

Sex ratios of loggerhead sea turtles *Caretta caretta* during the juvenile pelagic stage

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Abstract Sex ratios are a fundamental trait for species reproduction. In species with temperature-dependent sex determination (TSD), sex ratios are not necessarily even, which has important demographic consequences. We examined the sex ratio of juvenile pelagic stage loggerhead turtles *Caretta caretta* offshore Madeira Island, North Eastern Atlantic, using laparoscopy and histology. The overall sex ratio was 2:1 (F:M), significantly different from an even sex ratio. Although there was no apparent temporal variation, sex ratios among size classes were significantly different. The sex ratio of juveniles was compared with known sex ratios for the putative source rookery and found to be similar to the subadults' sex ratio, but significantly less female-biased than the hatchlings sex ratio. This suggests overestimation of hatchlings sex ratios and/or, less likely, differential mortality of females during the first months of life. Alternatively, the Madeira Island aggregation may be recruiting males from other geographical sources such as the Mediterranean and the Cape Verde.

Introduction

In sexually reproducing organisms, the sex ratio is an important factor for the determination of population growth. In most dioecious species, males and females are produced in approximately equal numbers, regardless of the mechanism of sex determination (Seger and Stubblefield 2002), to achieve balanced sex ratios (Fisher 1930). However, under some circumstances Fisher's principle may not hold, and 'extraordinary sex ratios' (i.e., different from 1:1) are produced (Hamilton 1967). Striking sex ratios patterns have been described in Hymenoptera (ants, bees and wasps) (West et al. 2002) and in species subject to environmental sex determination (ESD), namely temperature-dependent sex determination (TSD), among them many reptiles (Bull and Charnov 1989; Charnov and Bull 1989a; Ciofi and Swingland 1997), including marine turtles (Wibbels et al. 1987b; Wibbels 2003).

Although the pelagic or oceanic life stage of loggerhead marine turtles, *Caretta caretta*, entails a major part of the life cycle, little is known about this phase. This knowledge gap is largely a result of the logistical difficulty in addressing the juvenile pelagic stage of any hard-shelled sea turtle species whereby individuals leave the nesting beaches as hatchlings and develop in the open sea for many years before moving to inshore foraging grounds as subadults (Bolten 2003b). Therefore, the role and relevance of turtles within the oceanic environment is far from being understood, and the exact numbers, movements and behaviour of pelagic juveniles are poorly documented, with only sparse information available regarding the locations of developmental habitats. For example, knowledge of the loggerhead turtle pelagic juvenile phase is only available from three broad geographic areas: the Mediterranean, the North Atlantic and the North Pacific loggerhead

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populations (Musick and Limpus 1997; Bjørndal 1999; Bolten 2003a, b; Margaritoulis et al. 2003). For a species with a circum-global distribution, this dearth of information indicates a need to prioritise studies of these pelagic populations (Bjørndal 1999).

An opportunity to further our knowledge of pelagic juvenile loggerheads can be developed in the Macaronesian waters of the northeast Atlantic, which comprise the seas around and between the Madeira and the Azores Archipelagos, the Selvagens Islands and the Canary Islands. These waters are used by pelagic populations of several sea turtle species, but by far the most common species are loggerhead turtles (Brongersma 1995; Dellinger 2008). Breeding is historically referenced in the Canary Islands (López Jurado 2007).

Despite a paucity of historical records of juvenile loggerheads [summarized by Carr (1986)], large numbers have been reported from the Azores, Madeira and even between Madeira and mainland Portugal (Dellinger 2008). Juvenile loggerheads are also found alive (Hays and Marsh 1997) or stranded on the coasts of northern Europe (Brongersma 1972, 1982). These loggerhead turtles are almost exclusively (99%) originated from the nesting beaches of the east coast of the United States of America and Mexico (Bolten et al. 1998), arrive in Madeira waters as young post-hatchlings and juveniles, where they spend 6–12 years in an oceanic or pelagic life stage, and leave oceanic waters as they approach subadult (Bjørndal et al. 2000, 2001, 2003).

The current status for loggerheads is “Endangered” (www.iucnredlist.org), and the species are quoted in the Portuguese Regional Red Data Book (Oliveira et al. 2005), thus making it a priority for conservation. For conservation and management purposes, critical demographic parameters such as growth rates, survivorship, recruitment, age at first reproduction, per cent of animals reproductively active each year, age and duration of the reproductive life history and each population’s sex ratio are essential for the development of population models (Owens 1997) and are especially important for marine turtles since all species are threatened (Casale et al. 2006).

Currently, the loggerhead turtles sex ratios known for the western North Atlantic, which hosts one of the largest nesting aggregates in the world (Murphy and Hopkins-Murphy 1989), are discrepant. While hatchling productivity from rookeries in the southeastern United States indicate an highly female-skewed sex ratio (Mrosovsky and Provancha 1989), as high as 6:1 (F:M) (Hopkins-Murphy et al. 2003), the sex ratio of benthic immature loggerheads foraging along the eastern United States is still biased towards females but sex ratios reported for these rookeries are consistently 2:1 (F:M) (Wibbels et al. 1987a, 1991; Owens 1997).

To better understand and to clarify this apparent discrepancy in sex ratios, data on the oceanic phase are needed. Moreover, demographic models and management plans would benefit from data on the immature pelagic phase for sea turtle populations, since for population modelling purposes it is important to incorporate the sex ratios of young cohorts, as they can affect the future reproductive rate of the population.

In immature sea turtles, however, identification of sex to generate populations’ sex ratios using external morphology is not possible (Owens et al. 1978). Loggerheads are dioecious, and sexual dimorphism becomes evident only at adult life stages, with males showing longer tails than females and large developed claws on front flippers used to hold females during copulation (Owens 1997). Males have a shorter plastron, probably to accommodate the large muscular tail (Hughes 1974; Geldiay et al. 1982). Females, in contrast, do not develop any secondary sexual characteristics (Bolten et al. 1994). The age of first sexual maturity for young captive loggerheads was estimated to be 6–7 years (Uchida 1967), but estimates from captive-reared animals are believed to be misleading (Bjørndal and Zug 1995). Not all individuals mature at the same size even within the same population (Limpus et al. 1994a, b), and the age at sexual maturity for loggerheads in nature is now estimated to span 12–37 years (Frazer 1983; Heppell et al. 1996; Bjørndal et al. 2000, 2001) up to 20–63 years (Parham and Zug 1997). None of the phenotypic features that distinguish adults seems to be a reliable indicator of individual sex for juvenile pelagic stage loggerheads, and only one study in the Mediterranean Sea has ever critically assessed this topic (Casale et al. 2005).

Despite its invasiveness, laparoscopy is the most definitive sexing method for immature turtles (Wibbels 1999), although circulating testosterone has also been used and validated by laparoscopy (Diez and van Dam 2003; Braun-Mcneill et al. 2007; Blanvillain et al. 2008). For very small individuals such as hatchlings, histological validation is required (Mrosovsky and Benabib 1990). In this study, we have used laparoscopy and histology to investigate the sex ratios of loggerhead turtles during the pelagic stage in the eastern North Atlantic and compared it with known sex ratios for the other life stages of the same population so that more refined estimates of sex ratio at different stages of life history can be made.

Materials and methods

Study area description and general sampling

The Madeira Archipelago (Portugal) is located off the northwest coast of Africa (~33°N; 17°W), around

1,000 km from the European continent and 500 km from the African coastline (Fig. 1). Depths such as 1,000–2,000 m can be reached as close as 1–2 nautical miles from the coast. The Madeira Archipelago is included in the general North Atlantic circulation system, with the Canary current as the dominant surface current and the NE trades the dominant wind regimes. The island mass effect phenomena provide a sheltered leeward area on the south coast of these Islands (Caldeira et al. 2001), making potential spotting of sea turtles while basking easy during special warm and calm weather.

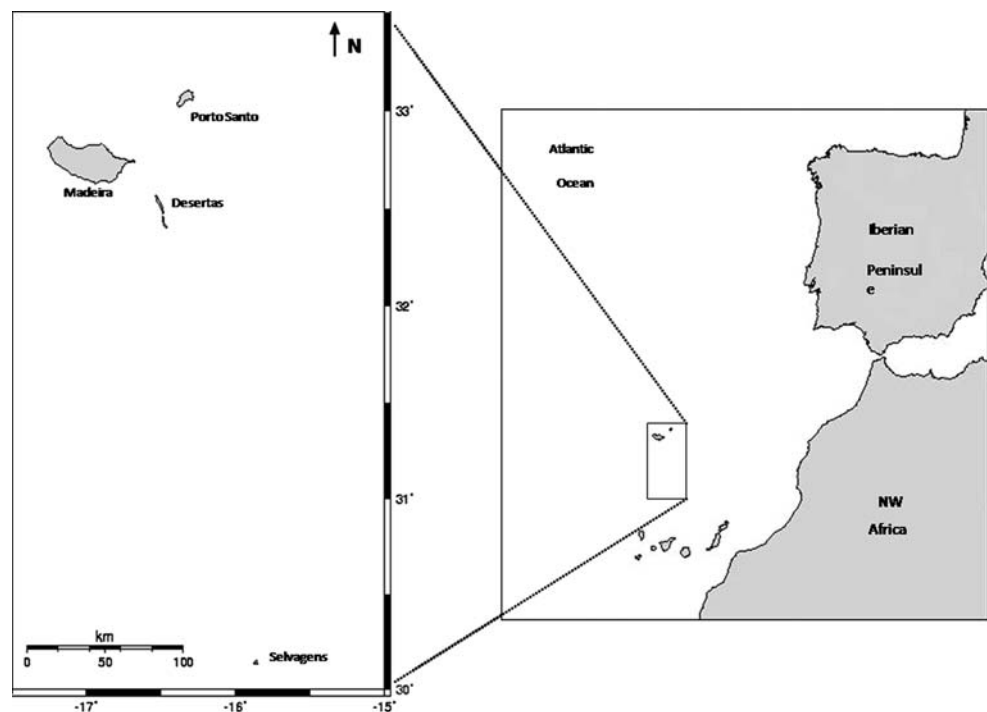
Marine turtles were captured between 2000 and 2006 offshore south Madeira Island. Sampling took place randomly, depending on weather conditions. Turtles were actively searched for by boat up to 10 nautical miles (~ 18 km) offshore and were captured without regard to their size or location by approaching them at slow speed from behind and scooping them up with a large dip-net (Dellinger et al. 1997). Between 2000 and 2006, at least 25 individuals were caught and sexed per year (2000: $n = 43$; 2001: $n = 56$; 2004: $n = 34$; 2005: $n = 36$; 2006: $n = 25$) except in 2002 and 2003 when sampling effort was limited and only 13 individuals were sampled altogether. Overall catching success was over 50% of the animals sighted. Following capture, all turtles were transported to land-based holding facilities where they were maintained overnight in tanks filled with seawater to prevent dehydration. Two large circular tanks (3 m diameter, 1.5 m water depth, $\sim 10,000$ l) and 4 smaller squared tanks ($\sim 1,000$ l) were

used to keep the turtles. All turtles were returned unharmed to their habitat after sampling and tagging procedures.

Each individual turtle was measured: Standard Straight Carapace Length (SCLnt), head width (HW), fore-flipper width (FFW), 1st claw length (CLW), right fore-flipper width (FFW), plastron-cloacae distance (PC), post-cloacae to tip of tail distance (PCL) and tail length (TL), weighted, photographed and tagged with an identifier during the holding period. Straight line measurements were taken to the nearest mm using Hagl f forestry calipers for large biometric measurements (e.g. SCLnt) and Vernier callipers for small biometric measurements (e.g. CLW). Over the curve measurements were taken using a flexible tailor's tape, and weight was taken with an electronic platform balance (Mettler Spider1s60Lst) (nearest 2 g).

From 2000 until June 2003, turtles were tagged on the trailing edge of each fore flipper with Monel style 681 tags (National Band and Tag Co., 721 York Street, PO. Box 430, Newport, Ky 41072-0430, USA, Archie Carr Center for Sea Turtle Research, University of Florida). From July 2003 until May 2005, turtles were additionally tagged with PIT tags [Passive Integrated Transponders, AVID FriendChipTM (AVID Identification Systems, Inc.)], inserted subcutaneously on the ventral side of the shoulder for small size animals (SCL < 30 cm) and for larger animals the region underneath the scales or between the digits of the dorsal surface of the right front flipper (Balazs 1999). From May 2005 onwards, turtles were PIT tagged only.

Fig. 1 Map showing the location of Madeira Archipelago, Portugal, in the North East Atlantic



Sex diagnosis: laparoscopy and gonad biopsy

Laparoscopies were performed using aseptic techniques the day following capture, allowing the animals to fast for a period of >18 h. Laparoscopy equipment consisted of a telescope (telescope 30°, diam. 2.7 mm, length 18 cm from Karl Storz) and a fibre optics projector and light cable, trocar and trocar sleeve, as well as standard surgical instruments. A biopsy forceps and a double cannula were used for biopsy sampling. Equipment was sterilized by soaking in a solution of 70% ethanol and povidone iodine (Betadine®) for 10–30 min prior and between each use, and surgical sterile gloves were used.

Laparoscopies were performed as previously described by Wood et al. (1983) and reviewed by Owens (1999) in order to determine sex, as well as to get video images of the gonad. Turtles were only subject to laparoscopy when the animals' health condition was considered safe to overcome the surgical procedure.

Sex was diagnosed macroscopically by the presence (ovary) or absence (testis) of follicles and overall gonad colouration. After this macroscopical observation, a biopsy forceps was inserted through the same incision in order to withdraw a small gonad biopsy. The gonad biopsy was fixed in Bouin's solution for histological processing. Video images were recorded whenever possible for posterior reference using a handycam attached to the endoscope ocular with an adaptor ring. The incision was sutured using surgical gut [sterile absorbable # 2-0 suture, 26 mm curved cutting needle (triangular point)], and one to two surgical knots and an antiseptic (Betadine®) were applied to the wound. If insufflation was needed, all the air was removed from the coelomic cavity before suturing by gently pressing the lower region of the plastron. The procedure usually took 15–25 min per individual. The animal was then left to rest for up to 24 h in individual dry receptacles (Wood et al. 1982) to allow monitoring of its recovery and then released back in the wild after the completion of all sampling procedures (biometry and tagging).

During the study period, only a few animals were not considered physically fit for surgery. Of the 224 laparoscopies performed, two turtles died subsequent to the surgery, giving a rate of <1% mortality. *Post mortem* examination of these two turtles was supervised by a veterinary but the cause of death was inconclusive. No vital organs were struck during trocar entry.

The gonad biopsy samples (~1 mm diameter) were embedded in paraffin, serially sectioned at 6 µm and stained using standard haematoxylin-eosin (H&E) procedures (Yasutake and Wales 1983; Bancroft and Stevens 1990). Two to three slides were prepared for each individual and observed under a compound light microscope for presence of follicles (ovary) or seminiferous tubules

(testis) (Yntema and Mrosovsky 1980; Merchant-Larios 1999). Intersex was assigned when the gonad exhibited both ovary and testis characteristics (Limpus et al. 1982).

Sex ratios and statistical analysis

Sex ratios were generated following laparoscopy, which was validated through histology. Chi-square analysis was employed to evaluate sex ratio differences among cohorts and among years across the capture period (2000–2006) and with sex ratios known for the western North-Atlantic rookeries (Wibbels 1999). Due to the small number of animals sexed in years 2002 and 2003 (6 and 7 turtles, respectively), these cohorts were pooled together for sex ratio analysis. Unknowns were excluded from the analysis. Values are expressed as mean ± standard deviation (SD). Biometrical parameters were tested for sexual dimorphism using analysis of covariance with SCLnt as covariate.

Results

Laparoscopic observations

Two hundred and twenty-four juvenile turtles were examined and subject to laparoscopy. SCLnt ranged from 171 to 687 mm (mean = 359 mm; SD = 107.4) (Fig. 2) and mass from 824 to 48,450 g (mean = 8,980 g; SD = 7,157.2).

The gonads are paired organs located in the coelomic cavity, caudal to the lungs and attached to the peritoneum overlying the paired kidneys. Under laparoscopic examination, juvenile males were identified by the smooth gonad, variable in colour from pale white to bright orange, often irrigated by a more or less developed capillary web (Fig. 3b, d). In contrast, juvenile females possessed a gonad with a granular aspect with more or less developed follicles easily visible with the naked eye (Fig. 3a, c).

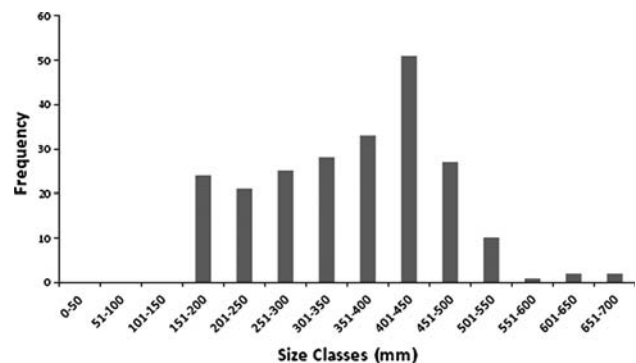
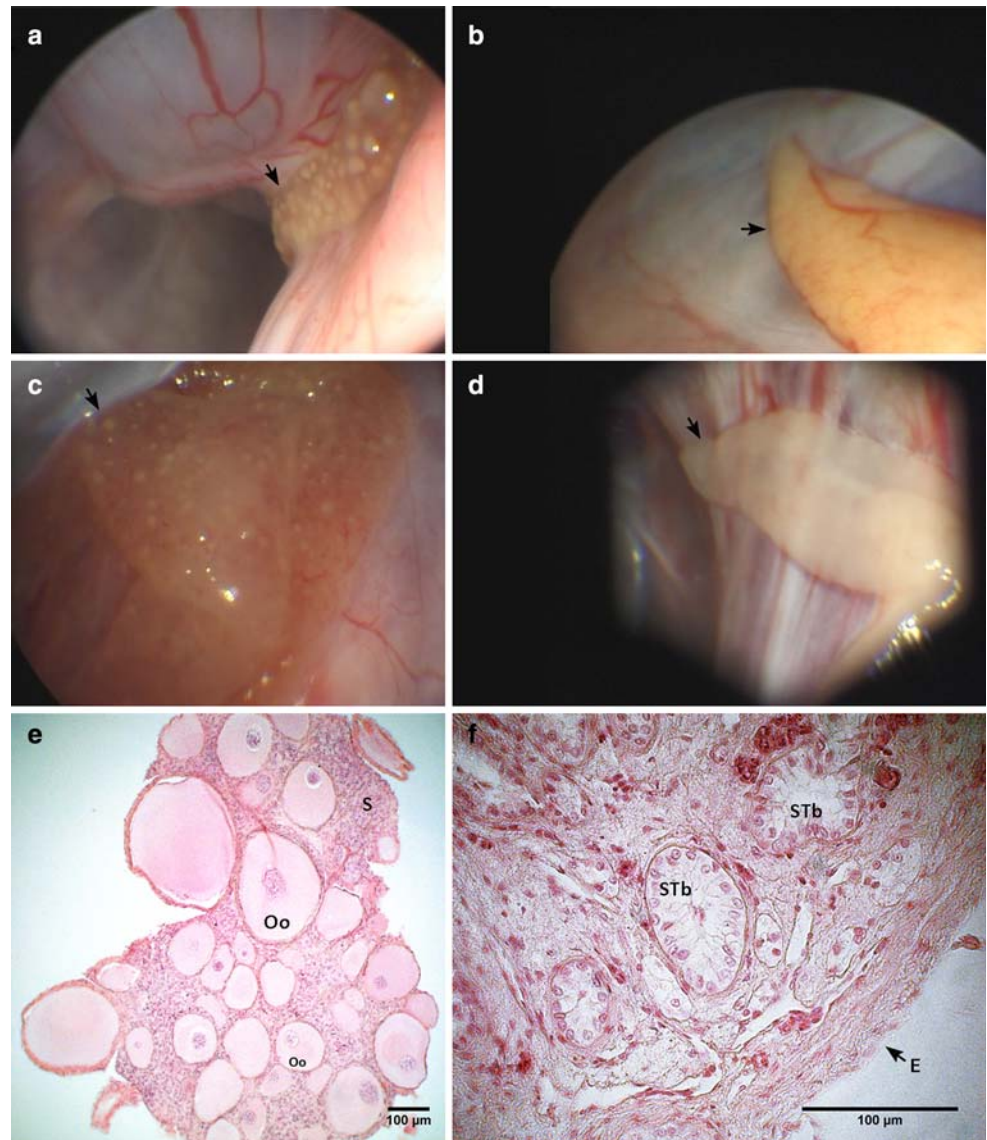


Fig. 2 Frequency distribution of size classes measured as straight carapace length until notch (SCLnt) of turtles subject to laparoscopy (2000–2006)

Fig. 3 Macroscopic (laparoscopy, **a–d**) and microscopic (histology, **e, f**) determination of sex. **a** and **c** Ovaries (*arrows*) are usually transparent, variable in size and shape, and showing many follicles of different sizes; note the overlapping, folded ovary in (**c**); **b** Testis (*arrows*) can vary from bright yellow to pale white, sometimes showing a capillary web; **d** testis are usually fusiform in shape, but note the folded testis (*right-down side*); **e** ovary showing oocytes (*Oo*) and stroma (*S*) and **f** testis showing seminiferous tubules (*STb*) and flattened epithelium (*E*) (6 μ m section, H&E stain, $\times 10$ and $\times 40$, respectively)



Females presented ovaries with non-expanded stroma, and no variation of macroscopical appearance was observed within sexes. No vitellogenic follicles, corpora lutea, corpora albicantia or atretic follicles were present. The oviduct/paramesonephric duct was commonly observed in females, but seldom in males.

One hundred and thirty-eight turtles were identified by laparoscopy to be females (61.3%), 69 were males (30.7%), and for 17 individuals, it was not possible to observe the gonads (7.6%; classified as undetermined). In undetermined animals, the trocar was not sharp or long enough to go through the several skin and muscle layers into the coelomic cavity in some of the larger turtles so that it was not possible to observe the gonads. Therefore, of 207 animals for which sex was positively determined, 66.7% were females, and 33.3% were males, a sex ratio of 2

females to 1 male [2:1 (F:M)]. This sex ratio is statistically significantly different from a 1:1 sex ratio ($\chi^2 = 23.0$, $df = 1$, $P < 0.0001$).

Histological validation

Gonad biopsies were obtained from 36.4% of the laparoscopies and included individuals 173–551 mm SCLnt (mean = 352 mm; SD = 87.6; $n = 82$). From the histological analysis, 52 specimens were identified as females (Fig. 3e) and 26 as males (Fig. 3f). In three specimens, laparoscopic and histological identification did not match. These samples were both female–male and vice versa. One individual was histologically assigned as intersex. These 4 samples were excluded from further analysis. Thus, of 78 biopsies, 66.7% were assigned females and 33.3% males,

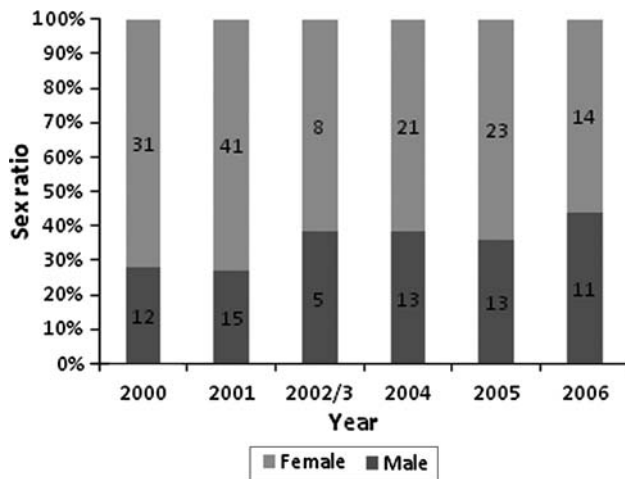


Fig. 4 Estimated sex ratios between 2000 and 2006; absolute number in each category is indicated on the bar (Note: 2002 and 2003 were pooled together because of low numbers)

i.e., an overall sex ratio of 2 females for each male [2:1 (F:M)]. This sex ratio is significantly different from a 1:1 sex ratio ($\chi^2 = 8.667$, $df = 1$, $P = 0.003$).

A 96.3% validation of the laparoscopic technique was obtained from histological results.

Estimation of sex ratios

The sex ratio from the laparoscopy pooled across all years was 2:1 (F:M). The sex ratios across years showed a consistent predominance of females (Fig. 4). Each year's cohort compared against the population 2:1 (F:M) sex ratio was not statistically significant, but were significantly different from a 6:1 (F:M) hatchling sex ratio (Table 1).

In order to assess possible age/size-dependent sex ratios, the sample was subdivided into 50 mm size classes (SCLnt) (Fig. 5; Table 2). Sex ratios were close to 1:1 at the smaller size classes (<299 mm SCLnt), but showed a bias towards females at the middle size classes (300–499 mm SCLnt); the largest size class (>500 mm SCLnt) presented a close to balanced sex ratio (see Table 2 for detailed statistical analysis and significance).

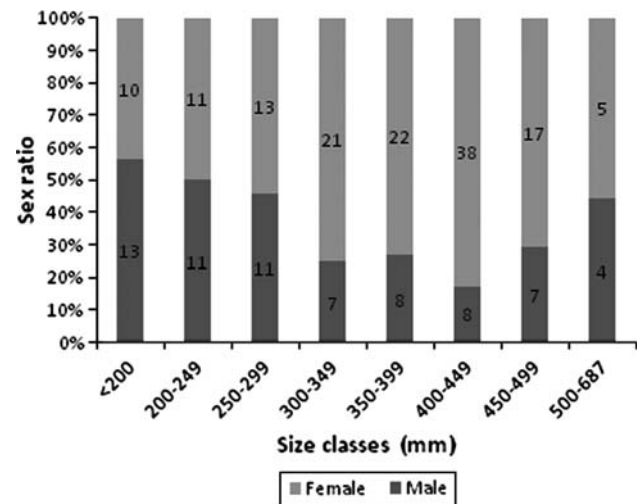


Fig. 5 Estimated sex ratios for each size classes (straight carapace length until notch, SCLnt); absolute number in each category is indicated on the bar

No statistically significant sex-specific differences were found for the biometrical parameters HW [$F(1,194) = 0.003$, $P = 0.95$, $r = 0.95$], FFW [$F(1,194) = 0.002$, $P = 0.97$, $r = 0.9$], CLW [$F(1,193) = 0.80$, $P = 0.37$, $r = 0.67$], PC [$F(1,185) = 1.96$, $P = 0.16$, $r = 0.80$], PCL [$F(1,185) = 0.47$, $P = 0.50$, $r = 0.03$] and TL [$F(1,194) = 3.60$, $P = 0.06$, $r = 0.68$].

Comparison of sex ratio between Eastern and Western North Atlantic

Statistical comparisons were made between the juveniles' sex ratio in Madeira and the sex ratios known for the hatchlings [6:1 (F:M)] and for the benthic immature [2:1 (F:M)] in the western North Atlantic as well as for an even sex ratio. The overall sex ratio is significantly different from a 1:1 sex ratio ($\chi^2 = 23.0$, $df = 1$, $P < 0.0001$). Moreover, the juvenile pelagic sex ratio [2:1 (F:M)] is significantly different from hatchlings [6:1 (F:M)] ($\chi^2 = 61.3$, $df = 1$, $P < 0.0001$), but not from benthic

Table 1 Sex ratios for the period 2000–2006 and significance values compared for a 1:1, 2:1 and 6:1 F:M sex ratios using χ^2 tests (* indicate significant results at $P < 0.05$)

Year	N (total)	N females	N males	Sex ratio	P (1:1)	P (2:1)	P (6:1)
2000	43	31	12	2.58	0.004*	0.450	0.011*
2001	56	41	15	2.73	0.001*	0.617	0.008*
2002; 2003	13	8	5	1.60	0.405	0.695	0.013*
2004	34	21	13	1.61	0.170	0.544	<0.0001*
2005	36	23	13	1.78	0.096	0.724	<0.0001*
2006	25	14	11	1.27	0.549	0.258	<0.0001*

Table 2 Sex ratios across size classes and significance values compared for a 1:1, 2:1 and 6:1 F:M sex ratios using χ^2 tests: 1 M (* indicates significant results at $P < 0.05$)

Size class	N (total)	N females	N males	Sex ratio	P (1:1)	P (2:1)	P (6:1)
<200	23	10	13	0.77	0.532	0.018*	<0.0001*
200–249	22	11	11	1	0.835	0.140	<0.0001*
250–299	23	13	11	1.18	0.683	0.194	<0.0001*
300–349	28	21	7	3	0.008*	0.350	0.105
350–399	30	22	8	2.75	0.011*	0.439	0.053
400–449	46	38	8	4.75	<0.0001*	0.022	0.547
450–499	24	17	7	2.43	0.041*	0.665	0.037*
500–687	9	5	4	1.25	0.739	0.480	0.010*

immatures [2:1 (F:M)] ($\chi^2 = 0.0001$, $df = 1$, $P = 1.0$) in the western North Atlantic.

Discussion

In this study, an overall sex ratio of 2:1 (F:M) was obtained by laparoscopy and confirmed by histology for the turtle populations visiting the Madeira Islands. Female-biased sex ratios as well as dynamic sex ratios have been documented for marine turtle species (Wibbels et al. 1987b; Wibbels 2003) and strongly non-equal population-wide primary sex ratios have been attributed, both empirically and theoretically, to environmental sex determination (Charnov and Bull 1989b). Moreover, when skewed sex ratios are observed, they are usually skewed towards females (Bull and Charnov 1989).

Not only was the sex ratio of juvenile loggerhead turtles female-biased, but also this sex ratio was consistently different from a 1:1 sex ratio for each of the 7 years examined. Some variation among years was observed, however, and although a slight trend for an increasing number of males from 2001 to 2006 can be seen, this is not statistically significant. This minor sex ratio variation is possibly due to yearly fluctuations in hatchling sex ratio production and to the relatively small sample size. Since juvenile pelagic loggerheads represent a condensation of several year cohorts of hatchling sex ratios, the variability of sex ratios observed each year at the nesting beaches is ‘buffered’ at larger size classes such as juveniles, and therefore sex ratios from hatchlings at beaches may not reflect the sex ratios of large juveniles about to enter their reproductive phase (Wyneken et al. 2006).

Although sex ratios reported for marine turtles are commonly strongly biased towards females (Wibbels 2003), some exceptions have been documented. The northern nesting subpopulation (distributed from the North Carolina/Virginia line to northeastern Florida) (Hopkins-Murphy et al. 2003) produces a balanced-hatchling sex

ratio, in contrast to the southern Florida female-skewed sex ratios (Turtle Expert Working Group 1998). In larger size classes of loggerhead turtles, balanced or male-skewed sex ratios have been found in the eastern Australian population, with male to female sex ratios of 1:0.41 (M:F) in the Great Barrier Reef and 1:0.54 (M:F) in Moreton Bay (Limpus and Limpus 2003), and a $\sim 1:1$ for the Mediterranean population (Casale et al. 2006). Therefore, long-term assessments of sex ratios are desirable in order to detect significant trends and mitigate confounding inter-annual effects, since wide variation in sex ratios of hatchlings leaving the beach is common (Wyneken et al. 2006).

The fact that the size distribution of the turtles sexed during this study appears to be representative of the pelagic population (Dellinger 2007) and since we can assume an equal probability of catching either sex (Diez and van Dam 2003), we believe that the 2:1 (F:M) sex ratio does reflect the true sex ratio of this juvenile pelagic population. Furthermore, no differential distribution patterns for juvenile males and females within the oceanic realm are known and thus a differential mortality due to different home ranges, where females could be more susceptible to increased mortality (e.g. due to human-related factors), is not plausible. Therefore, it is possible that the number of females leaving western Atlantic nesting beaches might have been overestimated.

A sex ratio of $\sim 2:1$ (F:M) is already present in the smallest loggerhead turtles recruited to Madeira waters (Fig. 5), statistically different from the hatchlings 6:1 (F:M) at the source rookery in the western Atlantic (Hopkins-Murphy et al. 2003). If this estimation of 6:1 (F:M) is correct, then the sex ratio in the <200 mm SCLnt size class suggests a strong female-biased mortality occurring within the first months of life, although there may be a contribution also of other factors such as different geographical distributions between sexes. Sex ratios across size classes show a steady tendency for an increase in female’s surplus until the 450 mm SCLnt size class. Once 450 mm SCLnt is reached, the proportion of females in Madeira waters seems

to decrease, which coincides with the size ranges that these animals are reported to start leaving the pelagic environment to enter neritic habitats (Bolten 2003b). Therefore, we speculate that females start the ontogenetic shift to neritic feeding grounds earlier than males.

Our data show that the pelagic juveniles sex ratio in the Madeira Islands is similar to that of the neritic adults in the western North Atlantic, but this is significantly different from the hatchlings sex ratio (Bolten et al. 1998). Causes for these differences can be attributed to possible differential mortality and/or behaviour-related differences between the sexes such as different migratory routes or developmental areas. However, given the strong agreement between the pelagic and the neritic sex ratios, we may speculate that past assessments of hatchling sex ratios may have been over estimated and may not accurately reflect the sex ratio of the hatchlings leaving the beaches and entering the ocean. It is also possible that juveniles sampled in the current study originated from different rookeries than previously assumed, with inputs from the Cape Verde Archipelago or from the Mediterranean nesting populations (Dellinger 2008). This hypothesis is based on the observation that one individual tagged in Madeira island as a juvenile in 1994 was seen nesting in Boa Vista Island in 2005 (Dellinger and Ferreira 2005). Since at the time Madeira's population assignment was made (Bolten et al. 1998) the Cape Verde rookery was little known, this rookery was not included in the mixed-stock analysis. This omission may have resulted in an overestimation of the contribution of US nesting beaches as the primary source for the Madeira population. A Cape Verde contribution would likely make the Madeira population sex ratio less female-biased, since the sex ratios found for Boavista Island hatchlings are in the order of ~2:1 (F:M) (Abella et al. 2007; Delgado et al. 2008).

Furthermore, a contribution from the Mediterranean should not be discarded. A male-biased or close to even juveniles sex ratio in the Mediterranean Sea (Casale et al. 2002, 2006) could account for the significant difference observed between the putative source-hatchling population in Eastern US and the juvenile population from Madeira, since migratory movements in and out of the Mediterranean have been reported for loggerheads (Camiñas 1995; Carreras et al. 2007). Thus, contributions from other rookery populations may explain a large part of the pelagic sex ratio we observed in Madeira and is supported by the fact that mixed-stock analyses of loggerhead juveniles indicate that cohorts from genetically distinct rookeries extensively mix in oceanic habitats. For instance, surveys of pelagic stage juveniles indicate no population structure among locations across the North Atlantic, and loggerhead turtles have progressively greater population structure as they advance in age and developmental stage (Bowen and Karl 2007).

According to Bowen and Karl (2007), the type of DNA used in genetic analysis can influence the conclusions regarding population genetic structures, and nuclear DNA or mitochondrial (mt) DNA alone can provide incomplete and misleading conclusions about population structure. In fact, the mtDNA surveys of North Atlantic juvenile turtles, taken alone, would indicate a single panmictic population, obscuring the true structure of subadults and nesting adults (Bowen and Karl 2007). A new genetic assessment of this population, including the individuals sexed in this study, should help to clarify the origin of the individuals and possibly the sex ratios found.

A complicating factor in the understanding of sex ratios of pelagic juveniles is the recently demonstrated pattern of migration from oceanic to neritic foraging grounds for Atlantic loggerheads. It has been assumed that this transition from an oceanic to neritic existence is a discrete ontogenetic niche shift. However, satellite tracking data demonstrated that this shift is both complex and reversible, with some individuals moving into coastal waters and then returning to the open ocean, sometimes for multiple years (McClellan and Read 2007). Hence, more than addressing dynamic sex ratios within a population in the oceanic environment, we may be addressing variable sex ratios from a mixed population.

Some studies suggest that green turtle *Chelonia mydas* hatchlings incubated at lower temperatures have reduced swimming ability, which would promote rapid dispersal to deeper offshore water, and which may affect their survival when entering the ocean and trying to escape predators on near-shore waters, prior to reaching the relative safety of the open sea (Booth et al. 2004; Burgess et al. 2006). Since females are produced at warmer incubation temperatures (Yntema and Mrosovsky 1980, 1982), we may expect that female hatchlings in general have a higher fitness than their cohort males. This finding, however, is in direct conflict with the hypothesis that a main part of differential mortality would occur at the earliest life stages, having more of an impact on female hatchlings. Additionally, incubation temperatures that are close to the upper extreme favour developmental abnormalities (Miller et al. 2003). Hence, we may speculate that female incubation temperatures at their upper extremes are more likely to produce hatchlings with abnormalities and greater chances for mortality. However, this is unlikely since the association of nest-site selection with environmental sex determination, characteristic of sea turtles, facilitates the maternal manipulation of offspring sex ratios towards females (Freedberg and Wade 2001).

Finally, hatchlings from south Florida and northern rookeries (Carolinass, Georgia and northern Florida) may encounter differential mortality rates when entering the ocean, thus altering the predicted contributions for the

pelagic sex ratios. The highly female-biased Florida population is probably more prone to higher mortalities when entering the Gulf Stream Current since they spend more time in near-shore waters and thus are more susceptible to capture and mortality (from predation) until they reach safer oceanic open waters (Hopkins-Murphy et al. 2003). This neritic differential mortality would increase the relative contribution of males from the northern US rookeries, thus decreasing the sex-ratio bias towards females.

Therefore, both an overestimation of female hatchlings leaving W. Atlantic nesting beaches and some level of increased mortality in rookeries that produce strongly female-biased sex ratios are the most plausible reason for the sex ratios observed in loggerheads within the North Atlantic, both for hatchlings and pelagic stage loggerheads. The reasons for this higher mortality rates, whether behaviour or physiological related, need to be further investigated.

Biometrical parameters and correlation with sex

All the biometrical parameters tested showed no sex-specific dimorphism in our juvenile population. This includes tail length and relative head size, which are reportedly sexually dimorphic in adult loggerhead turtles (Deraniyagala 1939; Pritchard and Trebbau 1984; Wibbels et al. 1987a, b).

Since histological criteria are considered the most accurate sex diagnosing method (Mrosovsky and Benabib 1990), the sex of a subset of the population sampled through laparoscopy was also subject to a gonad biopsy. A strong agreement between the histological and laparoscopic technique (96.3%) was found: from the 82 biopsies obtained and histologically examined only three did not match the sex identified through the laparoscopic technique (plus one individual that was assigned as intersex). The mismatched samples were female–male and vice versa and were likely due to errors during sample labelling or entering data into the database. Therefore, the overall misidentification rate was negligible, i.e., did not statistically influence the estimation of the pelagic juvenile sex ratio. Hence, in future studies, the sex of juvenile loggerheads can be accurately identified macroscopically using laparoscopy only.

The fact that all the animals were sampled during the warmer months might explain the absence of variation in gonad macroscopical appearance, namely regarding colour and granularity within the same sex, since gonads morphological appearance seems to be temperature-dependent and vary seasonally, as observed in captive-reared post-hatchlings (Wyneken et al. 2007). Given the lack of function of paramesonephric ducts in males, it is not surprising that they were seldom observed, since they tend to

regress in males but persist as the Müllerian duct in females (Miller and Limpus 2003). In contrast with pubescent females, juvenile females had ovaries with non-expanded stroma and no vitellogenic follicles, corpora lutea, corpora albicantia or atretic follicles, which is consistent with the expectations for the sexually immature status of these juvenile loggerheads (Limpus and Limpus 2003) and confirms the non-breeding condition expected for juvenile pelagic loggerheads. Therefore, we can state that only juvenile non-breeding individuals are present within Madeiran waters and that there is no reproduction in this region. According to Miller and Limpus (2003), during the years before the turtle reaches puberty the main morphological changes in the gonads result from growth of both the ovary and the testis. What triggers further gonad development for these juvenile loggerheads is not clear, but it likely occurs after the ontogenetic shift from oceanic to neritic foraging areas (Bolten 2003a).

Conclusions

Since conservation management for marine turtles is based on basic biological data and demographic trends for a given population, it is important to know the sex ratio of all life history stages, such as those generated in this study. Even a change of 1–2°C in incubation temperature can make a considerable difference to the sex ratio of the hatchlings (Mrosovsky and Yntema 1995; Hawkes et al. 2007), and a temperature change of 3°C or less could potentially shift sex ratios from all male to all females or vice versa (Wibbels 2003), making global warming a major conservation concern for TSD species. Therefore, it would be of the utmost importance to continue a comprehensive and long-term monitoring assessment of sex ratios in the pelagic stage that should incorporate periodic assessments of current sex ratios on the different size/age classes in order to ensure accuracy of population models. This should also integrate the geographically close archipelagos of the Azores and the Canary islands, as well the important foraging grounds off the Great Banks of Canada (North Atlantic Long Distance Waters) for a more clear perspective of demographic dynamics within the North Atlantic Ocean basin. Moreover, it should integrate a combination of genetic data for rookery-source assignment and sub-population distribution (Casale et al. 2006; Bowen and Karl 2007) as well as survivorship rates for the different age classes. Since resources for endangered species research and conservation are limited, they need to be used effectively and therefore, representative populations should be selected for intensive studies and long-term monitoring and used as ‘index’ populations (Bjorndal 1999). The juvenile loggerheads within Madeiran waters can be used as an

index population providing the baseline information for the pelagic stage in order to meet the current and future challenges of conservation and management of marine turtles.

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