Sex ratio estimations of loggerhead marine turtle hatchlings by incubation duration and nest temperature at Sirte beaches (Libya)

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SUMMARY: Hatchling sex ratios in loggerhead marine turtles (Caretta caretta) were estimated on the beaches near Sirte (Libya), using two methods: incubation duration and nest mean temperature during the middle third of the incubation period. Electronic temperature/humidity loggers were deployed at a total of 13 selected nests at Al-Ghbeba, Al-Thalateen, west of Al-Thalateen, Shash and Al-Arbaeen. The incubation period ranged from 47 to 58 days and average temperature ranged from 29°C to 31.8°C. The maximum temperature during this period increased to between 0.6°C and 3.5°C, while the mean temperature also increased during the middle third of the incubation period compared with the first third and continued to increase during the last third. As expected, this study showed that the temperature in the nest decreased with increasing depth of the nest. The results showed a female-dominated sex ratio at 85.4% on the basis of incubation duration and 70.4% on the basis of mean temperature. These findings support the reported highly female-skewed sex ratios in the Mediterranean and elsewhere.

Keywords: loggerhead turtle, hatchlings, sex ratio, incubation duration, nest temperature, Libya.

INTRODUCTION

Marine turtles (family Cheloniidae) are an example of a species that is diminishing in numbers as a result of the degradation of the living and nesting habitats, incidental catches, and pollution (Lutcavage et al. 1997). Significant resources are currently devoted to numerous conservation and management projects at nesting areas. One of most important and costly activities in management projects is the monitoring of beaches to protect nests and hatchlings from anthropogenic threats, such as coastal development, sand extraction.
and tourism, in addition to management of natural predation, to ensure high hatching success (Adam et al. 2007, Casale and Margaritoulis, 2010). Richardson (1999) stated that a complete study of all aspects related to nesting biology is essential for the selection and application of suitable marine turtle population conservation and management.

With the present trends and potential impacts of global warming, increased temperature can affect the sex ratio in marine turtles as well as in many other reptile species (Janzen and Paukstis 1991), as it is determined by the prevailing environmental conditions (mainly nest temperature) during the embryonic development (TSD for temperature-dependent sex determination). This means that warming temperatures could lead to a higher production of female hatchlings.

Sex determination is the initial event in which undifferentiated gonads opt for either ovarian or testicular differentiation. This process in most vertebrate species is genotypic (GSD for genotypic sex determination), leaving little scope for deviation from a balanced primary sex ratio (Zbinden et al. 2007). In marine turtles, sex depends on the proportion of time spent at specific temperatures during the thermosensitive period (TSP), which is the middle third stage of incubation (Mrosovsky and Pien 1991). The point at which a balanced sex ratio occurs is known as the pivotal temperature; more females result from temperatures above the pivotal temperature and more males from cooler temperatures (see Wibbels 2003 for a review).

For convenience, three major life-stages (hatchlings, juveniles, and adults) can be distinguished in marine turtles; different methods are used to investigate sex ratios at each of these stages (Casale et al. 2006). Hatchling sex ratio is obtained directly, through microscopic examination of hatchling gonads (Wood et al. 1983) sampled at nesting beaches (e.g. Yntema and Mrosovsky 1980), indirectly estimated as a function of a series of nest temperature records (e.g. Standora and Spotila 1985) or using additional variables associated with nest temperature, such as the incubation duration (e.g. Mrosovsky et al. 1999). Adults are easily differentiated because of their external sexual dimorphism, in particular, the most obvious characteristic is the large and muscular prehensile tail of adult males (Casale et al. 2005). Juvenile sex ratio is the most difficult to obtain, owing to both the at-sea sampling requirement and the absence of sexual dimorphism. Other methods for estimating sex ratio are to use blood hormonal levels (Owens et al. 1978), observation of gonads by laparoscopy (Wood et al. 1983), or necropsy of dead animals (Work 2000).

The determination of sex and hence the sex ratio of hatchlings is very significant basic information in marine turtle population dynamics (Casale et al. 2006, Zbinden et al. 2007). It should therefore be taken into account in any conservation planning of nesting beaches in order to conserve the "population's sexual structure" and act in an appropriate manner for the protection of these endangered reptiles, especially in the context of current global warming (Hawkes et al. 2009, Witt et al. 2010). Indeed, in species with TSD, the sex-determining pathway is extremely sensitive to temperature (Mrosovsky et al. 2009). The transitional range of temperature within which the complement of offspring sex shifts from 100% male to 100% female (or vice versa) is generally less than 2°C, and may be less than 1°C (Ewert et al. 1994), while the mean warming predicted on a scale of 100 years is 2°C (GIEC 2007).

Among the world's seven marine turtle species, three species are regularly observed in the Mediterranean. The loggerhead turtle, Caretta caretta, the most common species dispersed across all marine areas, has important nesting sites in Greece, Libya, Turkey and Cyprus (Margaritoulis et al. 2003, Casale and Margaritoulis 2010). The green turtle, Chelonia mydas, is restricted to the Levantine basin of the Mediterranean, with nesting sites in Turkey, Cyprus and Syria (Kasparek et al. 2001, Casale and Margaritoulis 2010). The leatherback turtle, Dermochelys coriacea, has no nesting population at all in the Mediterranean (Lesure et al. 1989), but enters the basin in small numbers from the Atlantic ocean (Margaritoulis 1986, Casale et al. 2003, Bradai et al. 2004, Casale and Margaritoulis 2010). Investigations of the sex ratio of three classes of loggerhead turtles (hatchlings, juveniles, and adults) have recently begun in the Mediterranean region, but the results cannot yet be considered conclusive at the regional population scale (Casale et al. 2006). The loggerhead hatchling sex ratios are estimated to be female-biased on most beaches (Godley et al. 2001, Öz et al. 2004, Zbinden et al. 2006, Witt et al. 2010).

All three marine turtle species mentioned above were reported in Libyan waters, but only the loggerhead turtle is a nesting species along the Libyan coast (Schleich 1987, Laurent et al. 1997, 1999, Hamza 2010). The importance of Libyan sandy beaches for marine turtle nesting was realized in 1995-1998 when national surveys at potential nesting beaches revealed a major rookery of loggerhead turtle nesting (Laurent et al. 1997). The results showed a substantial number of marine turtle nests laid each season, exceeding the number of nests in Cyprus and Turkey, and possibly equal to or higher than that in Greece (Laurent et al. 1997, 1999). The subsequent surveys in 2005-2008 indicated that nesting is largely concentrated mainly in four areas: the Gulf of Sirte, the region around Benghazi, some sandy beaches of Aljabal Alakhdar (Cyrenaica) and the region of Derna-Tubruk (Hamza 2010). In view of the nesting density and the large distribution range, these sites should be considered as a major Mediterranean nesting site for the loggerhead turtle.

Recalling the articles of the SPA protocol and the revised action plan on marine turtles in the Mediterranean, taking into account the new developments concerning conservation measures based on scientific groundwork, and considering the potential effects of
Sex ratio of loggerhead marine turtle hatchlings in Libya

The Libyan coast spans approximately 2000 km (Fig. 1). Most of this coast is still in pristine condition because of limited human activity and an undeveloped fishing industry compared with neighbouring countries (Laurent et al. 1997). Generally, the nesting season in Sirte starts in late May and continues until mid-August and sometimes early September (Hamza 2010). The fieldwork was conducted during the summer months of 2009 on the beaches of Al-Ghbeba (length 5.12 km, average width 65 m), Al-Thalateen (length 5.43 km, average width 55 m), west of Al-Thalateen (length 3.45 km, average width 35 m), Shash (length 4.85 km, average width 35) and Al-Arbaeen (length 8.54 km, average width 40 m). These beaches are located on the Gulf of Sirte, which has a total length of more than 800 km. Nesting and hatching activity were observed over the beaches as part of the long-term monitoring undertaken by the Libyan Sea Turtle Programme (LibSTP) since 2005. Each nest was located by walking on the beach and the precise GPS position was recorded in order to locate the nest and identify its first hatching emergence date. Sampled nest locations were chosen to fall within the mean distance from the sea line, based on same year data collected at each beach weeks prior to data logger placement. The monitored nests were protected by under-sand wire netting to deter predators (mainly red foxes, Vulpes vulpes). Nest distance from the sea was recorded from the nest location to the sea line. For the purpose of this study, incubation duration is defined as the period in days between observation of the newly laid nest and the first record of emergence, by either direct observation of hatchlings or their crawl tracks emerging from nests.

Temperatures in 14 loggerhead turtle nests were examined using a LogTag HAXO-8 electronic humidity and temperature data logger (LSTechnology, UK) from July to September 2009 on the selected beaches (when most clutches are in their thermosensitive stage for sex determination).

In order to cover the entire nesting site so that the study could be representative of the area, the data loggers were distributed to all beaches according to the nesting densities of 2009 and previous seasons at each beach (Hamza, 2010) as follows: 3 at Al-Ghbeba, 2 at Al-Thalateen, 2 at west of Al-Thalateen, 4 at Shash and 3 at Al-arbaeen. Within each nesting beach, nest sites were selected so that the data loggers were homogeneously spread along the beach length. Furthermore, the locations of studied nests were also chosen within the area of mean distance from the wave line, based on data of nests laid at the start of the same season. Due to logistical restrictions, there was a delay in 9 data logger placements, but in all cases temperature/humidity data loggers were placed into the centre of the nests before the start of the second third of the incubation period. A small hole was made adjacent to the egg chamber, without excavating the nest, 3-4 eggs were carefully removed in order to place the data logger and then they were returned to their initial positions, with the exact orientation. Temperatures and humidity at three levels (top, middle, and bottom) in two nests were also recorded. The temperatures were not initiated in one nest in Al-Ghbeba due to a malfunction of the data logger. This nest was later visited and the date of emergence was not accurately determined. Therefore, it was excluded from data analysis.

Additionally, temperature data loggers were buried adjacent to each nest (Approximately 1 m to the east at the same depth and the same distance from the sea) in order to study the effect of metabolic heating. All loggers were programmed to record a reading every 15 minutes.

The middle third of the incubation period was calculated on the basis of the incubation period mentioned above. Nest contents were excavated within a specific period after the first hatching emergence, as suggested by Adam et al. (2007); nest depths were measured and data loggers were retrieved. The total number of eggs (the number of eggs laid into the nest) and the hatching success were calculated by counting unhatched eggs, dead hatchlings in eggs, dead hatchlings in nests and empty eggshells (>50% complete) which were char-
caractéristiques des cuvettes enregistrées dans les nids et la grès (1

dans la table 2. L'information sur les températures et la relative rhum.

durant la deuxième moitié de l'incubation étaient celles de

Mrosovsky et al. (2002) adaptées au terrain. Les courbes de

sélectivité des mâles et des femelles comme une fonction de la

température moyenne pendant la deuxième moitié de l'incubation

durée était adaptée à la plage par ajout de 4 jours, ce qui

correspond à la différence entre la température de la plage et la

température de l'eau (Godfrey et Mrosovsky 1997) (fig. 3). Les équations

des deux courbes utilisées (après corrections) ont été calculées

et les valeurs exactes de la sex-ratio étaient déterminées.

La situation de l'étude de Mrosovsky et al. (2002) est basée sur

le fait que les tortues de Grèce et celles de Libye sont

partie de la même population méditerranéenne et ont la

même géographie. Ce choix est basé aussi sur le fait que la

température de pivot en méditerranéennes tortues est un


e RESULTS


durant la période de nidification 2009, 358 cuvettes ont

été enregistrées dans la zone d'étude (environ 25 km). La densité

enregistrée variait entre 9,02 cuvettes/km à Al-Thalatheen

plage à 1,65 cuvettes/km à Shash plage, avec une moyenne

de 14,45 cuvettes/km à toutes les plages. Les distances de

les cuvettes à la ligne des vagues ont été trouvées dans les limites

de la niche d'habitation de chaque plage de nidification durant la

précoce période de nidification de la même saison (tableau 1). Les

paquets d'informations sur la température et la relativité

de l'hygrométrie dans les cuvettes et la grès est présenté dans

les tableaux 3 et 4 respectivement.


durée de la période de nidification (chaque point de donnée de

température enregistré a été utilisé)

à 26,07°C (nest 9). Le maximum de la

température de la croissance pendant la période d'incubation

pour les 13 cuvettes a varié de 29,1°C à 32,1°C. La

température moyenne pendant la période d'incubation

écart type de la moyenne est de 3,5°C (nest 9: minimum de 29,3°C,

maximum de 34,6°C). La température moyenne de la

température de la plage de la troisième moitié de l'incubation

variait de 29°C (nest 10) à 31,8°C (nest 9). Le maximum de la

température de la croissance pendant cette période est de 3,5°C (nest 9: minimum de 30,2°C, maximum de 33,7°C). La

la température de la croissance pendant cette période est de 0,6°C (nest 3: minimum de 28,9°C, maximum de 29,5°C).

La température moyenne de la période de nidification durant la

troisième moitié de la période de nidification comparées avec

la troisième et continué à augmenter

après la troisième. Cela n'est pas le cas pour le

sable (contrôle), où l'air de la température contrôle l'

augmentation et diminution de la température de sol.

Concernant l'hygrométrie, il parait que l'air dans

les cuvettes et le sable adjacent était saturé avec de l'eau.

Cependant, il y avait une baisse de l'hygrométrie après

l'arrivée de l'eau. Concernant la température, il y avait

une diminution de l'humidité avant l'arrivée de l'eau.
Sex ratio of loggerhead marine turtle hatchlings in Libya

During the middle third of the incubation period, when sex is thought to be determined, the mean temperature difference between nest and sand was 0.48°C (n=13; -0.2-2°C).

The equation of sex ratio (% of females) as a function of temperature is as follows (Fig. 2):

\[ Y = \frac{100.06}{1 + \exp(188.78 - 6.37 \times X)} \]

where Y is the sex ratio and X is the temperature.

The equation of sex ratio (% of females) as a function of incubation duration is as follows (Fig. 3):

\[ Y = \frac{99.88}{1 + \exp(-103.34 + 1.82 \times X)} \]

where Y is the sex ratio and X is the incubation duration.

The sex ratios of hatchlings for all nests estimated from curve equations are shown in Table 6. The mean sex ratio based on average temperature during the middle third of incubation duration (T°) was 70.4% and 71.4%.

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**Table 3.** Mean temperature in study nests and adjacent sand during different incubation periods. IP, incubation period. The data logger in nest 7 stopped during the last third of the IP.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Total IP</th>
<th>First third IP</th>
<th>Middle third IP</th>
<th>Last third IP</th>
<th>Total IP</th>
<th>First third IP</th>
<th>Middle third IP</th>
<th>Last third IP</th>
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<td>31</td>
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<td>29.4</td>
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<td>31.3</td>
<td>30</td>
<td>30.6</td>
<td>32.2</td>
<td>31.1</td>
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<td>31.4</td>
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<td>29.2</td>
<td>30.3</td>
<td>31.5</td>
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<td>29.2</td>
<td>29.8</td>
<td>29.5</td>
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<td>29.4</td>
<td>30.3</td>
<td>31.8</td>
<td>29.4</td>
<td>29.1</td>
<td>29.6</td>
<td>29.4</td>
</tr>
</tbody>
</table>

**Table 4.** Mean humidity in studied nests and adjacent sand (control) during different incubation periods. IP, incubation period.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Total IP</th>
<th>First third IP</th>
<th>Middle third IP</th>
<th>Last third IP</th>
<th>Total IP</th>
<th>First third IP</th>
<th>Middle third IP</th>
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<td>100</td>
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<td>100</td>
<td>100</td>
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<tr>
<td>13</td>
<td>95.2</td>
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<td>85.7</td>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 5.** Mean temperature (°C) in different parts of the nest 13 during the incubation period. T, top; M, middle; B, bottom; TSP, thermosensitive period.

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation period</td>
<td>T 31.3</td>
</tr>
<tr>
<td></td>
<td>M 30.6</td>
</tr>
<tr>
<td></td>
<td>B 30.2</td>
</tr>
<tr>
<td>Before TSP</td>
<td>T 30.3</td>
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<tr>
<td></td>
<td>M 29.4</td>
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<td></td>
<td>B 29.1</td>
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<tr>
<td>TSP</td>
<td>T 31.3</td>
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<tr>
<td></td>
<td>M 30.3</td>
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<td></td>
<td>B 30</td>
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<tr>
<td>After TSP</td>
<td>T 32.2</td>
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<tr>
<td></td>
<td>M 31.8</td>
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<td></td>
<td>B 31.3</td>
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</tbody>
</table>

![Fig. 2.](image-url) Percentage of females as a function of temperature (Derived from Mrosovsky et al. (2002) and adapted to the field).

\[ Y = \frac{100.06}{1 + \exp(188.78 - 6.37 \times X)} \]

\[ Y = \frac{99.88}{1 + \exp(-103.34 + 1.82 \times X)} \]
the mean sex ratio based on incubation duration (ID) was 85.4%.

The sex ratio ranged between 5% (with the T° method) or 8% (with the ID method) and 100%, but sex ratio between nests did not vary significantly (Kruskal-Wallis test, H=17.469, P=0.133).

Of the 13 nests, 11 were predicted to produce more females if the estimation was based on incubation duration. If based on temperature during the second third of incubation duration, 10 were predicted to produce more females and three were predicted to produce more males. Comparison of the two methods indicates that sex ratios are not significantly different (t=1.725841, df=12, p-value=0.110007). Comparison of the two methods for each beach taken separately also shows that there is no significant difference; p-values of the t-test for the beaches of Al-Ghbeba, Al-Thalateen, west of Al-Thalateen, Shash and Al-Arbaeen were respectively 0.5, 0.5, 0.47, 0.64 and 0.23.

Furthermore, the analysis of the results of the sex ratio estimated by the two methods shows that there was no difference among the five beaches (Kruskal-Wallis test, H=7.154, p-value=0.128 for the T° method and H=6.629, p-value=0.157 for the ID method).

Similarly, the analysis of sex ratio results for the nests laid in early July and those laid in late July showed no significant differences (Kruskal-Wallis test, H=0.021, p-value=0.885 for the T° method and H=0.663, p-value=0.415 for the ID method).

**DISCUSSION AND CONCLUSION**

Sex ratio estimations and their biological and ecological implications are clearly a complex issue. The threats to nesting beaches are increasing and funding for conservation programmes may not be keeping pace, so it is becoming increasingly important to make informed decisions and draw up appropriate strategies (Kaska et al. 2006). Studies, knowledge and monitoring of hatching’s sex ratios should be a part of this process.

The results of the present study were in agreement with the common pattern of loggerhead marine turtle sex ratios in the Mediterranean region, which is female-dominated (Hanson et al. 1998, Kaska et al. 1998, 2006, Godley et al. 2001, Öz et al. 2004, Rees and Magaritoulis 2004, Witt et al. 2010, Rees et al. 2013). Although methodologies and timescales of studies vary, this tendency of female production is accepted to be globally predominant (Wibbels 2003). However, the sex-ratio estimated (% females) in this study (70.4% and 85.4% of females) should be considered as maximum because the few nests laid during June, which were not studied because the field sites were inaccessible during that month, had a cooler temperature regime than July when most clutches are laid. Therefore, it would seem wise to pay particular attention to the few possible male-producing clutches laid at the start of the season.

The measurements of temperature at the different parts of the nest (top, middle and bottom) allowed us to claim that the mean temperature in the central part of the nest is representative of the whole nest sections. Recording only central parts of the nests therefore allowed us to save more data loggers to be used at other nests and yielded the best estimation of sex ratios.

**Table 6.** Information on nests, incubation duration and temperature data with the estimated sex ratio (%♀). ID, incubation duration.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Clutch size</th>
<th>Emergence success</th>
<th>Distance from sea (m)</th>
<th>ID</th>
<th>T° (Middle third ID)</th>
<th>Sex ratio (%♀) from ID</th>
<th>Sex ratio (%♀) from T°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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However, this finding might be not applicable to other Mediterranean nesting sites with different physical and biological characteristics (e.g., soil type, humidity and vegetation cover).

In this study, the daily mean temperature of sand during the entire incubation period was 0.48°C (range: –0.2-2.2), which is lower than the corresponding mean in loggerhead nests at the same depths. This figure is lower than those of northern Cyprus and Turkey (range: 1.1-1.9°C) (Kaska et al. 1998), Zakynthos (Greece) (mean: 0.6°C, CI: 0.25) (Zbinden et al. 2006) and Tunisia (mean: 2°C, range:1-2.9) (Jribi et al. 2012). Taking into consideration that we measured the nest temperature at the centre of the clutch and that it has been shown, however, that temperature in loggerhead clutches is not evenly distributed (Godfrey et al. 1997, Hanson et al. 1998, Kaska et al. 1998, Booth and Astill 2001, Houghton and Hays 2001) and that the amount of metabolic heating is higher in the centre than at the sides of the clutch (Godfrey et al. 1997, Booth and Astill 2001), our results are likely to have overestimated the amount of metabolic heating experienced by the average egg. This potential bias is not likely to affect the sex ratio as this heat increase is negligible (Zbinden et al. 2006). Results of our study on an area with a large nesting potential support the conclusion of Zbinden et al. (2006) with loggerhead clutches of Zakynthos, which is the largest among Mediterranean populations (Margariotulis et al. 2003), that metabolic heat production has no significant feminizing effect on Mediterranean loggerheads.

Although a female-biased primary sex ratio for hatching production was found in this study by two methods, the temperature method was more accurate than the incubation duration method, because the latter was based on the relationship between incubation duration and the temperature during the entire development period. Therefore, it is less accurate, as it is indirect and based not only on the middle third of the incubation duration. The other thirds may confound results in case of within-clutch heterogeneous temperature regimes. More studies would be needed in the next few years to investigate any inter-annual differences in sex ratio of hatchlings occurring at these nesting beaches.

The coverage of other nesting sites in Libya, which might have different local climatic conditions, is necessary and the factors that may alter the sex ratio, such as inundation and predation, should be considered and further assessed. Indeed, nest inundation has been shown to increase incubation duration and has been suggested to possibly play a significant role in masculinization of turtle hatchlings (Rees and Margaritoulis 2004). Data analyses should take incubation duration of inundated nests into account. LibSTP nest management activities aim to reduce the number of nests inundated by the sea, as this can be an important cause of nest mortality. Nests are relocated away from the dangerous surf zone and installed in zones which are thermally inclined to the production of female turtles.

Thus, the number of male hatchlings may be reduced slightly but the overall number of hatchlings is greatly increased (Rees and Margaritoulis 2004).

Nest predation is also known to have effects on the sex ratio of hatchlings that emerge from the nest and reach the sea. The longer the incubation period, the greater the chances of nests being predated. Kaska et al. (2006) stated that smell and activities of the first group(s) of hatchlings may provide clues for predators about the location of a nest, and those emerging towards the end of the hatching duration are threatened most. Nest predation is a major threat at Sirte beaches (Hamza and Elghmati 2006), especially when conservation practices are not fully in place, and it also has an important indirect effect on the loggerhead sex ratios.

It was planned to extend the current study in 2010 to include important nesting beaches of the Misuratah province, but during of the conflict in Libya in 2011 it was not possible to retrieve the data loggers from Misuratah office or to conduct any field work because of the security situation and the suspicion of the risk of landmines in the study area. Until this threat is cleared any future fieldwork is not feasible.

The civil conflicts can seriously halt conservation efforts for marine turtles in Libya unless the international community help the new authorities to conduct actions in the field and to restructure the conservation sector of the country to safeguard the remains of the best marine turtle nesting, wintering and feeding habitats in the Mediterranean.

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