of regression after nesting season were found earlier in the year, in May and June 2024. The individual T23/24 stranded on 18.05.24 had possibly nested in the nesting season of the previous year 2023, since it seems unlikely that it completed nesting and the post-nesting migration at this time of the year. In the case of T59/24, stranded on 30.06.24, it is unknown whether this individual had nested in the previous season 2023, or in the nesting season of the same year 2024. While the end of June would be an early finding, it is possible that the post-nesting migration of this individual started earlier than average. One possible explanation for this could be an advanced beginning of nesting season in the Mediterranean in response to rising SSTs (Mazaris et al., 2008), which could also shift the post-nesting migration to an earlier date. However, since it is unknown when and where this female nested, this hypothesis remains untested. Of particular interest is the adult female T104/24, stranded in October 2024, as an external flipper tag allowed to trace back the nesting history of this animal and revealed that it had nested in the same nesting season in Kyparissia Bay, Greece (ARCHELON, personal communication 2024). The ovaries were well-developed and showed large, vitellogenic follicles that were not ovulated during the nesting season, and would presumably have been absorbed over the next year, along with ovarian scars from ovulated follicles and atretic follicles (Miller and Limpus, 2003). The presence of this individual in the northern Adriatic Sea three months after being tagged while nesting in Greece confirms the connectivity between Greek nesting rookeries and the northern Adriatic Sea, which has been demonstrated in several previous studies (e.g. Baldi et al., 2023a; Lazar et al., 2004; Zbinden et al., 2008).

Due to the high energy expenditure during reproduction, female loggerhead turtles do not nest every year (Hays et al., 2010). Female turtles in quiescence are characterized by ovaries with expanded stroma, but absence of large, vitellogenic follicles, while bearing some smaller follicles and ovarian scars from previous nesting seasons (Miller and Limpus. 2003). In the present study, one female turtle stranded in September 2024 (T93/24) was assessed to be mature, in quiescence. Miller and Limpus (2003) described quiescent females as females that did not breed in the previous season, and will also not breed in the upcoming season. Female loggerhead turtles in Greek nesting grounds have been estimated to nest on average every two years (Hays et al., 2010), which would imply that the quiescent period may be shorter in the Mediterranean subpopulation, given that vitellogenesis is estimated to start about seven to eight months prior to the migration to the breeding area (Wibbels et al., 1990).

The process of reproduction is less energetically costly for male turtles, and adult males can reproduce annually (Hays et al., 2010). The two adult males that were found stranded in June 2024 showed gonads with mature development. The flaccid appearance of testes and epididymides, as well as the month of stranding, indicate that these adults were in quiescence. Mediterranean male turtles return from their breeding sites between May and June (Casale et al., 2018), which coincides with the presence of the two males in the northern Adriatic Sea. However, it cannot be said if these individuals bred in the season of the same year, or the previous one, as the remigration interval of Mediterranean males is 1-1.8 years (Casale et al., 2018). As male turtles may breed annually, the transition of testes and emididymides from regression after nesting season to quiescent is more rapid than in females, and not further distinguished (Miller and Limpus, 2003). According to Wibbels et al. (1990), spermatogenesis in loggerhead turtles requires about nine months, and maximum spermatogenesis occurs during courtship. In Mediterranean males that will breed in the next season, this would correspond an initiation of spermatogenesis between July and August, giving that the peak mating season in the Mediterranean is between April and May (Casale et al., 2018, Schofield et al., 2013; Schofield et al., 2017). If the two mature males would have prepared to breed in the coming season, the beginning of their spermatogenesis would therefore have started shortly after they were found stranded. Due to the advanced DCC the testes of these individuals were not suitable for histology, so that the stage of spermatogenesis could not be determined to confirm the reproductive status.

Pérez et al. (2010) reported male hawksbill turtles with early spermatogenic activity, but a tail development that was insufficient for the fertilization of females, which they therefor classified as pubescent. This suggests that the development of these reproductive features does not necessarily occur synchronous, and that males that are anatomically not yet able to reproduce may already show spermatogenic activity. However, there are no thresholds for loggerhead sea turtles which define at which tail length, or CCL/tail length ratio, male individuals can be considered mature. The two mature males in the present study had a CaTT of 5 cm (T41/24, CCL = 64 cm, CCL/CaTT ratio = 0.078) and 18 cm (T46/24, CCL = 77 cm, CCL/CaTT ratio = 0.234). However, 5 cm seems to be a small tail length for successful copulation, since Rees et al. (2013) found that only a CaTT of ≥ 6 cm (named "TL" in their study) can be used for differentiating between male and female turtles. Therefore, research focusing on the correspondence of gonad development and tail length are strongly necessary to better to make reliable inference on reproductive maturity in male loggerhead turtles.

No mature animals with gonads in breeding condition were found along the Veneto coastline between January 2024 to February 2025. However, it is possible that the three mature females that were preparing to breed would have stayed in the Veneto region for nesting, given the presence of two nests in 2021 (Pietroluongo et al., 2023). In particular, T20/24 was found on 18.05.24, which is relatively late for the start of breeding migration, as the peak mating period in the Greek breeding areas takes place between April and May (Casale et al., 2018; Schofield et al., 2013; Schofield et al., 2017). For successful reproduction, mature individuals of both sexes must co-occur in time and space. In June 2024, two mature males (T41/24 and T46/24) and one mature female (T59/24) were found, and male T46/24 and female T59/24 stranded in relatively close proximity (zone 3 and zone 5), however, all individuals were in regression after nesting season or quiescence, and there were no reports on nesting along the northern Adriatic coastline during that period. Successful nesting and hatching require the availability of a suitable nesting beach, which in the Veneto region constitutes a challenge due to the highly urbanized coast and human activity on sandy beaches (Pietroluongo et al., 2023).

Histological analysis confirmed the maturity level of five female and one male turtle. While the prepubescent female T01/25 showed numerous small oocytes in early developmental stages, the oocytes of the pubescent female T16/24 showed an increase in size, and the development of a zona pellucida, as well as a more differentiated theca. Both individuals did not show evidence of vitellogenesis according to the criteria from Pérez-Bermúdez et al. (2012), but it must be noted that the ovarian section of the pubescent individual was highly affected by autolysis which impaired the analysis of the intracellular oocyte components. Mature females were characterized by the presence of follicles of different sizes and development stage, including large follicles with evidence of yolk deposition. The male turtle T64/24 showed immature testes without spermatogenic activity in the seminiferous tubules, confirming the prepubescent gonad development assessed macroscopically. The results showed that histology is useful to confirm the assessment based on gonad gross morphology, however the quality of results is highly affected by DCC.

#### 4.2.1 Gonad development, CCL and age classes

Individuals with mature gonads had a significantly larger mean CCL (mean 77.36 cm) than prepubescent (mean 57.29 cm) and pubescent (mean 65.35 cm) turtles, which suggests that larger turtles are more likely to be mature. Nevertheless, the overlap in CCL of individuals with gonads in different development stages indicates that size is an inaccurate predictor of maturity in loggerhead sea turtles. This is congruent with results from studies on loggerhead sea turtles in the western North Atlantic and North Pacific, kemp's ridley sea turtles, green sea turtles and hawksbill turtles (Avens et al., 2015; Ishihara and Kamezaki, 2011; Craven et al., 2019; da

Silva Fabrício et al., 2019; Pérez et al., 2010), and underlines the variability in size at sexual maturity (SSM) in loggerhead sea turtles (Avens et al., 2015).

"Prepubescent" was the most frequent gonad developmental stage in both males (47.4%, n = 9/19) and females (38.8%, n = 19/49), and these individuals showed a large range of CCL (29 cm to 82 cm). Given that the average size of loggerhead turtles nesting in the Mediterranean is considered to be 79.1 cm CCL (Casale et al., 2018), and the threshold for adult individuals in this study was > 70 cm, the presence of individuals with > 70 cm CCL and prepubescent gonad development (25%; n = 5/20) is surprising. One possible explanation is that large individuals with prepubescent gonads do not belong to the Mediterranean subpopulation, but have a different genetic origin, since Mediterranean loggerheads are smaller than other populations, and mature at a smaller size (Casale et al., 2018; Tiwari and Bjorndal, 2000). Another possibility is an incorrect assessment of gonad maturity in these animals, either due to the decomposition of the animals, or subjectivity in assessment. One difficulty in macroscopic assessment of gonad maturity is the differentiation between adult individuals in quiescence and immature individuals, for which it is possible that some mature turtles were incorrectly assessed to be prepubescent or pubescent.

Individuals with pubescent gonads ranged between 46 and 83 cm (mean 65.35 cm). During puberty, the gonads develop from an immature, prepubescent state to the mature form (Miller and Limpus, 2003). Limpus (1990) assumed that puberty in loggerhead sea turtles from Australia lasts about 10 years, however this development stage is poorly studied in sea turtles. Pubescent loggerhead turtles in the North Pacific had a size range of 63.6 to 85.3 cm SCL (Ishihara and Kamezaki, 2011), and based on the size at which individuals with pubescent gonads outnumbered prepubescent individuals, the size at puberty was estimated as 66.0 cm SCL. While this value is comparable to the mean CCL of pubescent turtles in the present study, the mean SCLs of pubescent North Pacific loggerhead turtles were 74.5 cm for male and 74.4 cm for female individuals, and therefore larger than individuals from the northern Adriatic Sea.

The CCL of individuals with mature gonads ranged between 64 cm to 89 cm, at a mean size of 77.36 cm. These values are smaller than reported for mature loggerhead turtles from the North Pacific (size at maturity: 82.1 cm; SCL range 73.8 to 91.9 cm; males: mean 83.8 cm, females: mean 82.1 cm) (Ishihara and Kamezaki, 2011), which confirms that Mediterranean loggerheads mature at a smaller size than populations from different origins (Casale et al., 2018; Tiwari and Bjorndal, 2000). The smallest individuals presenting gonads with mature characteristics were a male of 64 cm (T41/24), in a quiescent period, and a female of 68 cm (T20/24) in a condition preparing to breed. These individuals were smaller than the suggested threshold of > 70 cm for adult turtles, however, also Guarino et al. (2020) found a mature male loggerhead turtle in Campania (western Mediterranean Sea, Italy) with a CCL of 65 cm and a mature female with a CCL of 69.5 cm. Females as small as 63 cm CCL have been found nesting in Cyprus and Turkey (Broderick et al., 2003; Margaritoulis et al., 2003, and references therein), suggesting that the presence of a mature female with 68 cm CCL is realistic. In the present study, the gonads of the two smallest mature individuals were too decomposed for histological examination, for which no confirmation of the macroscopic maturity assessment was possible.

The level of gonad development did not correspond to the age classes defined for this study ( ≤ 40 cm = juvenile; 41 – 70 cm = sub-adult; > 70 cm = adult), as 64.7% of the sub-adult individuals had prepubescent or mature gonads (n = 22/34), and 50% of the adult individuals had prepubescent and pubescent gonads (n = 10/20). All individuals ≤ 40 cm CCL had prepubescent gonads (n = 5/5). While misidentification is a plausible explanation for some cases, these results nevertheless underline that size at maturity, and therefor also the size at puberty, marking the transition between prepuberty and maturity, are highly variable among individual loggerhead sea turtles (Avens et al., 2015).

4.2.2. Implications for conservation

SSM is an important aspect for population dynamics and conservation. For sea turtles, SSM is often derived from the size of nesting females, which however leaves uncertainty about dynamics in males (e.g. Avens et al., 2015; Stewart et al., 2007). Margaritoulis et al. (2022) reported a decreasing size of neophyte females (new recruits) on Zakynthos (Greece), one of the major rookeries for Mediterranean loggerhead sea turtles, over a 38-year monitoring period. Female body size is related to clutch size, and therefore the reproductive output, for which a declining size in size of nesting females might have substantial effects for the populations' demography and conservation (Margaritoulis et al., 2022). The smallest CCLs of a mature male (T41/24) with 64 cm CCL and a mature female with 68 cm CCL (T20/24) are therefore important findings towards understanding trends in SSM for the Mediterranean loggerhead sea turtle population and shows how stranding data can complement information collected from nesting beaches.

#### 4.2.3. Study limitation

The most comprehensive and detailed description of sea turtle gonads in different developmental stages based on gonad gross morphology has been compiled by Miller and Limpus (2003), however, only few studies have dealt with gonad maturity in sea turtles based on macroscopic (e.g. da Silva Fabrício et al., 2019; Ishihara and Kamezaki, 2011; Pérez et al., 2010) or microscopic assessment (e.g. da Silva Fabrício et al., 2019; Failla et al., 2019; Guarino et al., 2020; Pérez et al., 2010; Pérez-Bermúdez et al., 2012). This stresses the importance of further studies with larger sample sizes to better understand gonad development and the correlation with turtle size in loggerhead sea turtles and other turtle species. Histological analysis allows a more detailed insight into gonad development, which is particularly relevant for cryptic life stages such as quiescence, that might be misinterpreted as immature gonad tissue, for which future studies are strongly necessary.

### 4.3. Beginning of tail elongation

The tail length is the main sexual dimorphism found in adult sea turtles (Casale et al., 2005). Adult male turtles have longer tails than adult females, for the purpose of fertilizing the eggs during courtship, and the onset of tail elongation is associated with puberty (Ishihara and Kamezaki, 2011; Owens, 1997).

In the present study, the tail measurements PT, CTL and CaTT were analyzed for their possible correlation with CCL and Sex. For PT and CaTT, the respective GLMs and GAMs indicated that males have significantly longer tails than females, and that their tails show a significantly stronger growth. For both measurements the respective models explained 80.23% and 80.7%, and 43.62% and 46.9% of the deviance, which suggests that changes in PT and CaTT are well explained by CCL and Sex. Although the GAM analyzing the effect of CCL and Sex on the CTL measurement suggested a significantly different relationship between CCL and CTL for males and females, this model only explained 33.9% of the data deviance. The respective GLM did not find a significant interaction between Sex and CCL and the reduced GLM only looking at main effects of Sex and CCL on CTL explained only 26.9% of the deviance in the data. These results indicate that PT and CaTT are more suitable than CTL to differentiate male and female turtles based on their tail lengths. This reflects findings from Casale et al. (2005), who concluded that the increase in distance between the cloaca and the tip of the tail was independent from sex.

The CCL values at which males began to develop significantly longer tails was estimated as 50.2 cm for PT and 56.2 cm for CaTT. These values contradict findings from Casale et al. (2005), who analyzed the tail length of 2631 loggerhead sea turtles and concluded that tail elongation in males begins at a CCL around 70 cm, while full maturity would be reached between 75 to 80 cm. However, it must be noted that the exact origin of the turtles used in that study is not clear, since they were collected in the waters around Italy, which suggests that some of them might also stem from a different loggerhead sea turtle subpopulation than the Mediterranean, with different growth dynamics. In a different study on Mediterranean loggerhead turtles foraging in Amvrakikos Gulf (Greece), tail elongation was found to begin between 60 to 65 cm SCL, and clear differences between males and females were evident at 75 cm SCL (Rees et al., 2013). The authors suggested that a tail length (CaTT; termed "TL" in their study) of $\geq$ 6 cm is indicative of male sex. In the present study, two individuals were found to be females based on their gonads, however they had CaTT measurements of 8 cm (T59/24; CCL 74 cm) and 10 cm (T103/24; CCL 86 cm), and would have incorrectly been assessed as males if only this criterion would have been used for sex determination. Ishihara and Kamezaki (2011), studying loggerhead sea turtles in the North Pacific, used a linear regression model to find the point of divergence between male and female tail elongation, which was determined to be 65.8 cm SCL. This size coincided with the size at puberty in this population (66 cm SCL). In the present study, the size at puberty ranged between 43 to 86 cm, and the estimated beginning of tail elongation of 50.2 cm (PT) and 56.2 cm (CaTT) falls inside this range. It should be noted that the tail elongation models in this study were based on a sample with low number of male observations available (n = 31). Moreover, tail measurements in stranded individuals can be affected by DCC if the animal is bloated or the cloaca is protruded, which may influence the results. Continued research with larger sample sizes aiming to understand whether the beginning of tail elongation coincides with the beginning of puberty in Mediterranean loggerhead turtles are therefore necessary.

# 5. Conclusion

The present study shows that the northern Adriatic Sea is inhabited by loggerhead sea turtles of variable sizes and different stages of reproductive maturity. Based on CCL, most individuals belonged to the sub-adult age class (41 to 70 cm; 61%), followed by adult-sized individuals (> 70 cm; 21.9%), while only 13.4% of the strandings were juveniles (≤ 40 cm CCL). However, the analysis of the gonads showed that the age classes based on CCL did not necessarily correspond to the level of gonad development.

The stranding data revealed an evident seasonality in stranding trends, with most turtles stranded in summer (64.8%) and the least in winter (3.3%). Individuals stranded in summer had a significantly larger CCL than those stranded in spring, which might be related to the seasonal breeding migration of mature adults, that can be expected to be of larger size. However, the high occurrence of prepubescent individuals during summer months could not be explained by this, and further studies are needed to understand temporal trends in stranding frequencies. The uneven distribution of strandings along the Veneto coastline with a peak in zone 6 and 7 may be related to anthropogenic factors, however further research is needed to draw meaningful conclusions.

Most of the analyzed individuals were classified as prepubescent (41.2%), 25% showed pubescent gonad development and 20.6% were assessed to be mature, which demonstrates the importance of the northern Adriatic Sea for loggerhead sea turtles in different stages of gonad development. The presence of mature individuals in different phases of their reproductive cycles moreover highlights that this region is a crucial habitat for adult individuals that prepare for breeding or recover from a reproductive season. The flipper tag that identified T104/24 as having nested in the same year in Greece (ARCHELON, personal communication 2024) provided a very interesting insight into the process of gonad regression in females after nesting season and emphasized moreover the value of CMR and collaboration among research institutions in understanding the biology of migratory individuals such as sea turtles. The low number of males in comparison to females might indicate that the assumed female-biased hatchling production on Mediterranean nesting beaches translates into a similar sex ratio at later life stages, however, might also be due to a different habitat preference of male and female turtles and further research is needed.

The smallest individuals with mature gonads were a male of 64 cm CCL and a female of 68 cm CCL. Although below the average size of nesting females in the Mediterranean (79.9 cm), comparable sizes were reported for adult individuals in the Mediterranean. As minimal SSM in sea turtles is usually inferred from the smallest size of nesting females in a population, this demonstrates the informative value of strandings complementing other techniques, in particular for male individuals that are commonly less studied than females. Nevertheless, the large CCL overlap of individuals with different gonad development levels emphasizes that size and maturity are not necessarily related and that SSM can be highly variable inside the same population.

This study confirmed the sexual dimorphism in sea turtles regarding the tail length, and estimated that in males, the tail begins to shows a stronger elongation than in females at a CCL between 50.2 cm (PT) and 56.2 cm (CaTT). CTL was found to be less useful to differentiate between the sexes. While it is known that a sufficiently developed tail is necessary for successful reproduction in males, a threshold for maturity is not available for loggerhead sea turtles and the small sample size of only two mature males in the present study did not allow to draw meaningful conclusions.

The present study provides the first analysis of reproductive maturity of loggerhead sea turtles in the northern Adriatic Sea, based on gonad gross morphology and histology. While it constitutes a first overview of the population demography in that regard, the decomposition of the carcasses limited the possible analyses and allowed only a sub-sample of the individuals to be assessed. This emphasizes the need for further research using standardized assessment of gonad development to better understand reproductive maturity of loggerhead sea turtles.

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# Appendix

## Appendix A: Sea Turtle Necropsy Form

SCHEDA NECROSCOPIE TARTARUGHE MARINE

**ID:**

**· ANIMAL HISTORY**

| | | | | |
|---|---|---|---|---|
| **Stranding date** | | **Recovery date** | | **Recovery operator** |
| **Necropsy date** | | **Necropsy operator** | | |

| | | | |
|---|---|---|---|
| **Modality of discovery** | stranding | Capture with net type: | adrift |
| **Place of discovery** (Municipality, Province, Locality) | | | |
| **Geographic coordinates** (Format: 45.NNNNNN, 12.EEEEEE) | | | |
| **Temperature of conservation** | room temperature | refrigerated | frozen |

| | | | | | |
|---|---|---|---|---|---|
| **DCC at stranding** | 1 | 2 | 3 | 4 | 5 |
| | Live/Fresh dead (2h) | Fresh carcass (24h) | Moderate decomposition | Advanced decomposition | Mummified or skeletal remains |

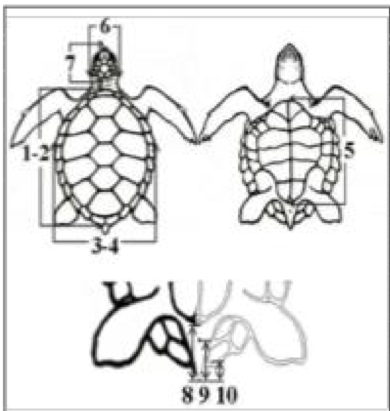
**· STRANDING DETAILS**

| | | | |
|---|---|---|---|
| **Species** | *Caretta caretta* | *Chelonia mydas* | *Dermochelys coriacea* |
| **Tags** | Position: | Code: | Origin: |

**· STATO DI CONSERVAZIONE**

| | | | | | |
|---|---|---|---|---|---|
| **Decomposition Conservation Code** | 1 | 2 | 3 | 4 | 5 |
| | Live/Fresh dead (2h) | Fresh carcass (24h) | Moderate decomposition | Advanced decomposition | Mummified or skeletal remains |

**· MORPHOMETRIC DATA**




| | | |
|---|---|---|
| 1.**CCL** n-t (cm) | 2.SCL n-n (cm) | **Weight** (kg) |
| 3.CCW (cm) | 4.SCW (cm) | |
| 5.CPL (cm) | 6.HW (cm) | 7.HL (cm) |
| 8.PT (cm) | 9.CTL (cm) | 10.CaTT (cm) |
| ANTERIOR LIMB: (cm) | POSTERIOR LIMB: (cm) | |

**1-2:** curved/straight carapax length;
**3-4:** curved/straight carapax width;
**5:** plastron length;
**6:** head width;
**7:** head length;
**8:** plastron–tip of the tail;
**9:** cloaca–tip of the tail;
**10:** carapax–tip of the tail

| | |
|---|---|
| Report the presence of wounds and/or anomalies on the figure and describe them on the right;<br>Also note the presence of tar, oil, ropes, fishing lines, debris, damage from propellers, etc. | |

**· EXTERNAL EXAM**

| | |
|---|---|
| **1) Carapax** (fractures, scars, bruises…) | **2) Plastron** (fractures, scars, bruises…) |
| **3) Flippers** (scars, fishing lines, amputations...) | **4) Skin in axillary/inguinal areas** (wounds, scars...) |
| **5) Head, eyes, beak and oral cavity** (fractures, bruises, scars, foreign bodies, bleeding…) | **6) Cloaca** (lacerations, bleeding, leakage of intestinal content…) |

| | | | | |
|---|---|---|---|---|
| **Epibionts** | Species: | Distribution: | 0-25% | 25-50% |
| | | | 50-75% | 75-100% |
| **Parasites** | Species: | Distribution: | Quantity (indicate total number if possible): | |

**· NUTRITIONAL CONDITION**

| | | | |
|---|---|---|---|
| Optimal (with the presence of hepatic lipidosis) | good | moderate | poor |

**· INTERNAL EXAM**

| | |
|---|---|
| 1) **Muscles** (atrophy, hemorrhages, hematomas, discolorations) | 2) **Thyroid gland and thymus** (lesions, dimensions)<br><br>peso tiroide: gr peso timo: gr |
| 3) **Pericardium, heart and large vessels** (effusions, myocardial lesions, vessel wall lesions, parasites)<br><br>weight of heart: g | 4) **Coelom and coelomic cavity** (body wall, effusions, lesions, adhesions, organ topography)<br><br>volume of effusion: ml |
| 5) **Liver** (dimensions, lesions of the capsule and/or parenchyma, consistency, gallbladder)<br><br>weight: kg | 6) **Esophagus** (content, mucosa, foreign bodies/marine litter)<br><br>volume of content: |

| | |
|---|---|
| 7) **Stomach** (contents, wall, mucosa, foreign bodies/marine litter, parasites)<br><br>volume of content: | 8) **Intestine** (contents, mucosa, foreign bodies/marine litter, parasites)<br><br>volume of content: |
| 9) **Spleen and pancreas**<br><br>weight of spleen: g | 10) **Gonads** (Macro description and possible presence of eggs):<br><br>**MALE** / **FEMALE**<br>Size (testis length or mean follicle/egg diameter) (cm): |
| 11) **Bladder** (contents, wall, mucosa, parasites)<br><br>volume contenuto: (ml) | 12) **Kidneys** (capsule, parenchyma, perirenal area) |
| 13) **Adrenal gland**<br><br>dimensions: (cm) | 14) **Trachea and bronchi** (wall, mucosa, luminal fluids, parasites) |
| 15) **Lungs** (air content, fluids, lesions in the parenchyma, penetrating wounds) | 16) **Eyes** |
| 17) **Salt glands** | 18) **Brain** |

- **NOTES**

- **MAIN CONCLUSIONS**