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Sex ratios of juvenile loggerhead sea turtles *Caretta caretta* in the Mediterranean Sea

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ABSTRACT: Species with environmental sex determination may show sex ratios that differ from 1:1; therefore, sex ratio is an important variable when studying the population dynamics of these species. For instance, when estimating population size and productivity, sex ratio would be a required parameter. For endangered species, such as sea turtles, this is particularly important in order to understand the possible effects of human impacts and conservation measures. Unfortunately, only adult sea turtles show evident external sexual dimorphism; sex ratios are more difficult to obtain for the juvenile class, which represents the largest part of a population. Here we present the first extensive assessment of the sex ratio of juvenile loggerhead turtles *Caretta caretta* in the Mediterranean Sea. A total of 310 dead turtles from 4 different areas were sexed by direct examination of gonads, the most reliable sexing method. Females comprised 54.2% of the whole sample, and no significant differences were observed among study areas. However, this value cannot be ascribed to a single population, because specimens from different nesting sites—both within and outside the Mediterranean—share the same foraging areas, and each population may potentially contribute a different sex ratio. Although we recorded an unbiased sex ratio, our results were compatible with a hypothetical scenario in which Mediterranean nesting beaches produce a majority of females when other information from the region (distribution, mixed stocks and sex ratios) was considered.

KEY WORDS: Sex ratio · *Caretta caretta* · Mediterranean · Juvenile sea turtles

INTRODUCTION

As in many other reptiles (Janzen & Paukstis 1991), the sex of sea turtles is determined by the temperature to which an embryo is exposed during its development. The point at which a balanced sex ratio occurs is known as the pivotal temperature; in sea turtles, more females result from temperatures above the pivotal temperature and more males from cooler temperatures (see Wibbels 2003 for a review).

As a general consequence, skewed sex ratios are more common in species with temperature-dependent sex determination (TSD) than in species with a genotypic sex determination (Bull 1980). For this reason, the sex ratio must also be considered in population dynamics of species with TSD. Important parameters such as

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population size and productivity can only be correctly estimated if the sex ratio is known. Since all species of sea turtles are threatened, this is particularly important for our understanding of how they may respond to both anthropogenic effects and conservation measures. Unfortunately, sex ratio is not easy to assess in sea turtles, and this leads to increased uncertainty in population models. An understanding of natural sex ratios and possible sex ratio variability among species and populations is also important in our understanding of the evolutionary basis of environmental sex determination (Mrosovsky 1994).

For convenience, 3 major life-stages (hatchlings, juveniles, and adults) can be distinguished in sea turtles; a different method is used to investigate sex ratios at each stage. Hatchling sex ratio is obtained directly, through examination of gonads of hatchlings sampled at nesting beaches (e.g. Yntema & Mrosovsky 1980), or indirectly, estimated from nest temperature (e.g. Standora & Spotila 1985) or other variables associated with nest temperature such as incubation duration (e.g. Mrosovsky et al. 1999). Although hatchlings can be sampled on land, other life stages must be sampled at sea. Adults are easily differentiated because of their external sexual dimorphism (Casale et al. 2005a). Juvenile sex ratio is the most difficult to obtain, owing to both the at-sea sampling requirement and the absence of sexual dimorphism. Sex ratio can be determined by blood hormonal dosage (Owens et al. 1978), observation of gonads by laparoscopy (Wood et al. 1983), or necropsy (Work 2000) in the case of dead animals. Juveniles represent the greatest part of the population and a pool of many cohorts, so an assessment of their sex ratio is of particular importance.

Investigations of the sex ratio of all 3 classes of loggerhead turtles (hatchlings, juveniles, and adults) have recently begun in the Mediterranean region, but so far lack results that can be considered as conclusive at the population scale. Hatchling sex ratio was skewed towards females in estimations from Cyprus, Turkey (Kaska et al. 1998, Godley et al. 2001a) and Greece (Rees & Margaritoulis 2004, Zbinden et al. 2006). Available data on incubation duration (shorter incubation periods result from higher nest temperatures, suggesting a female bias, and vice versa) from monitored nesting beaches in the Mediterranean suggest a heterogeneous situation. Female-biased sex ratios are probably common in most years and at most sites, but balanced or even male-biased sex ratios probably occur in at least some years and at some sites (Godley et al. 2001b). These data show that about 53% of monitored nests are laid in beaches where incubation temperatures are probably above the pivotal temperature. The nesting situation in Libya remains unclear. An unknown, but perhaps substantial, number of nests are laid in Libya along a very extensive tract of coast (Laurent et al. 1998a). The only incubation duration data available from Libya suggest a female-biased sex ratio, but this may not be representative as it was based on only 5 nests (Margaritoulis et al. 2003). The Libyan coast is the southernmost turtle nesting area in the Mediterranean, and the high temperatures required for a female-biased sex ratio are theoretically possible. In summary, existing data are insufficient to facilitate estimation of the overall sex ratio of Mediterranean loggerhead hatchling production; however, they are suggestive of a female predominance.

For adults, the only data available for the Mediterranean also suggest a predominance of females (77%; Casale et al. 2005a). A preliminary study of juveniles in the central Mediterranean estimated a sex ratio of ~1:1 (55% females), but this was based on an indirect approach (blood testosterone levels) and could not be considered as conclusive (Casale et al. 1998).

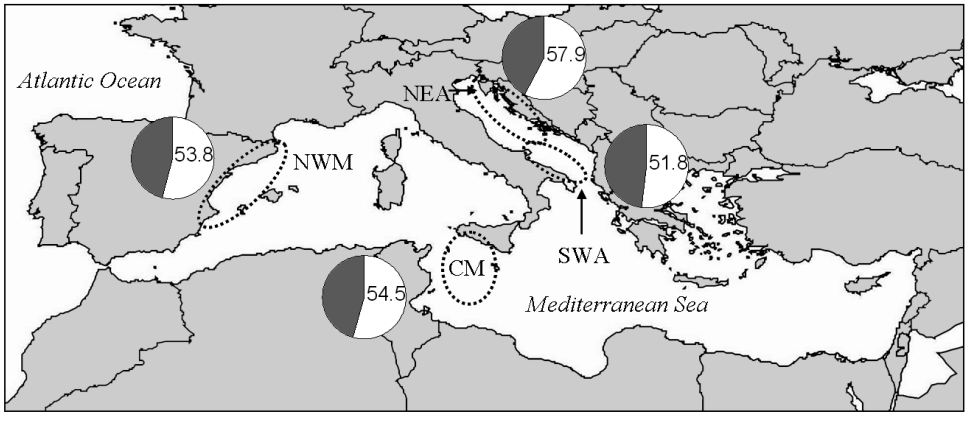

Fig. 1. Sampling areas. NWM: north-west Mediterranean; CM: central Mediterranean; SWA: south-west Adriatic; NEA: north-east Adriatic; % female *Caretta caretta* observed in each area is shown

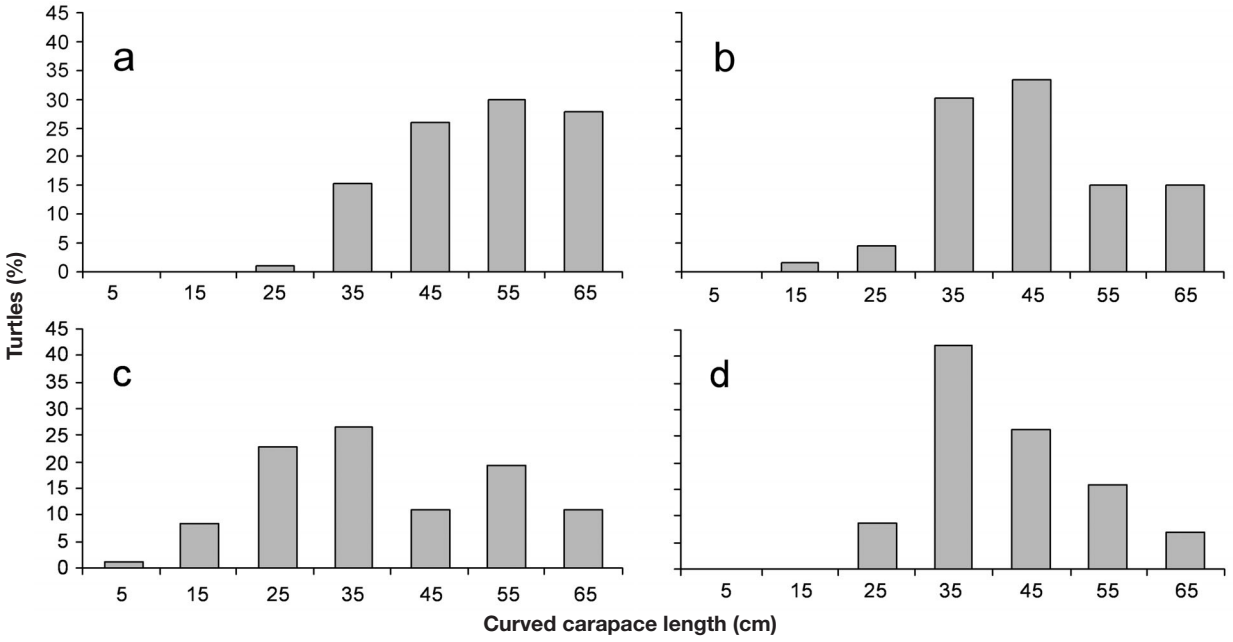

Fig. 2. *Caretta caretta*. Size distribution (cm curved carapace length notch-to-tip) of turtles in 4 study areas: (a) north-west Mediterranean (n = 104), (b) central Mediterranean (n = 66), (c) south-west Adriatic (n = 83), (d) north-east Adriatic (n = 57); total n = 310

The present study aimed to provide the first estimations of sex ratios of juvenile loggerhead turtles from different Mediterranean areas using the most reliable method: direct examination of gonads.

MATERIALS AND METHODS

We examined 310 dead immature loggerhead sea turtles captured by fishing gear (longline, trawl, static net), found floating at sea or stranded between 1986 and 2005. Turtles were collected from 4 different areas of the Mediterranean Sea (Fig. 1): the north-west Mediterranean (Spain, Catalonia and Valencia regions; n = 104); central Mediterranean (Italy; n = 66); the south-west Adriatic (Italy; n = 83); and the north-east Adriatic (Croatia and Slovenia; n = 57).

Sex was determined through direct examination of the gonads, which exhibit sex-specific differences in both external morphology and histology (e.g. Yntema & Mrosovsky 1980, Wyneken 2001, Wibbels 2003). In most cases gonad morphology was examined during necropsy, while in some cases via removal of gonads for examination under a dissecting microscope and in other cases through histological analysis.

In order to assess possible age/size-dependent sex ratios, we arbitrarily subdivided the sample into 2 size classes: ≤40 and >40 cm curved carapace length notch-to-tip (CCL n-t) (Bolten 1999).

Confidence intervals (CI) for sex ratios were calculated using the method for binomial distribution (Zar 1999).

RESULTS

The overall proportion of females was 54.2% (CI 95%: 48.5 to 59.8%; n = 310), with no significant difference observed among the 4 study areas ($\chi^2 = 0.51$; $p = 0.92$; n = 310) (Fig. 1). In 3 of the areas, sex ratios of the 2 size classes (≤40 and >40 cm CCL n-t) did not differ significantly. In the north-west Mediterranean, a lower proportion of females was observed among small specimens (29.4%) than among larger turtles (58.6%) (Fisher's exact test; $p < 0.05$; n = 104). The size distribution (cm CCL n-t) of combined sexes from each area is shown in Fig. 2.

DISCUSSION

When investigating the sex ratio of juveniles, it should be taken into account that different populations may share a common foraging area. Thus, the observed sex ratio might actually represent that of different contributions of these mixing populations.

The basin-wide distribution of loggerhead sea turtles originating from various Mediterranean and Atlantic nesting sites remains poorly understood.

At present, the following information is available from the 4 areas sampled in this study. The western Mediterranean is frequented by both Mediterranean and Atlantic specimens (Laurent et al. 1998b). The central Mediterranean is frequented by turtles from Greece (Margaritoulis et al. 2003), Cyprus (A. Broder-

ick & B. Godley pers. comm.), and the Atlantic (Laurent et al. 1998b). The southern Adriatic seems likely to be frequented by oceanic-stage juveniles, probably from adjacent Greek nesting sites (Casale et al. 2005b), but turtles from the Atlantic can reach this area too (Manzella et al. 1988, Argano et al. 1992). The northern Adriatic is frequented by turtles from Greece (Margaritoulis et al. 2003, Lazar et al. 2004). Therefore, the overall juvenile sex ratio recorded in this study is likely to be a combination of sex ratios from several Atlantic and Mediterranean populations.

Since the sex ratio of Atlantic specimens entering the Mediterranean is probably male-biased (Casale et al. 2002), the sex ratio of ~1:1 reported here would imply that the sex ratio of Mediterranean juveniles, or at least of those from some nesting sites, is female-biased. This scenario would explain why a higher proportion of males was observed among small specimens in the north-west Mediterranean but not among those from other study areas. In fact, based upon the modal size of loggerhead sea turtles captured in the Azores (around 30 cm CCL n-t; Bjorndal et al. 2000), we can assume that Atlantic specimens enter the western Mediterranean at a small size. They then disperse into the central Mediterranean (Laurent et al. 1998b) and perhaps even further eastwards, reaching a larger size during this dispersal (a sea turtle from the Gulf of Mexico was re-encountered in the mouth of the Adriatic Sea at a size of 43.5 cm CCL n-t; Manzella et al. 1988). The absence of Atlantic specimens among adults observed in the Mediterranean (Laurent et al. 1998b) suggests that these turtles eventually leave this region at a certain size. These Atlantic specimens would be predominantly males, and their departure from the Mediterranean agrees well with the observed female-biased sex ratio among Mediterranean adults (77%; Casale et al. 2005a). It is interesting that this adult sex ratio is similar to the hatchling sex ratios estimated from the 2 most important nesting sites assessed in the Mediterranean (Margaritoulis et al. 2003), which were also those closest to our study areas: Zakynthos (75%; Zbinden et al. 2006) and Kyparissia Bay (70%; Rees & Margaritoulis 2004) (western Greece). This also supports the hypothesis that the observed ~1:1 sex ratio of juveniles in our study might not be exclusively indicative of the Mediterranean stock, but may also include juvenile loggerhead sea turtles of Atlantic origin.

Our study demonstrates that investigation of the sex ratio of species with a complex life history, such as sea turtles, is not a simple task. This is particularly true when individuals from different nesting sites share the same marine habitats, at least for a portion of their life-cycle. Hence, in addition to collecting simple data on sex, interpretation of sex ratios requires additional knowledge on distribution, movement and origin of sea turtles.

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