

SIZE AT MALE MATURITY, SEXING METHODS AND ADULT SEX RATIO IN LOGGERHEAD TURTLES (*CARETTA CARETTA*) FROM ITALIAN WATERS INVESTIGATED THROUGH TAIL MEASUREMENTS

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Tail length is the main secondary sexual characteristic of adult sea turtles. In order to assess the size at which sexual dimorphism in this character becomes evident, six different measurements of tail length were collected or calculated from 2631 *Caretta caretta* specimens found in the waters around Italy. These data show that an average male develops a longer tail at a size of 70 cm and attains sexual maturity at a size of 75-80 cm Curved Carapace Length. Studies of adult sex ratio based on tail measurements should therefore be restricted to specimens >75 cm. The distance from the cloaca to the posterior margin of the carapace appears to be the most effective measurement for sexing turtles of this size among the six characteristics investigated. In the sample, females are estimated to comprise 61% of the specimens >75 cm.

Key words: male sexual maturity, Mediterranean Sea, sexing method, sexual dimorphism

INTRODUCTION

The loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758), is globally classified as an endangered species (Hilton-Taylor, 2000). Population sub-structuring and genetic isolation – at least at the female level (Schroth *et al.* 1996, Laurent *et al.* 1998) – make Mediterranean loggerheads vulnerable to numerous anthropogenic threats, particularly high fishing effort. Therefore, understanding the population dynamics and demography of these animals are important for any effective regional conservation strategy. In this context, sex ratio and age/size at maturity are among the most important demographic parameters. In the Mediterranean, a few investigations on sex ratio at sea have been carried out on juveniles (Casale *et al.*, 1998; Casale & Freggi, in press; Lazar *et al.*, in press) but no data are available for adults. Size at maturity of females can be inferred from data on nesting females (Margaritoulis *et al.*, 2003), but size at maturity of Mediterranean males is unknown.

Different methods are available to obtain sex ratios in hatchling, juvenile and adult sea turtles (review in Wibbels, 2003). Although it requires the sampling of turtles at sea, adult sex ratio is commonly regarded as relatively easy to obtain because adults are sexually dimorphic. In particular, the most obvious characteristic is the large and muscular prehensile tail of adult males (Wibbels, 1999). However, using tail length for diagnosing the sex makes an important, but often implicit assumption: i.e. a turtle with a 'short' tail is more likely to be a female than an immature male. This assumption can be satisfied if the sample includes only turtles above

the average size of adult males or the average size in which males begin to show an evidently longer tail. Unfortunately, average or even minimum size of nesting females are usually used as threshold size of specimens in such studies, because size at maturity of males is much more difficult to know. Hence, this lack of knowledge bears the potential risk of underestimating the number of males, particularly if they mature at a size larger than the average or minimum size of nesting females.

A second problem of using tail length for diagnosing sex is to have a meaningful measurement and a rigorous threshold value. On the contrary, usually thresholds are arbitrary and both they and measurements vary depending on the researcher. Furthermore, these methods are apparently not based on any specific study. The most widespread measures – used with constant arbitrary thresholds differing between researchers – are 'Plastron Tip of Tail' and 'Carapace-Tip of Tail'; furthermore, according to Bolten (1999) adult males would have a higher 'Plastron-Tip of Tail' to 'Cloaca-Tip of Tail' ratio than adult females.

The aim of the present study, based on a large sample, is to (1) detect the size at which sexual dimorphism in tail length becomes evident, and thus the size at male sexual maturity; (2) investigate which type of measure and threshold value are the most appropriate ones for sexing adult/maturing turtles; and (3) provide an adult sex ratio for the study area.

MATERIALS AND METHODS

As part of a research and conservation programme (Argano, 1992) we collected biometric data from 2631 live *Caretta caretta* specimens ranging from 17 to 109

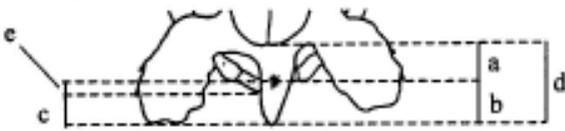


FIG. 1. Tail measurements. Measured: a, Plastron-Cloaca; b, Cloaca-Tail; c, Carapace Tail. Calculated: d, Plastron-Tail; e, Carapace-Cloaca. See materials and methods for details.

cm CCLn-t from 1981-2003. These turtles were found in different circumstances (incidentally caught by longline, trawl, nets, and other fishing methods; taken from the sea surface by hands or handled tools; stranded; found while nesting; seized; unknown origin). These data included curved carapace length notch to tip (CCLnt; Bolten, 1999) and three measurements of tail: (1) distance from the posterior margin of the plastron to cloaca (Plastron-Cloaca; Fig. 1a), (2) distance from cloaca to the tip of the tail (Cloaca-Tail; Fig. 1b), (3) distance from the posterior margin of the carapace to the tip of the tail (Carapace Tail; Fig. 1c). From these three tail measurements we calculated three other values: (4) distance from the posterior margin of the plastron to the tip of the tail (Plastron-Tail; Fig. 1d), (5) distance from the

posterior margin of the carapace to the cloaca (Carapace-Cloaca; Fig. 1e), and (6) Plastron-Tail to Cloaca-Tail ratio (Plastron-Tail/Cloaca-Tail). In relationships between carapace length and tail parameters, for each 5-cm carapace length class we calculated the upper value including 99% of the sample as mean + 2.33 SD; we used only classes with a reasonable number of specimens (min = 48).

Ten turtles were certainly adult females, because they were found while nesting or they were recaptured at sea with tags identifying them as females which nested previously (D. Margaritoulis, pers. comm.). These specimens were excluded from the sex ratio estimation, because their sampling was not independent of their sex. 95% Confidence Intervals of sex ratios were calculated according to the method for binomial distributions (Zar, 1999).

RESULTS AND DISCUSSION

All parameters except 'Plastron-Tail/Cloaca-Tail' showed a strong difference between specimens above and below 65 cm CCLnt (Fig. 2). From the 65-70 size class onward, more specimens with high values were

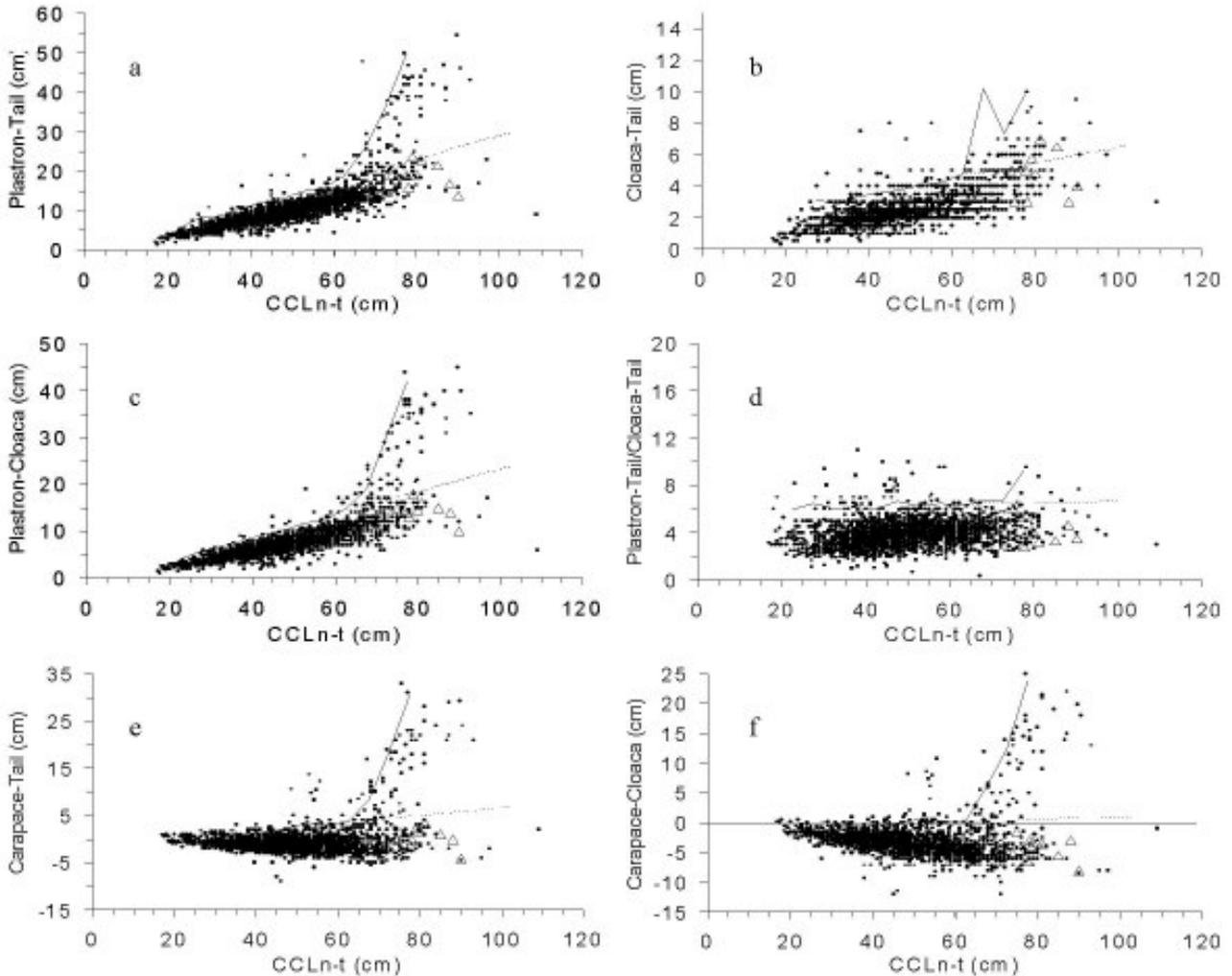


FIG. 2. Relationships between tail-related measures and carapace length ($n=2631$). Solid line includes 99% of the sample (see text). Dotted line is its tendency in the range >65 cm based on values in the range <65 cm. Triangles represent adult females.

observed than expected from the distribution in the previous size classes. This suggests that an average Mediterranean male begins to develop an elongated tail around a size of 70 cm CCLn-t and that full maturity is probably attained around 75-80 cm, the first size class in which tail lengths reach maximum values. Therefore, in the Mediterranean, only loggerheads with CCLn-t >75 cm should be used for sex ratio estimates based on tail measurements.

Limpus (1990) found that in Heron Island Reef (Queensland) the average loggerhead female starts breeding at a size slightly smaller than the average size of nesting females. The means observed in Greece, hosting the main known nesting sites in the Mediterranean, are around 83 cm CCLn t (Margaritoulis *et al.*, 2003). If the rule above applies to the Mediterranean populations too, present findings do not suggest a strong difference between male and female size at maturity.

The six tail parameters investigated show different characteristics affecting their efficacy for sexing turtles. 'Plastron-Tail' (the whole 'tail'; Fig. 2a) can be separated into two measures: 'Plastron Cloaca' and 'Cloaca Tail'. On the basis of specimens known to be adult females and the lack of an evident clustering, the increasing of 'Cloaca-Tail' observed in large-size specimens appears to be independent of sex (Fig. 2b), in contrast to 'Plastron-Cloaca' (Fig. 2c). Thus, 'Plastron-Cloaca' seems to be a better indicator of sex than 'Cloaca Tail' and 'Plastron Tail'. Generally, measures considering the cloaca (like 'Carapace-Cloaca') are expected to be better indicators of maleness than those considering the tip of the tail.

Probably for the same reason, the ratio based on the tip of the tail ('Plastron-Tail' to 'Cloaca-Tail'; Fig. 2d) does not show any clustering in the large size class and the specimens known to be adult females distribute across most of the range; so this ratio is unlikely to be useful for diagnosing sex.

'Plastron Cloaca' (Fig. 2c) (and 'Plastron Tail'; Fig. 2a) is positively correlated to carapace length in all size classes; in presumed males (higher cluster) this relationship would just have a different degree. Hence, a sexing threshold for this measurement should be a size dependent variable, and not a constant, otherwise large females would be wrongly diagnosed as males. On the contrary, it seems that 'Carapace Tail' (Fig. 2e) remains constant and 'Carapace Cloaca' (Fig. 2f) even decreases as size increases, except for presumed males, where it would change in a positive relationship. So, using only one threshold for the whole large size class would be appropriate with these two measures, which are so preferable to 'Plastron Cloaca'. Good candidates as sexing thresholds are the values 5 and 0 cm for 'Carapace Tail' and 'Carapace Cloaca' respectively (Figs. 2e, 2f), which include 99% of the immature size sample as well as all known adult females of the sample. Moreover, 'Carapace Cloaca' seems to be preferable to 'Carapace Tail' because of the considerations made above concerning tail vs. cloaca, the negative correlation with size in

immatures and adult females in contrast to the positive correlation in presumed adult males, and the very convenient threshold. This threshold means that only males would have a cloaca protruding beyond the carapace, and for the field work this implies that no measurements at all need to be taken, making data collection very easy. Hence, 'Carapace Cloaca' appears to be the best of the six tail parameters taken into consideration. It is interesting that its threshold value (0 cm; Fig. 1e, 2f) is probably not arbitrary, but may have a biological reason, because possible injuries of the distal part from the cloaca (the true tail) are less harmful than ones on the proximal part, and females would have no advantage balancing the disadvantage of exposing a vulnerable part beyond the protection of the carapace. On the other hand, males have the fundamental need to be able to mate.

Although measuring tail length cannot be so accurate as laparoscopy in diagnosing sex of large specimens (i.e. those thought to be adults), nonetheless it is easy to apply in any field condition and can provide a large amount of data, provided that a good method is used. In summary, present results suggest 'Carapace Cloaca' as the best of the six tail parameters taken into consideration, and in the Mediterranean it should be used on specimens larger than 75 cm only.

With the method above a female proportion of 61% is calculated for specimens larger than 75 cm found at sea in the study area ($n=69$). However, adult sex ratio can be biased by sex specific patterns of reproductive migration, and to avoid this bias it is preferable to estimate sex ratios during nonmigratory periods only (Wibbels, 2003). So we calculated sex ratios for two periods: a 'warm' one (Apr-Sep), when reproductive migration occurs, and a 'cold' one (Oct-Mar), when reproductive migrations are unlikely to occur. The resulting sex ratios were 53.8% (95% CI: 39.5-67.8%; $n=52$) for the warm period and 76.5% (95% CI: 50.1-93.2%; $n=17$) for the cold one. Although the two sex ratios are not significantly different (Fisher exact test: $P=0.15$; $n=69$) possibly due to the small sample size, we conservatively considered the cold period sex ratio more representative of the real one, which would be likely to be skewed towards females.

This finding is apparently in contrast with unbiased sex ratios recently reported by two studies on loggerhead turtle juveniles in the Mediterranean (Casale & Freggi, in press; Lazar *et al.*, in press), but this difference could be explained by male-biased juveniles coming from the Atlantic (Casale *et al.*, 2002).

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REFERENCES

- Argano, R. (1992). Sea turtles and monk seal in Italian seas: conservation and perspectives. *Bollettino del Museo dell'Istituto di Biologia dell'Università di Genova* **56-57**, 113-135.
- Bolten, A. B. (1999). Techniques for measuring sea turtles. In *Research and Management Techniques for the Conservation of Sea Turtles*, 110-114. Eckert, K. L., Bjørndal, K. A., Abreu-Grobois, F. A. and Donnelly M. (Eds.). Washington, DC: IUCN/SSC Marine Turtle Specialist Group Publication No. 4.
- Casale, P. & Freggi, D. (in press). Sex ratio of loggerhead turtle (*Caretta caretta*) juveniles in the central Mediterranean through direct observation of gonads. In *Proceedings of the Twenty-third Annual Symposium on Sea Turtle Biology and Conservation, Kuala Lumpur, 17-21 March 2003*. Miami: NOAA Technical Memorandum.
- Casale, P., Gerosa, G., Argano, A., Barbaro, S. & Fontana, G. (1998). Testosterone titers of immature loggerhead sea turtles (*Caretta caretta*) incidentally caught in the Central Mediterranean: a preliminary sex ratio study. *Chelonian Conservation and Biology* **3**, 90-93.
- Casale, P., Laurent, L., Gerosa, G. & Argano, R. (2002). Molecular evidence of male-biased dispersal in loggerhead turtle juveniles. *Journal of Experimental Marine Biology and Ecology* **267**, 139-145.
- Hilton-Taylor, C. (2000). *2000 IUCN Red list of threatened species*. Gland: IUCN.
- Laurent, L., Casale, P., Bradai, M. N., Godley, B. J., Gerosa, G., Broderick, A. C., Schroth, W., Schierwater, B., Levy, A. M., Freggi, D., Abd El-Mawla, E. M., Hadoud, D. A., Gomati, H. E., Domingo, M., Hadjichristophorou, M., Kornaraky, L., Demirayak, F. & Gautier, Ch. (1998). Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. *Molecular Ecology* **7**, 1529-1542.
- Lazar, B., Lackovic, G., Tvrtkovic, N. & Tomljenovic, A. (in press). Histology of gonads and preliminary sex ratios in juvenile loggerhead sea turtles, *Caretta caretta*, in the eastern Adriatic Sea. In *Proceedings of the Twenty-third Annual Symposium on Sea Turtle Biology and Conservation, Kuala Lumpur, 17-21 March 2003*. Miami: NOAA Technical Memorandum.
- Limpus, C. J. (1990). Puberty and first breeding in *Caretta caretta*. In *Proceedings of the Tenth Annual Workshop on Sea Turtle Biology and Conservation*, 81-83.
- Richardson, T. H., Richardson, J. I. and Donnelly, M. (Compilers). Miami: NOAA Technical Memorandum NMFS-SEFC-278.
- Margaritoulis, D., Argano, R., Baran, I., Bentivegna, F., Bradai, M. N., Caminas, J.A., Casale, P., De Metrio, G., Demetropoulos, A., Gerosa, G., Godley, B., Houghton, J., Laurent, L. & Lazar, B. 2003. Loggerhead turtles in the Mediterranean Sea: present knowledge and conservation perspectives. In *Loggerhead Sea Turtles*, 175-198. Bolten, A. B. & Witherington B. (Eds.). Smithsonian Institution Press.
- Schroth, W., Streit, B. & Schierwater, B. (1996). Evolutionary handicap for turtles. *Nature* **384**, 521-522.
- Wibbels, T. (1999). Diagnosing the sex of sea turtles in foraging habitats. In *Research and Management Techniques for the Conservation of Sea Turtles*, 139-143. Eckert, K. L., Bjørndal, K. A., Abreu-Grobois, F. A. and Donnelly M. (Eds.). Washington, DC: IUCN/SSC Marine Turtle Specialist Group Publication No. 4.
- Wibbels, T. (2003). Critical approaches to sex determination in sea turtles. In *The Biology of Sea Turtles, vol. II*, 103-134. Lutz, P. L., Musick, J. A. and Wyneken J. (Eds.). Boca Raton: CRC Press.
- Zar, J. H. (1999). *Biostatistical Analysis, Fourth edition*. Upper Saddle River: Prentice-Hall Inc.

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