CONSERVATION BIOLOGY OF THE LARGEST POPULATION OF THE LOGGERHEAD SEA TURTLE IN THE MEDITERRANEAN

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Judith Zbinden

von Rüschegg (BE)

Leiter der Arbeit:
Prof. Dr. R. Arlettaz
Abteilung Conservation Biology
Zoologisches Institut der Universität Bern

Prof. Raphaël Arlettaz
Conservation Biology
Zoological Institute
University of Bern
CH-3012 Bern Switzerland

CONSERVATION BIOLOGY OF THE LARGEST POPULATION OF THE LOGGERHEAD SEA TURTLE IN THE MEDITERRANEAN

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Judith Zbinden

von Rüschegg (BE)

Leiter der Arbeit:
Prof. Dr. R. Arlettaz
Abteilung Conservation Biology
Zoologisches Institut der Universität Bern

Von der Philosophisch-naturwissenschaftlichen Fakultät angenommen.

Der Dekan:
Prof. Dr. P. Messerli

Bern, 21. Juni 2006

Contents

Introduction

Chapter one

Global warming may further bias hatchling sex ratio in Mediterranean sea turtles: a call for smaller conservation units

Submitted

Chapter two

Metabolic heating in Mediterranean loggerhead sea turtle clutches

Journal of Experimental Marine Biology and Ecology (in press)

Chapter three

High frequency of multiple paternity in the largest population of Mediterranean loggerhead sea turtles

Manuscript

Chapter four

Post-nesting migrations and foraging areas of loggerhead sea turtles from a major Mediterranean nesting area

Manuscript

Chapter five

Spatial behaviour of satellite-tracked loggerhead sea turtles during the internesting period at the main Mediterranean breeding area

Manuscript

Acknowledgements

Curriculum Vitae

Introduction

Human activities are affecting and in many cases threatening literally all ecosystems nowadays. Whilst the ecology of many terrestrial and freshwater vertebrates is relatively well-studied, our knowledge of marine species generally lags behind. Many marine organisms suffer from overexploitation by fisheries, whilst others are indirectly affected by fishery bycatch. Populations of large marine charismatic vertebrates, such as whales, dolphins, seals, seabirds and sea turtles, are especially vulnerable to accidental catch (Lewison *et al.*, 2004). Species which furthermore have to go to land for reproduction, for example marine turtles, face additional threats as their coastal breeding habitat is progressively deteriorated or disturbed.

Loggerhead sea turtles (*Caretta caretta*) are nowadays threatened in most parts of the world. Knowledge of their complex life cycle, which includes a number of ecologically distinct phases during ontogenetic development, is essential to identify their conservation needs (Meylan & Meylan, 1999). Upon emergence from their nests and leaving the beach, hatchlings enter an oceanic phase that is believed to last at least several years. During the early oceanic phase, turtles are passively drifting with large currents, which may span entire ocean basins. When having reached a certain size, loggerhead sea turtles perform a dramatic habitat shift to neritic waters (waters above the continental shelf), where they forage on benthic prey. They may inhabit various neritic habitats until they reach maturity, probably at an age of 30 or more years. It has been hypothesized that they adhere to the habitat where they reached maturity as adults. Females typically reproduce every few years, while males may reproduce annually. Both sexes migrate to the nesting areas to mate. Nesting areas are usually situated several hundred or thousand kilometres from the foraging areas. Females (and probably to a lesser extent males) show a high degree of philopatry to their natal area for reproduction. Female natal homing leads to strong population structure, with isolation of individual nesting colonies. The exact mechanisms of imprinting and navigation remain only partly understood, but are likely to involve orientation according to the earth magnetic field. Females deposit several large clutches of eggs in nests excavated about half a meter deep in the sand at intervals of roughly two weeks. During this internesting period, they stay at sea in the vicinity of the nesting beach. Incubation of clutches takes between 40 and 80 days, depending on sand temperature. Incubation temperature also determines the sex of hatchling sea turtles. In sea turtles in general, natural mortality is very high early in life and low in later life stages. This is reflected in a high sensitivity of population growth rate to subadult and adult mortality rates compared to fecundity or mortality in the earlier life stages.

Loggerhead sea turtles occur in all temperate and tropical oceans. While the other six extant sea turtle species nest mostly on tropical beaches, the distribution of nesting habitat of the loggerhead sea turtle reaches well into temperate zones. The loggerhead sea turtle is listed as an endangered species by the IUCN. Compared to that of other sea turtle species, commercial exploitation of loggerhead sea turtles has never played an important role in most areas. However, because many of the species' nesting areas occur in regions with dense human populations, coastal development might be a particularly critical issue for this species. Reduction in the survival rates of immature and adult turtles caused by fishery bycatch is often responsible for sea turtle population declines. Juveniles in their oceanic phase are heavily affected by longlines. Individuals having shifted to the neritic zone suffer from being accidentally caught in trawling and gill nets. The bycatch problem has long been ignored due to past research and conservation focusing primarily on the terrestrial life stages. Hence, even basic knowledge on distribution and ecology of turtles at sea remains sparse.

Sea turtles appeared very early on Earth, with the oldest fossil records dating from the Cretaceous period. Sea turtles have thus been confronted with profound ecological challenges in the past. Although their worldwide distribution might prevent them from biological extinction, loggerhead sea turtles might go regionally extinct if the present conditions persist or further deteriorate.

Loggerhead sea turtles in the Mediterranean represent a distinct management unit with a rather uncertain future. This meta-population is relatively small with an average of roughly 5'000 clutches deposited yearly on the known nesting beaches of Greece, Turkey and Cyprus. Various sources of information suggest that juveniles frequent the western Mediterranean basin while subadults and adults, i.e. individuals which have switched to a neritic habitat, are mostly found in the eastern basin (Margaritoulis et al., 2003). Casual information indicates a large population decline before systematic monitoring started in the 1970ies (Margaritoulis, 1982). As the Mediterranean is one of the world's tourism hotspots, many nesting beaches are affected by human encroachment. Although no quantitative population dynamics model exists, the current levels of fishery bycatch are thought to be incompatible with the long-term protection of Mediterranean sea turtles.

This situation, together with a scarcity of basic scientific knowledge about the biology of Mediterranean loggerhead sea turtles which could lead to tailored, efficient conservation actions, elicited the development of the present PhD study. Our investigations were carried out on the largest known population of loggerhead sea turtles in the Mediterranean, in the Bay of Laganas on the Ionian Island of Zakynthos (Greece). Here, nesting activity has been monitored for over two decades by the Greek NGO ARCHELON, The Sea Turtle Protection Society of Greece.

The thesis consists of two main parts. The first part (three chapters) is devoted to the potential effects of the ongoing global warming on Mediterrean loggerhead sea turtles. The second part (two chapters) deals with the spatial behaviour of loggerhead sea turtles at sea. Each chapter represents a publishable paper with one chapter (chapter two) being in press, whereas another one (chapter one) is submitted.

In **chapter one**, I tested the hypothesis of small-scale temperature and related hatchling sex ratio variation within the nesting habitat of the Bay of Laganas. Indirect methods, namely sand temperature measurements at nest depth and incubation duration data, both used for determining hatchling sex ratio, showed that the nesting area is very heterogeneous in terms of hatchling sex ratios produced. This has direct implications for setting new conservation priorities for this population. The habitat producing predominantly male hatchlings warrants particular attention, this because males are not only the rare sex, but also likely to decline under a global warming scenario. The fine-scale variation in sand temperature conditions could potentially act as buffer in the face of climatic change, enabling the population to keep producing a certain sex ratio, this without necessitating a change in natal homing behaviour.

The **second chapter** is tightly connected to the first one. Here I investigated whether sand temperatures at nest depth consistently differ from actual nest temperatures due to metabolic heating produced by the clutch. Metabolic heating was evident in most clutches, but the level of extra heat produced was low during the middle third of incubation, when sex is determined.

The **third chapter** is related to chapter one and two in that the changes in sex ratio may potentially affect the mating system, with possible dramatic consequences on population dynamics. The aim was to assess the current level of multiple paternity in the population and to investigate whether multiply fathered clutches have better survival than clutches fertilized by one male. We found a very high rate of multiple paternity. Clutch hatching success was related to the number of fathers detected. In addition, the number of fathers detected in a clutch was positively correlated with female size.

Unbiased information about the whereabouts of turtles at sea is urgently needed to locate main foraging areas so as to implement spatially-explicit, tailored action plans to mitigate fishery-bycatch related problems. In the **fourth chapter**, I investigated the post-nesting migrations and locations of foraging grounds in seven adult females equipped with satellite transmitter. They dispersed to two main areas, namely the north Adriatic Sea and the north African coast. Moreover, we witnessed extended seasonal migrations in two individuals originally foraging in the north Adriatic Sea.

The satellite transmitters additionally provided location information during the internesting period, i.e. during the period when females lay their successive clutches. This is presented in the **fifth chapter**. Our tracking data allowed scrutinizing female habitat use in relation to offered zones of protection from disturbance from turtle-watching tourism. Turtles generally stayed within the restricted area of the bay during the internesting period, but did not seem to spend more time in the highly protected area.

Field work was carried out in cooperation and under a research agreement with ARCHELON. A large part of the project was funded by the MAVA Foundation for the Protection of Nature. We thank them for their collaboration and for funding.

Lewison RL, Crowder LB, Read AJ, Freeman SA (2004) Understanding impacts of fisheries bycatch on marine megafauna. *Trends in Ecology and Evolution*, **19**, 598-604.

Margaritoulis D (1982) Observations on loggerhead sea turtle *Caretta caretta* activity during three nesting seasons (1977-1979) in Zakynthos, Greece. *Biological Conservation*, **24**, 193-204.

Margaritoulis D, Argano R, Baran I *et al.* (2003) Loggerhead turtles in the Mediterranean Sea: Present knowledge and conservation perspectives. In: *Loggerhead sea turtles* (eds Bolten AB, Witherington BE), pp. 175-198. Smithsonian Institution, Washington.

Meylan AB, Meylan PA (1999) Introduction to the evolution, life history, and biology of sea turtles. In: *Research and management techniques for the conservation of sea turtles* (eds Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M), pp. 3-5. IUCN/SSC Marine turtle specialist group publication no. 4

Chapter one

Global warming may further bias hatchling sex ratio in Mediterranean sea turtles: a call for smaller conservation units

Judith A. Zbinden, Dimitris Margaritoulis, Christina Davy and Raphaël Arlettaz

Submitted

Global warming may further bias hatchling sex ratio in Mediterranean sea turtles: a call for smaller conservation units

Judith A. Zbinden[1], Christina Davy[2], Dimitris Margaritoulis[3] and Raphaël Arlettaz[1]

[1] Zoological Institute, Division of Conservation Biology, Baltzerstrasse 6, 3012 Bern, Switzerland
[2] Department of Biology, University of Western Ontario, London, ON, N6A 5B7, Canada
[3] ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, 10432 Athens, Greece

Key words: Conservation and management units, Temperature-dependent sex determination (TSD), Incubation duration, Mediterranean, Zakynthos, Greece, *Caretta caretta*

Corresponding author:
Judith Zbinden
Zoological Institute
Division of Conservation Biology
Baltzerstrasse 6
CH-3012 Bern
Tel.: +41 31 631 31 53
Fax: +41 31 631 45 35
judith.zbinden@nat.unibe.ch

Running head: Loggerhead hatchling sex ratios

Abstract

Monitoring and understanding the influence of sex ratio on population dynamics is becoming imperative due to global warming in threatened species with temperature-dependent sex determination such as sea turtles. We used two approaches (sand temperature profiles and clutch incubation duration) to estimate hatchling sex ratios produced on the individual nesting beaches of Zakynthos (Greece), the largest known nesting population of loggerhead sea turtles (*Caretta caretta*) in the Mediterranean. The overall primary sex ratio of Zakynthos was female-biased in 2002 and 2003 (68% and 75% females, respectively). Interestingly, we found clear differences in estimated hatchling sex ratios between two groups of beaches with over 70% difference in the proportion of females produced between the coldest and warmest beach. Such pronounced differences in hatchling sex ratio production between adjacent beaches, which cannot be explained by geographic climate variation, could potentially act as an avenue for behavioral reaction of sea turtles to global warming. Such a mechanism seems plausible because female site fidelity amongst the individual beaches of Zakynthos is weak. Whether or not females will lay more clutches on the cooler beaches with future climate warming, particular attention should be paid to the management of these beaches which produce more males, as this sex is expected to become even rarer in the future due to global warming.

Introduction

Sex ratio is a crucial demographic parameter affecting the viability of populations, and sex ratio figures amongst the determining factors in calculations of both demographic and genetic effective population size (Caughley & Gunn, 1996; Wedekind, 2002). Sex determination in most species is genetic, leaving little scope for deviations from the evolutionary stable balanced sex ratio (Krebs & Davies, 1993). Although evolutionary theory holds regardless of the mode of sex determination, primary sex ratios in species with environmental sex determination are often far from balanced (Mrosovsky, 1994; Marcovaldi *et al.*, 1997; Wibbels, 2003). While this parameter is of little conservation concern in species with genetic sex determination (except in extremely small populations), species with environmental sex determination may be vulnerable to disturbances in the environmental sex determining factors.

Sea turtles are amongst the taxa whose sex is determined environmentally, specifically by the temperature prevailing in the middle third of the incubation period, with warmer temperatures producing females and colder temperatures resulting in males (Mrosovsky & Pieau, 1991). In sea turtle populations where primary sex ratio was investigated, it is often highly female-biased (Mrosovsky, 1994; Wibbels, 2003).

Even assuming high heritability of the pivotal temperature T_p (the temperature producing a 1:1 sex ratio) and/or behavioral adaptations affecting clutch temperature, evolution is unlikely to be fast enough to compensate for temperature changes caused by current global warming. The extent to which female sea turtles can adapt their behavior to higher temperatures (e.g. by moving to colder nesting beaches, digging deeper egg chambers or changing the seasonal distribution of clutches) is probably limited. Philopatry inhibits shifts of nesting areas in ecologically relevant time frames (Bowen *et al.*, 1994). Variation in egg chamber depth is restricted by female morphology. A seasonal shift in the nesting season correlated to increasing sea surface temperatures off the nesting beach has been demonstrated for a population of Atlantic loggerhead sea turtles (*Caretta caretta*) (Weishampel *et al.*, 2004). However, unless the seasonal distribution of precipitation shifts as well, early clutches are expected to be adversely affected by rain and storms during spring in most climates, in particular in the Mediterranean. That a lack of males might become an actual problem for sea turtle populations is demonstrated by a high rate of infertility (22% of clutches) among leatherback sea turtle (*Dermochelys coriacea*) clutches in Malaysia, which has been attributed to an insufficient number of males in the population – a highly probable explanation given that in the past mainly clutches incubating during the warmer part of the nesting season had been protected (Chan, 1991). Sex ratios might become a key factor for the future conservation of sea turtles, and a clear understanding of hatchling sex ratios and the degree to which they vary is essential for effective conservation of threatened populations.

Variations in hatchling sex ratios are expected over latitudinal scales and such large-scale variation has been reported for the eastern US (Mrosovsky, 1987) and Brazil (Marcovaldi *et al.*, 1997; Baptistotte *et al.*, 1999). Because of female philopatry, the beaches producing markedly different sex ratios should be viewed as separate management units in these cases irregardless of the sex ratio produced. Differences in incubation temperature that were not associated with the latitudonal locations of the respective beaches have been reported for the green turtle (*Chelonia mydas*) population of Ascension Island (Hays *et al.*, 1999; Hays *et al.*, 2001; Hays *et al.*, 2003). Furthermore, these differences in temperature result in estimates of hatchling sex ratios ranging from 54% females on the coldest beach investigated to >99% females on the warmest beach studied (Godley *et al.*, 2002). Even though those beaches are within approximately 20km of each other, there is a strong tendency for individual females to nest consistently on one of the beach groups of this area (Mortimer & Portier, 1989).

The first aim of this study was to estimate the sex ratio of loggerhead sea turtle hatchlings produced in the Bay of Laganas, Zakynthos, Greece (hereafter referred to as Zakynthos). Zakynthos hosts the largest known population of sea turtles in the Mediterranean, accounting for roughly one fourth of all clutches (Margaritoulis *et al.*, 2003). Our second aim was the resolution of small-scale patterns of offspring sex ratio production, continuing the preliminary findings of Margaritoulis (2005). The nesting area of Zakynthos is composed of six distinct but adjacent beaches (Fig. 1). Individual females frequently alternate between beaches for successive egg layings within a season (ARCHELON internal reports, unpublished data). The six beaches differ considerably in physical characteristics; raising the possibility that hatchling sex ratio differs amongst them. Such a situation could potentially open new avenues for turtle females to react to global warming.

Materials and methods

Study area

The Island of Zakynthos is situated in the Ionian Sea in the eastern Mediterranean (Fig. 1). The climate is highly seasonal both in terms of temperature and rainfall, with high temperatures and generally low precipitation during the summer months, while autumn and winter are characterized by lower temperatures, heavy rains and storms. The south-facing Bay of Laganas contains the six discrete nesting beaches Marathonissi (MAR), East Laganas (LAG), Kalamaki (KAL), Sekania (SEK), Daphni (DAP) and Gerakas (GER); the opening of the bay is ca. 12km wide (Fig. 1). Loggerhead sea turtles nest on Zakynthos from the second half of May to the first half of August (Margaritoulis, 2005), and the shallow bay is used as a mating and internesting habitat (chapter five). Apart from a small nesting aggregation on the neighboring island of Kephalonia, the nesting beaches of Zakynthos constitute the northernmost loggerhead nesting area in the Mediterranean (Margaritoulis *et al.*, 2003).

Nest data collection

We collected data on the number of nests laid as part of a long-term monitoring program conducted by ARCHELON, The Sea Turtle Protection Society of Greece (Margaritoulis, 2005). This program included monitoring of all beaches on a daily basis during the entire season. Most egg chambers were not physically located. Instead, clutches were identified from track characteristics on the laying date (defined as the morning after they were laid). As most nests were left unprotected and not externally marked, we measured the distance from the estimated clutch location to two flanking marker poles set up at the back of the beaches, as well as the distance from the clutch to the sea. These measurements were done on surveys starting at first light and allowed us to locate and identify the nest during hatching. Hatching was determined by the observation of tracks during the daily transects of the beach. We considered the morning the first hatchling tracks were recorded from a nest as that nest's hatching date. The incubation duration (ID) was defined as the number of days from the laying date to the nest's hatching date. We excavated clutches fourteen days after first hatching and categorized their contents (Miller, 1999). We counted the number of eggs that had developed to over about 75% of incubation [determined by estimating the relation of the volume of the yolk sac to the volume of the embryo (Miller, 1985)]. These were assumed to have contributed to metabolic heating. A team led by at least one person with appropriate training and experience conducted data collection.

Sex ratio estimates

Since the sex of sea turtle hatchlings cannot be assessed from external morphology, we used two indirect methods to estimate hatchling sex ratios: 1) measurements of sand temperature at nest depth and 2) incubation durations of clutches.

Sand temperature. Sand temperature may be an appropriate proxy for estimating sea turtle hatchling sex ratio (Mrosovsky, 1994; Godfrey &

Mrosovsky, 1999), and has been applied in a number of studies (Mrosovsky, 1994; Godfrey *et al.*, 1997; Godfrey *et al.*, 1999). Other studies, e.g. Godley et al. (2001a), based their sex ratio estimates on clutch temperature instead. Inferring hatchling sex ratio estimates from sand temperature is complicated by the fact that developing embryos may produce additional heat (metabolic heating). However a comparison of within-nest temperatures with that of the surrounding sand indicated that metabolic heating was negligible (0.20 ± 0.20°C, N=20 clutches) in the study area during the middle third of development (chapter two). The main advantage of measuring sand instead of nest temperature is that data can be collected over the entire season with a minimal investment in equipment and effort. This method seemed therefore suitable for this study, as we required estimates of sex ratio for six different beaches.

The effect of temperature on sex determination is classically defined by two parameters: the pivotal temperature, T_p, and the transitional range of temperature (TRT), the range in which both sexes are produced (Mrosovsky & Pieau, 1991). Values for T_p and TRT were inferred from a laboratory study on eggs from the nesting population of Kyparissia Bay, Peloponnese (Mrosovsky *et al.*, 2002), which is about 80 km away from the Bay of Laganas. Since we measured sand temperature, we corrected the T_p value obtained from egg temperature measurements in the lab by the estimated amount of evaporate cooling (0.4°C), resulting in a field T_p of 29.7°C. Since Mrosovsky *et al.* (2002) estimated the TRT to be around 1.5°C, we considered that 29.0–30.5°C produces clutches containing both sexes.

In 2003 we programmed «Tinytalk» data loggers (accuracy ± 0.3°C; Gemini Dataloggers, UK) to record temperature at 1 h intervals and placed them at five haphazard locations on each beach, within an area encompassing ca 90% of nests. The loggers had been calibrated by the manufacturer and their precision tested by placing them next to each other. We placed data loggers at a depth of 39 cm, the average middle depth of loggerhead sea turtle clutches in Zakynthos (ARCHELON internal reports, unpublished data). Due to data storage capacity, data loggers were exchanged midway through the season. Therefore, each temperature profile was constructed from measurements by two successive data loggers. Mean daily temperature (from midnight to midnight) was used for analysis.

Incubation duration. Incubation durations are also used to estimate hatchling sex ratio (Marcovaldi *et al.*, 1997; Godley *et al.*, 2001b). The validity of this approach has been confirmed by histological sexing of hatchlings from nests of known incubation duration (Godfrey *et al.*, 1997; Mrosovsky *et al.*, 1999). We collected data on incubation duration during 2002 and 2003 from a representative sample of clutches on each beach, and used the relationship between hatchling sex ratio and incubation duration estimated by Mrosovsky *et al.* (2002) to convert observed durations to sex ratio. Lab incubation durations were corrected by the estimated time gap between hatching and emergence of 4 days (Godfrey & Mrosovsky, 1997). Thus, we considered that observed incubation durations of ≤55 days produced only females, 56 days produced 80% females, and 57 days produced an unbiased sex ratio. 58 days was assumed to produce 10% females and ≥59 days only males. To estimate sex ratio for an individual beach, each nest on the beach was placed in the appropriate incubation duration/sex ratio category and we calculated beach sex ratio based on the proportion of nests in a class. Finally, we estimated an area sex ratio (overall ratio for the entire nesting area) from beach values accounting for the proportions of nest numbers on a beach.

To evaluate the variation in incubation durations reflecting differences amongst beaches (as opposed to other factors that may influence this variable), we tested the factor «beach» in a general linear model together with the date a clutch was laid (laying day), the location of the nest in relation to the sea (distance to sea) and the number of eggs that had developed to over 75% of incubation. Interactions between all factors were included in the initial model. We performed this analysis with data from 2003 where sample sizes were generally higher.

Statistical analysis. Statistical treatment consisted of analysis of variance (ANOVA) followed by Tukey-Kramer HSD post-hoc tests. They were performed with the program JMP IN © (version 4.04, SAS Institute).

Results

Sand temperature profiles

Seasonal trends in temperature profiles were similar within beaches (Fig. 2b). Temperatures rose sharply from the onset of the nesting season until mid-June, and then increased gradually until the second half of September. Temperatures then declined to levels similar to those measured in spring. Sand temperature range during measurements was about 7°C across the season, but only 2°C during the crucial period for sex determination (Fig. 2). The general pattern was chiefly affected by storms, visible in Fig. 2b as dramatic declines in temperature lasting about one week.

Sand temperature variation within a beach was high, reaching over 2°C on some beaches (Fig. 2b). The location of profiles in relation to the T_p (and by extension TRT) indicates that KAL, SEK, DAF and to a lesser extent GER were characterized by temperatures resulting in female-biased sex ratios, whereas MAR and LAG produced male-biased sex ratios. Comparison of the temperature profiles of these two beaches suggests that a higher proportion of males was produced on MAR than on LAG (Fig. 2).

Incubation duration

Incubation durations were generally longer in 2002 than in 2003 (Fig. 3), which was predictable given the unusually high precipitation in summer 2002. The estimated area sex ratio for Zakynthos was biased towards females, which accounted for an estimated 68% and 75% of hatchlings, in 2002 and 2003, respectively.

In the original model we found no significant effect of distance to sea or any interaction between factors on incubation duration. We ran a new model with only «beach», laying date and the number of eggs that had developed to over 75% of incubation as factors. This model explained nearly 60% of the variation in incubation duration (ANOVA, $F_{7,\,277} = 56.66$, $p < 0.001$, $R^2 = 0.59$), with the factor «beach» alone explaining about 57% of the variation (partial R^2). Laying date and the number of eggs that had developed to over about 75% of incubation were negatively correlated with incubation duration. There were significant differences in incubation durations between the beaches MAR and LAG, which formed one group, and KAL, SEK, DAP and GER forming the other. Within the latter group, the only two beaches with statistically different incubation durations were KAL and SEK ($N = 328$, Tukey-Kramer HSD post-hoc test: $q^* = 2.87$, $p < 0.05$). Estimates of sex ratios from incubation durations established that MAR and LAG produced a male-biased and the other beaches a female-biased sex ratio (Fig. 3).

A comparison of June-August monthly air temperatures on Zakynthos for the years 2002 and 2003 with those of the previous 18 years revealed that temperatures during these two seasons were representative for the temperature conditions of the area (data not shown).

Discussion

We feel that the most interesting result of this study is the finding of pronounced spatial thermal variation (resulting in hatchling sex ratio differences of up to over 70%) within a nesting area, which would, under all traditional criteria be defined as one management unit. This finding is in some ways very similar to the situation for green turtles of Ascension Island (Godley *et al.*, 2002). However, the range of hatchling sex ratios produced is apparently much larger in Zakynthos loggerheads (75-85%) than in Ascension Island green turtles (ca. 46%). Moreover, females of

Ascension Island seem to be bound more strongly to a given beach group within the area than loggerheads of Zakynthos (although a quantitative analysis is lacking for the latter). This is consistent with generally higher levels of gene flow in the loggerhead than in the green turtle (Bowen & Avise, 1996).

The estimated hatchling sex ratio of the entire area of Zakynthos was female-biased in both years, however still produced a considerable proportion of male hatchlings. In northern Cyprus, the proportion of female hatchlings (inferred from incubation duration data, backed up with intra-nest temperature measurements and direct sexing of hatchlings found dead) is much higher (89-99%) than on Zakynthos (Godley *et al.*, 2001a; Godley *et al.*, 2001b). There is also evidence that the loggerhead hatchling sex ratio of Kephalonia is female-skewed (Houghton & Hays, 2001). Although rough summary information on incubation durations exist for other Mediterranean nesting areas (Margaritoulis *et al.*, 2003), we consider such data inadequate for inference of hatchling sex ratios, since using an average incubation duration will generate inappropriate estimates if prevailing thermal conditions in a given habitat are not homogenous. Zakynthos is the northernmost major nesting population in the Mediterranean and therefore has a relatively cool boundary climate. Therefore it is unlikely that any other sizeable nesting aggregation in the Mediterranean produces a strongly male-biased sex ratio. Assuming no contemporary gene flow between Atlantic and Mediterranean loggerhead sea turtles (Bowen *et al.*, 1993; Laurent *et al.*, 1993) and male-mediated gene flow amongst regional populations of loggerheads, as shown for the green turtle (Karl *et al.*, 1992; FitzSimmons *et al.*, 1997), the males produced in Zakynthos might be crucial for the entire Mediterranean loggerhead metapopulation.

There were significant differences in incubation durations among beaches, which translated into marked differences in the estimated hatchling sex ratios. This result is supported by sand temperature data of the given beaches. However, given the rather high intra-beach sand temperature variation, five locations did not enable us to assess the overall amplitude of temperature conditions within an entire beach. For this reason, we generated only qualitative sex ratio estimates from this data. The much higher number of locations sampled by incubation durations – although less accurate per sample - can much better account for this situation. Differences in incubation duration amongst beaches explained the by far largest proportion (by over an order of magnitude) of the explained variation in incubation duration. While laying date and the number of embryos contributing to metabolic heating explained some of the residual variation, they probably did not impact much on sex ratio variation, given that incubation duration is determined by temperature throughout incubation, whereas for sex determination, only the middle third of development is relevant (Mrosovsky & Pieau, 1991). Nests laid at the start of the season experienced much lower incubation temperatures during the first part of their development than during the time period relevant for sex determination (Fig. 2b). Similarly, the amount of metabolic heating was considerable during the last stage of incubation, but not during the critical middle third of development (chapter two). As neither seasonal distribution of nests, egg chamber depth or clutch sizes seem to differ significantly among the beaches of Zakynthos (ARCHELON internal reports, unpublished data), differences in the physical nature of the individual beaches, such as albedo (Hays *et al.*, 2001) or beach orientation and inclination, seem to be the crucial determinants of hatchling sex ratio differences in Zakynthos.

The pronounced differences in hatchling sex ratio within a single geographically small nesting area demonstrate that hatchling sex ratios can vary dramatically on extremely small scales. The wide range of incubation conditions found in Zakynthos might possibly act as a buffer against climate change. Two beaches (MAR and to a lesser extent LAG) crucial for present male production will continue to produce males under moderate warming scenarios. This new information could be integrated into current protection plans to further increase their effectiveness. So far,

(in fall

the most important beach in terms of nest numbers (SEK) has been afforded the best protection. Unfortunately, over the last three decades, about half of the nesting habitat of LAG has been destroyed by development and tourism, whereas hatchlings from MAR apparently suffer from disorientation caused by artificial lighting from tourist resorts (personal observation). We have demonstrated that these two beaches are producing a large proportion of the male hatchlings of Zakynthos, and probably of the entire Mediterranean metapopulation. Since they produce the sex that is foreseen to decline under global warming, MAR and LAG warrant better protection than they currently receive.

The implications of our results are not limited to Zakynthos, or to the Mediterranean. Many sea turtles nesting sites may not show as much variation as those in our study, particularly in the case of continuous uniform beaches. However, the existence of extremely small-scale yet significant variation reported here suggests that the possibility of differences in sex ratios within single nesting areas should not be discounted. In the case of Zakynthos, where individual female site fidelity is not restricted to an individual beach, females may have the opportunity for behavioural reaction to global warming. It is conceivable that female turtles will lay more clutches on colder beaches with an increase in temperature. The appeal of this possible scenario is that it requires no change in female site fidelity. Continued monitoring of clutch numbers on the individual beaches will show whether loggerheads do indeed take advantage of such a situation to maintain production of male hatchings in the future.

As a precautionary principle, the potential substructure of nesting areas with regards to hatchling sex ratio should be considered when developing conservation strategies. Whether or not females will lay more clutches on the cooler beaches with future climate warming, particular attention should be paid to the management of male-producing beaches.

Acknowledgements

The major part of this research was funded by a grant from the MAVA Foundation, to which we are extremely grateful. We would like to thank K. Grimanis, H. Strantzalos and A. Rees (ARCHELON) for support. B. Godley (Marine Turtle Research Group, University of Exeter) as well as A. Aebischer (Zoological Institute, University of Bern) contributed to the study. Special thanks to the ARCHELON volunteers and field assistants A. Bishop, P. Bradshaw, L. Coe, C. Dean, L. Emo, K. Heglund, A. Lazou, A. Reed and M. Sturt. The comments of two anonymous reviewers substantially ameliorated the quality of the manuscript.

References

Baptistotte C, Scalfoni JT, Mrosovsky N (1999) Male-producing thermal ecology of a southern loggerhead turtle nesting beach in Brazil: implications for conservation. *Animal Conservation*, **2**, 9-13.

Bowen BW, Avise JC (1996) Conservation genetics of marine turtles. In: *Conservation genetics - case histories from nature* (eds Avise JC, Hamrick JL), pp. Chapman and Hall, New York.

Bowen BW, Avise JC, Richardson JI, Meylan AB, Margaritoulis D, Hopkins-Murphy SR (1993) Population structure of loggerhead turtles (*Caretta caretta*) in the northwestern Atlantic Ocean and Mediterranean Sea. *Conservation Biology*, **7**, 834-844.

Bowen BW, Kamezaki N, Limpus CJ, Hughes GR, Meylan AB, Avise JC (1994) Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. *Evolution*, **48**, 1820-1828.

Caughley G, Gunn A (1996) Conservation biology in theory and practice. Blackwell Science, Cambridge.

Chan EH (1991) Sea turtlles. In: *The state of nature conservation in Malysia* (eds Kiew R), pp. 120-134. Malayan Nature Society, Kuala Lumpur.

FitzSimmons NN, Limpus CJ, Norman JA, Goldizen AR, Miller JD, Moritz C (1997) Philopatry of male marine turtles inferred from mitochondrial DNA markers. *Proceedings of the Natural Academy of Sciences USA*, **93**, 8912-8917.

Godfrey MH, Barreto R, Mrosovsky N (1997) Metabolically-generated heat of developing eggs and its potential effect on sex ratio of sea turtle hatchlings. *Journal of Herpetology*, **31**, 616-619.

Godfrey MH, D'Amato AF, Marcovaldi MA, Mrosovsky N (1999) Pivotal temperature and predicted sex ratios for hatchling hawksbill turtles from Brazil. *Canadian Journal of Zoology*, **77**, 1465-1473.

Godfrey MH, Mrosovsky N (1997) Estimating the time between hatching of sea turtles and their emergence from the nest. *Chelonian Conservation and Biology*, **2**, 581-585.

Godfrey MH, Mrosovsky N (1999) Estimating Hatchling Sex Ratios. In: *Research and Management Techniques for the Conservation of Sea Turtles* (eds Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M), pp. IUCN/SSC Marine Specialist Group Publilcations,

Godley BJ, Broderick AC, Downie JR *et al.* (2001a) Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. *Journal of Experimental Marine Biology and Ecology*, **263**, 45-63.

Godley BJ, Broderick AC, Glen F, Hays GC (2002) Temperature-dependent sex determination of Ascension Island green turtles. *Marine Ecology Progress Series*, **226**, 115-124.

Godley BJ, Broderick AC, Mrosovsky N (2001b) Estimating hatchling sex ratios of loggerhead turtles in Cyprus from incubation durations. *Marine Ecology Progress Series*, **210**, 195-201.

Hays GC, Ashworth JS, Barnsley MJ *et al.* (2001) The importance of sand albedo for the thermal conditions on sea turtle nesting beaches. *Oikos*, **93**, 87-94.

Hays GC, Broderick AC, Glen F, Godley BJ (2003) Climate change and sea turtles: 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Change Biology*, **9**, 642-646.

Hays GC, Godley BJ, Broderick AC (1999) Long-term thermal conditions on the nesting beaches of green turtles on Ascension Island. *Marine Ecology Progress Series*, **185**, 297-299.

Houghton JDR, Hays GC (2001) Asynchronous emergence by loggerhead turtle (*Caretta caretta*) hatchlings. *Naturwissenschaften*, **88**, 133-136.

Karl SA, Bowen BW, Avise JC (1992) Global Population Genetic Structure and Male-Mediated Gene Flow in the Green Turtle (*Chelonia mydas*): RFLP Analyses of Anonymous Nuclear Loci. *Genetics*, **131**, 163-173.

Krebs JR, Davies NB (1993) An Introduction to Behavioural Ecology. Blackwell Science, Oxford.

Laurent L, Lescure J, Excoffier L *et al.* (1993) Etude Génétique des relations entre les populations méditerranéenne et atlantique d'une tortue marine (*Caretta caretta*) à l'aide d'un marqueur mitochondrial. *Compte Rendu de l'Académie des Sciences, Paris*, **316**, 1233-1239.

Marcovaldi MA, Godfrey MH, Mrosovsky N (1997) Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. *Canadian Journal of Zoology*, **75**, 755-770.

Margaritoulis D (2005) Nesting activity and reproductive output of loggerhead sea turtles, *Caretta caretta*, over 19 seasons (1984-2002) at Laganas Bay, Zakynthos, Greece: The largest rookery in the Mediterranean. *Chelonian Conservation and Biology*, **4**, 916-929.

Margaritoulis D, Argano R, Baran I *et al.* (2003) Loggerhead turtles in the Mediterranean Sea: Present knowledge and conservation perspectives. In: *Loggerhead sea turtles* (eds Bolten AB, Witherington BE), pp. 175-198. Smithsonian Institution, Washington.

Miller JD (1985) Embryology of marine turtles. In: *Biology of Reptilia, Development A* (eds Gans C, Billett F, Maderson PFA), pp. 269-328. John Wiley & Sons, New York.

Miller JD (1999) Determining clutch size and hatching success. In: *Research and management techniques for the conservation of sea turtles* (eds Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M), pp. 124-129. IUCN/SSC Marine Turtle Specialist Group, Washington DC.

Mortimer JA, Portier KM (1989) Reproductive homing and internesting behavior of the Green turtle (*Chelonia mydas*) at Ascension Island, south Atlantic Ocean. *Copeia*, **4**, 962-977.

Mrosovsky N (1987) Pivotal temperature for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. *Canadian Journal of Zoology*, **66**, 661-669.

Mrosovsky N (1994) Sex Ratios of Sea Turtles. *The Journal of Experimental Zoology*, **270**, 16-27.

Mrosovsky N, Baptistotte C, Godfrey MH (1999) Validation of incubation duration as an index of the sex ratio of hatchling sea turtles. *Canadian Journal of Zoology*, **77**, 831-835.

Mrosovsky N, Kamel S, Rees AF, Margaritoulis D (2002) Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. *Canadian Journal of Zoology*, **80**, 2118-2124.

Mrosovsky N, Pieau C (1991) Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia*, **12**, 169-179.

Wedekind C (2002) Manipulating sex ratios for conservation: short-term risks and long-term benefits. *Animal Conservation*, **5**, 13-20.

Weishampel JF, Bagley DA, Ehrhart LM (2004) Earlier nesting by loggerhead sea turtles following sea surface warming. *Global Change Biology*, **10**, 1-4.

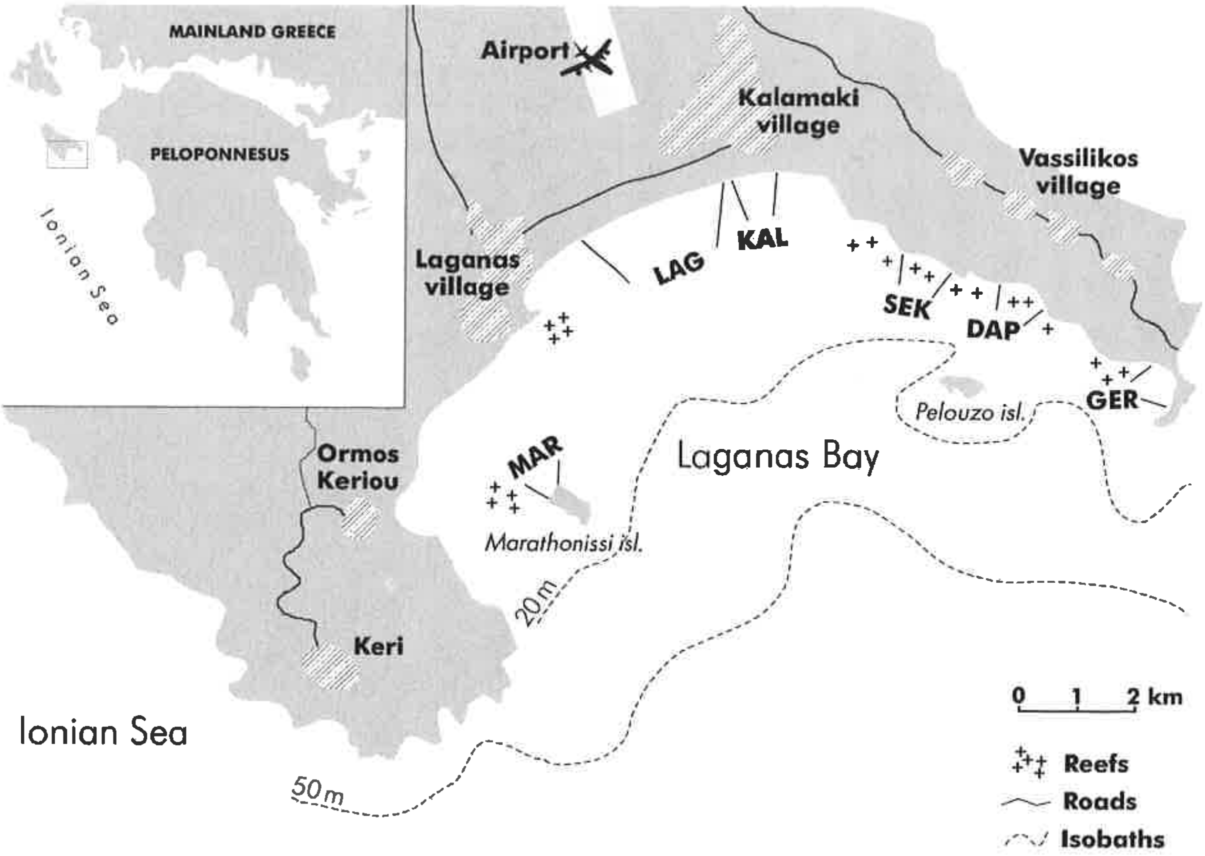
Wibbels T (2003) Critical approaches to sex determination in sea turtles. In: *The Biology of Sea Turtles Volume II* (eds Lutz PL, Musick JA, Wyneken J), pp. 103-134. CRC Press, Boca Raton.

Figure Legends

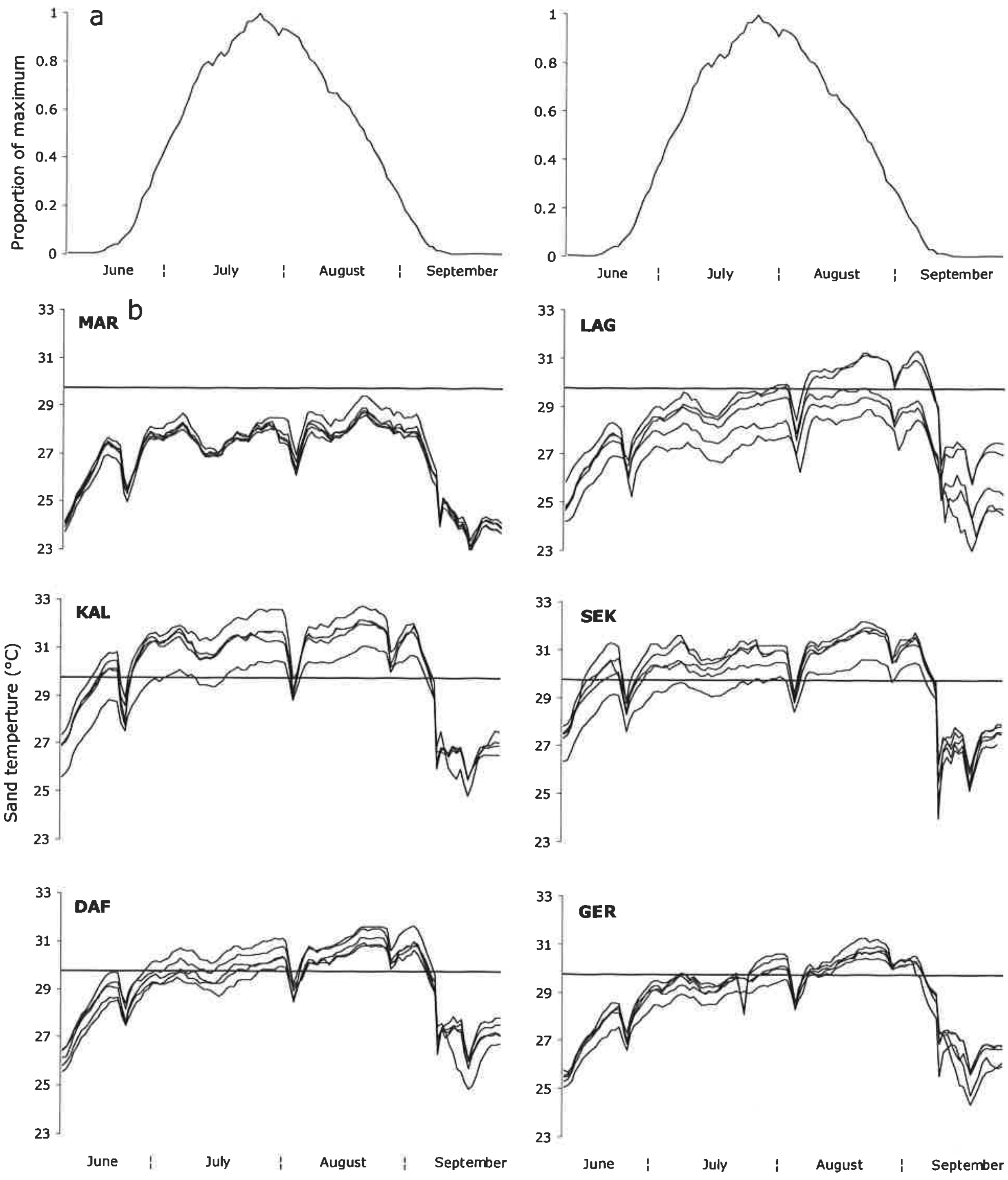
Fig 1. Map of the Bay of Laganas with the six distinct nesting beaches (MAR = Marathonissi, LAG = East Laganas, KAL = Kalamaki, SEK = Sekania, DAP = Daphni, GER = Gerakas).

Fig. 2. A. Proportion of nests in the thermo-sensitive stage for sex determination with respect to date (2003). The graph is shown twice for easier interpretation to the temperature profiles. B. Temperature profiles (daily average values) obtained in 2003 on the six beaches. For KAL, due to loss of a data logger, one of the five profiles only exists for the first half of the season. The horizontal line indicates the pivotal field temperature (T_p).

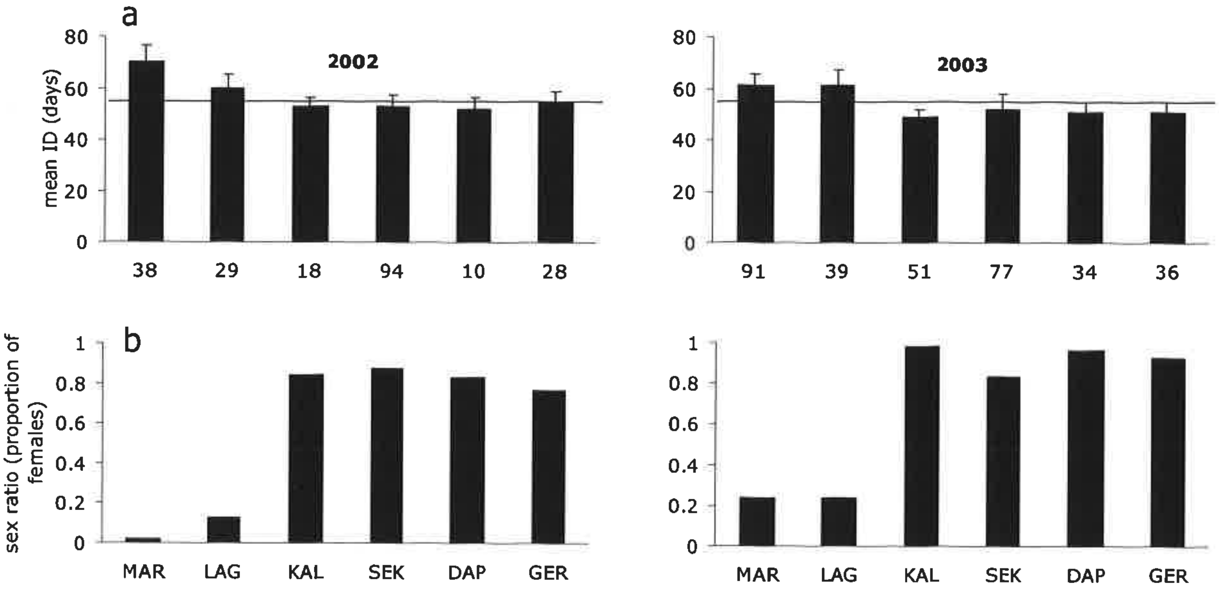
Fig. 3. Mean (+SD) incubation durations (ID, a) and derived sex ratio estimates (b) for the individual beaches in 2002 and 2003. Sample size is indicated at the base of each column, whereas the pivotal field incubation duration is indicated by a horizontal line.



Zbinden et al. Fig. 1



Zbinden et al. Fig. 2



Zbinden et al. Fig. 3

Chapter two

Metabolic heating in Mediterranean loggerhead sea turtle clutches

Judith A. Zbinden, Dimitris Margaritoulis, and Raphaël Arlettaz

Journal of Experimental Marine Biology and Ecology (in press)

ELSEVIER

Journal of Experimental Marine Biology and Ecology xx (2006) xxx – xxx

Journal of
EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Metabolic heating in Mediterranean loggerhead sea turtle clutches

Judith A. Zbinden [a,*], Dimitris Margaritoulis [b], Raphaël Arlettaz [a]

[a] *Zoological Institute, Division of Conservation Biology, Baltzerstrasse 6, CH-3012 Bern, Switzerland*
[b] *ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, GR-10432 Athens, Greece*

Received 23 August 2005; received in revised form 1 November 2005; accepted 26 January 2006

Abstract

Offspring sex ratio is an important demographic parameter and, given its determination by incubation temperature in sea turtles, might be a key factor for their conservation under climate warming. An appealing approach to estimate hatchling sex ratios is to measure sand temperatures at nest depth and deduce hatchling sex ratios from a beforehand-established relationship of hatchling sex ratio and sand temperature. Such estimates will only be accurate though if metabolic heat produced by the embryos is considered. Judging whether metabolic heating has a potential effect on hatchling sex ratios without actually measuring temperature within clutches would greatly facilitate monitoring protocols. We tested for a relationship between the amount of metabolic heating and the number of developed embryos as well as clutch size in the largest known loggerhead sea turtle (*Caretta caretta*) population of the Mediterranean on Zakynthos (Greece). Temperatures were measured within 20 nests as well as at a reference site in the sand at nest depth. Metabolic heating was detected, but only during the last third of the incubation period did nests heat up considerably (1.6°C on average) above the temperature of the surrounding sand. During the middle third of incubation, when sex is determined, the amount of metabolic heating was negligible. The amount of metabolic heating during the last third of the incubation duration was significantly correlated to the number of offspring developed to at least about 75% of incubation duration. This factor explained nearly 50% of variation in metabolic heating. Metabolic heating was also significantly correlated to clutch size. Given that clutch size within the Mediterranean is largest in Zakynthos loggerheads, we conclude that metabolic heating can be ignored in the estimate of hatchling sex ratios in Mediterranean loggerhead populations. These results thus provide the basis for a feasible monitoring of hatchling sex ratios in the loggerhead sea turtle in the Mediterranean.

Keywords: *Caretta caretta*; Hatchling sex ratio; Mediterranean; Metabolic heating; Temperature-dependent sex determination

1. Introduction

Increase of temperature in reptilian clutches over that of the surrounding environment is a consequence of high metabolic activity during development and restricted thermal conductivity of the surrounding medium. Sea turtles bury sizeable clutches of large eggs in sandy beaches. The large amount of living tissue in these clutches combined with the relatively low thermal conductivity of the medium (air) between the eggs suggests that sea turtle clutches heat up during development above the temperature of the surrounding sand. Indeed metabolic heating has long been proposed to occur in sea turtle clutches (Carr and Hirth, 1961). Although the embryonic development of sea turtles has been studied in detail (Miller, 1985), only recently the availability of small data loggers has made it feasible to measure temperature within clutches during the entire

---

* Corresponding author. Tel.: +41 31 631 31 53; fax: +41 31 631 45 35.

E-mail address: judith.zbinden@nat.unibe.ch (J.A. Zbinden).

0022-0981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2006.01.021

incubation duration with minimum disturbance to natural conditions. It seems that–as expected–detectable amounts of metabolic heating are a general characteristic of sea turtle clutches and that metabolic heating becomes evident around halfway through development and raises towards hatching (Godfrey et al., 1997; Booth and Astill, 2001; Broderick et al., 2001; Godley et al., 2001; Glen and Mrosovsky, 2004).

Metabolic heating in sea turtles is not merely an interesting topic in itself, but given the temperature-dependent sex determination of sea turtle embryos (Mrosovsky and Pieau, 1991), understanding what factors determine the amount of metabolic heating will improve monitoring protocols for these endangered species. Since sexing is not possible from external morphology in hatchling sea turtles, hatchling sex ratio estimates are traditionally inferred through indirect parameters (sand and within-nest temperatures or clutch incubation durations), based on their correlation with hatchling sex ratios established through histology of sacrificed hatchlings (Godfrey and Mrosovsky, 1997; Mrosovsky et al., 1999; Mrosovsky et al., 2002). Inferring to hatchling sex ratios through sand temperature measurements is a particularly appealing approach for several reasons, e.g., data can be collected with a minimum of working force. Meaningful hatchling sex ratio estimates can, however, only be obtained if a number of factors are considered, a major point of consideration being the average amount of metabolic heating. If metabolic heating is considerable, average clutch temperatures will systematically differ from sand temperatures at nest-depth. Indeed it has been suggested for several sea turtle populations that metabolic heating has a feminizing effect on hatchling sex ratios (Broderick et al., 2001; Glen and Mrosovsky, 2004). Therefore, to infer from sand temperature data to accurate hatchling sex ratio estimates, the amount of extra heat produced during the middle third of development must be known. Unfortunately, measuring metabolic heating is not all that simple, and monitoring hatchling sex ratios for a wide range of populations would be facilitated could metabolic heating be estimated from known population characteristics. It is likely, e.g., that the mass of developing tissue (i.e., the number and size of embryos) determines at least part of this variation. Two studies have indeed found correlations between the amount of metabolic heating and developed embryos/clutch sizes, both for the green sea turtle (*Chelonia mydas*). Broderick et al. (2001) found the amount of metabolic heating correlated positively with both clutch size and the number of hatchlings plus embryos that died during development in the Ascension Island population. However, the relationship they found may be driven by a few data points and should thus be interpreted with caution, as the authors state themselves. Booth and Astill (2001) also found that a surrogate of metabolic heating positively correlated with clutch size in Australia. However, their analysis is based on a very small sample (five clutches).

We investigated patterns of metabolic heating in Zakynthian loggerheads, the largest known population of the species in the Mediterranean (Margaritoulis et al., 2003) with a twofold goal: firstly, we simply aimed at estimating the amount of metabolic heating in Zakynthian loggerhead clutches as a prerequisite to estimate hatchling sex ratios from sand temperatures in this important nesting area. Secondly, we aimed to test for a reliable relationship between metabolic heating and developed embryos/clutch size, which could simplify estimates of metabolic heating in loggerhead populations within the Mediterranean and possibly on a larger scale.

2. Materials and methods

2.1. Study area

The Island of Zakynthos is situated in the Ionian Sea in the eastern Mediterranean. The climate is highly seasonal both in terms of temperature and rainfall, with high temperatures and almost inexistent precipitation during the summer months, while autumn and winter are characterized by lower temperatures, heavy rains and storms. The south-facing Bay of Laganas contains six discrete nesting beaches; the opening of the bay is ca. 12km. Loggerhead sea turtles nest from the second half of May until the first half of August (Margaritoulis and Rees, 2003). Apart from a small nesting aggregation on the neighbouring Island of Kephalonia, the nesting beaches of Zakynthos constitute the northernmost loggerhead nesting area in the Mediterranean (Margaritoulis et al., 2003).

2.2. Data collection

The study was conducted on one of the beaches of the Bay of Laganas [Gerakas beach (37°42`N, 20°59`E)], during 2003 and 2004. Clutches we investigated were laid between 15 June and 24 July. To measure clutch temperature, we placed a temperature logger («Tinytalk», accuracy±0.3 °C; Gemini Dataloggers, UK) into 20 nests (eight in 2003 and twelve in 2004). We programmed loggers to record temperature at 1 h intervals. Their precision was confirmed by comparing temperature measurements taken with loggers placed next to each other. We inserted data loggers into the clutch in the early morning following the night a clutch was laid (i.e., within 2 to 6 h of the clutch being laid), temporarily removing part of the eggs from the egg chamber and placing the logger in the approximate centre of the clutch. This methodology was chosen based on a preliminary study where loggers were dropped while turtles were laying eggs, as we noticed that the device often rolled to the side of the clutch.

This was done at first light when the air temperature was still rather cold and humid and care was taken to replace sand in the original order and to work fast. Therefore, we have no reason to suspect that the positioning procedure or the data logger itself (the electronic device fits into a photographic film canister) influenced the incubation conditions of the clutches. Additionally, we measured sand temperature at average nest depth for Zakynthos (39 cm, ARCHELON internal reports, unpublished data) at one location within the same beach, at a distance of no more than 500 m from any study nest, for reference in both years of the study.

Nests were monitored daily by observation of tracks in order to detect hatching. The morning the first hatchling's track was recorded was considered as hatching date. The incubation duration (ID) was defined as the number of days from the date after the night a clutch was laid until the date of observation of the first hatchling tracks. We excavated nests 14 days after initial hatching, retrieved data loggers, categorized nest contents and determined hatching success (Miller, 1999). Additionally to the overall count of dead embryos, the ones having reached at least about 75% of incubation duration were counted separately. All fieldwork was conducted during surveys by ARCHELON, The Sea Turtle Protection Society of Greece.

2.3. Data analysis

Nest temperature data of the time period between actual hatching and emergence, the latter being referred to as «observed hatching», estimated on 4 days (Godfrey and Mrosovsky, 1997), had to be excluded. Because sand temperature at nest depth can vary between nearby locations (i.e., the location of the study clutch and the location where sand temperature was measured), metabolic heating at any moment could not be simply calculated as the difference between clutch temperature and sand temperature.

Instead, as a first step, we determined the difference between clutch temperature and sand temperature per day for each day of the incubation duration. In a second step, the day-to-day change in this parameter was calculated. The baseline difference between nest temperature and sand temperature was considered as the average value of the difference between clutch temperature and sand temperature before its day-to-day change exceeded 0.3 °C (the precision of data loggers). We calculated metabolic heat per day as the difference between clutch temperature and sand temperature subtracted by the baseline difference as described above. To visualize the pattern of metabolic heating over the entire incubation period, we calculated the mean amount of metabolic heating during each 2%

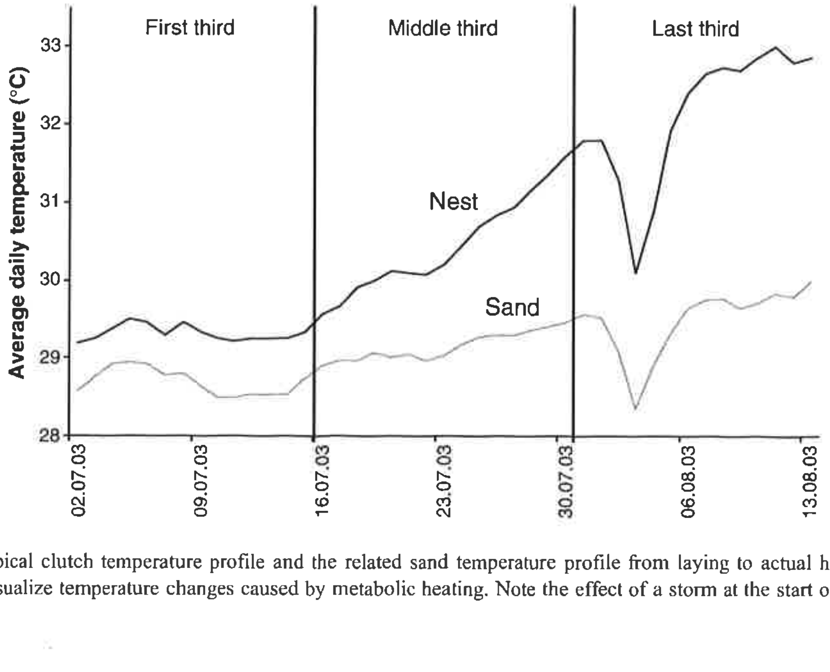


Fig. 1. Example of a typical clutch temperature profile and the related sand temperature profile from laying to actual hatching (4 days prior to observed hatching) to visualize temperature changes caused by metabolic heating. Note the effect of a storm at the start of August.

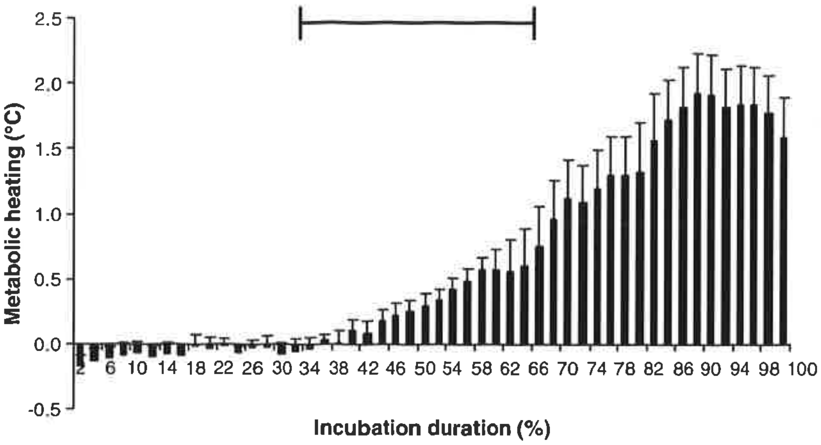


Fig. 2. Mean metabolic heating measured in eight clutches of 2003 according to relative advancement (%) of incubation. Error bars indicate SE. The horizontal bar depicts the second third of the incubation period.

period of the incubation duration. We tested for a relationship between the amount of metabolic heating and clutch size as well as the number of embryos that developed to at least about 75% of incubation duration (i.e., number of hatchlings plus number of late-stage dead embryos). To get an idea about the generalization of our results, we compared the data point of average metabolic heating vs. average clutch size of Cyprus loggerheads (Godley et al., 2001) to our data.

Apart from investigating metabolic heating, we verified the relationship between observed incubation duration and nest temperature—a basis to deduce hatchling sex ratio estimates from incubation durations.

Statistical analyses were performed with the program JMP IN© (version 4.04, SAS Institute Inc.).

3. Results

3.1. Metabolic heating

Clutch temperatures closely followed the course of sand temperatures during the first third of incubation as illustrated in the example (Fig. 1). The increasing discrepancy between nest and sand temperature afterwards is attributed to metabolic heating. The pattern of metabolic heating of the eight nests of 2003 (in 2004, sand temperature was recorded only from 12 July onwards due to loss of a data logger) over the entire incubation duration is shown in Fig. 2. Over the entire incubation period, these nests increased their temperature by, on average, 0.60±0.25 °C (±SD) over that of the sand. During the first third of development, no metabolic heating could be detected (−0.06±0.10 °C). During the middle third of development, the 20 clutches increased their temperature by, on average, 0.20 °C (±0.20) above that of the surrounding sand. During the last third of incubation, metabolic heating accounted for on average 1.64 °C (±0.56) in all clutches studied. Because metabolic heating during the middle third of development was negligible, we only tested the effect of eggs developed to at least about 75% of incubation duration and clutch size on metabolic heating during the last third of development. Since the number of eggs developed to at least about 75% of incubation duration were highly correlated to clutch size ($F_{1,18}=72.11$, $p<0.001$, $R^2=0.80$), we tested each factor separately. The amount of metabolic heating was significantly correlated to both factors, although the number of eggs developed to at

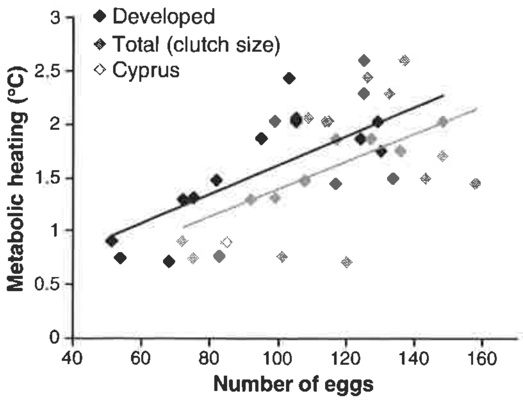


Fig. 3. Relationship between the amount of metabolic heating during the last third of development and the number of eggs developed to at least about 75% of incubation duration (metabolic heating = 0.013 × developed eggs + 0.27, black symbols) and clutch size (metabolic heating = 0.013 × clutch size + 0.12, grey symbols). The data point from Cyprus (grey outlined symbol) is an average of 15 clutches taken from Godley et al. (2001).

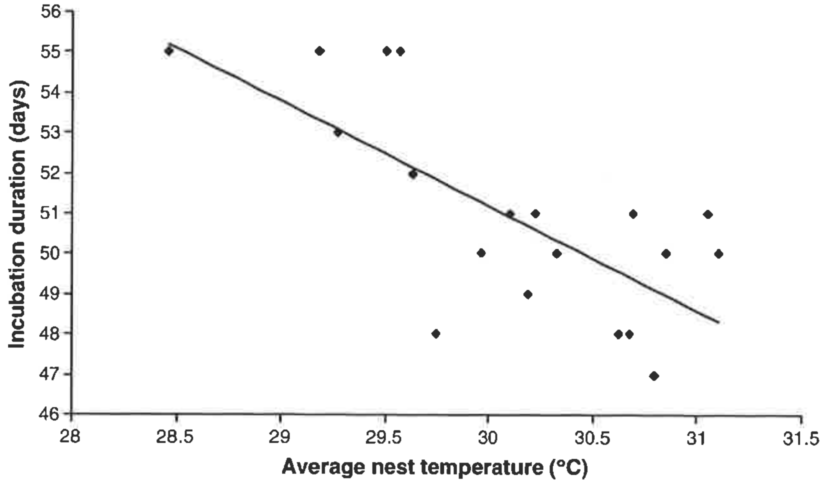


Fig. 4. Relationship between observed incubation duration and mean nest temperature for all study nests ($n=20$). Regression equation: Incubation duration $=-2.40\times$ mean nest temperature $+122.46$.

least about 75% of incubation duration explained more of the variation ($F_{1,18}=15.22$, $p=0.001$, $R^2=0.46$, Fig. 3) than clutch size did ($F_{1,18}=7.41$, $p=0.014$, $R^2=0.29$, Fig. 3). The data point from Cyprus fits rather well into the relationship (Fig. 3). The amount of metabolic heating is significantly higher than in loggerhead sea turtle nests of northern Cyprus during the final third of development ($t_{33}=-4.09$, $p<0.001$), although not during the middle third ($t_{33}=0.076$, $p=0.94$).

3.2. Relationship between incubation duration and nest temperature

The negative correlation of incubation duration with average within-nest temperature was highly significant ($F_{1,19}=18.16$, $p<0.001$, $R^2=0.502$, Fig. 4), as was the correlation of incubation duration with average temperature during the middle third of development ($F_{1,19}=9.74$, $p=0.0059$, $R^2=0.35$).

4. Discussion

A better understanding of how sea turtle sex ratios are affecting population dynamics is needed to predict the effect of climate change on turtle populations. In the meantime it seems wise to monitor hatchling sex ratios. The fact that we found nearly half the variation in metabolic heating explained by the amount of developed eggs has implications for development of monitoring protocols for hatchling sex ratio. Tentatively, we suggest that whether or not metabolic heating is a significant factor shaping hatchling sex ratios does not have to be tested for each nesting area. Instead, the amount of metabolic heating in a given nesting area can be judged based on the results of a conspecific population and differences in the average number of developed embryos. It is obvious that this notion is based on rather little data and should certainly be verified in other populations before being widely applied. The fact that no such relationship was found in Cyprus loggerheads despite a comparable sample size is not necessarily challenging our result, but might simply be the outcome of low variation in factors. The most obvious difference between loggerhead clutches of Cyprus and Zakynthos seems the difference in clutch size. Whereas an average clutch in Zakynthos contains between 110 and 130 eggs, average clutches in northern Cyprus comprise only 75 to 83 eggs (Margaritoulis et al., 2003). The fact that we found a significant difference in metabolic heating between the data sets of Zakynthos and Cyprus with less metabolic heating detected in Cyprus is thus not surprising. The growth of embryo mass over time increasing with incubation time (Ackerman, 1997) closely matching the increase in metabolic heating underlines the close relationship between metabolic heating and embryo mass.

Although we could explain a large proportion of the variation in metabolic heating by number of developed embryos, what other factors are at play remains unresolved. It is likely that the conductivity of the surrounding sand plays a role in determining the amount of metabolic heating within the clutch (Speakman et al., 1998). Incubation of turtle eggs under controlled conditions could substantially increase our understanding of factors determining the amount of metabolic heating, but are restricted due to the conservation status of these species.

Experimental incubation of alligator eggs, a species whose sex is likewise determined by temperature, has revealed that eggs incubated in a clutch produced different sex ratios than those incubated individually; a result that was explained by differences in metabolic heating between the two treatments (Ewert and Nelson, 2003).

Our interpretation is based on results obtained from metabolic heating during the last third of development, whereas it is during the middle third that sex is determined (Mrosovsky and Pieau, 1991). The congruent pattern of metabolic heating over the incubation duration (this study; Broderick et al., 2001; Godley et al., 2001) suggests that average metabolic heating during the middle third of incubation can be reliably extrapolated from the amount of metabolic heating during the last third of the incubation duration. A further point of consideration is that we measured temperature in only one location of the clutch. It has been shown, however, that temperature in loggerhead clutches is not uniform for all eggs in a clutch (Godfrey et al., 1997; Hanson et al., 1998; Kaska et al., 1998; Booth and Astill, 2001; Houghton and Hays, 2001). Specifically, Godfrey et al. (1997) and Booth and Astill (2001) found the amount of metabolic heating higher in the centre than on the side of the clutch. Thus our results are likely to overestimate the amount of metabolic heating experienced by the average egg. This potential bias is not affecting the qualitative result that the average amount of metabolic heating during the middle third of development is negligible in our study.

Loggerhead clutches of Zakynthos being the largest amongst Mediterranean populations (Margaritoulis et al., 2003), we conclude that metabolic heating has no significant feminizing effect on Mediterranean loggerheads. Loggerhead clutches in other ocean basins are likely differently affected by metabolic heating, since Mediterranean loggerheads lay larger clutches with relatively small eggs compared to their body size than, e.g., Atlantic loggerheads (Tiwari and Bjorndal, 2000). It is thus likely that the specific relationship between developing embryos/clutch size and metabolic heating is different for other population groups.

Although sand temperatures and incubation durations remain indirect methods and as such potentially inaccurate, our results eliminate some of the uncertainty in the deduced hatchling sex ratio estimates.

Acknowledgements

The major part of this research was funded by a grant from the MAVA foundation, to which we are extremely grateful.

We would like to thank K. Grimanis and A. Rees (ARCHELON) for support. We are grateful to B. Godley (Marine Turtle Research Group, University of Exeter) as well as A. Aebischer and M. Schaub (Zoological Institute, University of Bern) for their contributions to the study. Special thanks go to H. Freeman, C. Nienhuis and L. Southern for field assistance. The comments of two anonymous reviewers substantially ameliorated the quality of the manuscript. **[SS]**

References

Ackerman, R.A., 1997. The nest environment and the embryonic development of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC Press, Boca Raton, pp. 83–106.

Booth, D.T., Astill, K., 2001. Temperature variation within and between nests of the green sea turtle, *Chelonia mydas* (Chelonia: Cheloniidae) on Heron Island, Great Barier Reef. Australian Journal of Zoology 49, 71–84.

Broderick, A.C., Godley, B.J., Hays, G.C., 2001. Metabolic heating and the prediction of sex ratios for green turtles (*Chelonia mydas*). Physiological and Biochemical Zoology 74, 161–170.

Carr, A., Hirth, H., 1961. Social facilitation in green turtle siblings. Animal Behaviour 9, 68–70.

Ewert, M.A., Nelson, C.E., 2003. Metabolic heating of embryos and sex determination in the American alligator, *Alligator mississippiensis*. Journal of Thermal Biology 28, 159–165.

Glen, F., Mrosovsky, N., 2004. Antigua revisited: the impact of climate change on sand and nest temperatures at a hawksbill turtle (Eretmochelys imbricata) nesting beach. Global Change Biology 10, 2036–2045.

Godfrey, M.H., Mrosovsky, N., 1997. Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2, 581–585.

Godfrey, M.H., Barreto, R., Mrosovsky, N., 1997. Metabolically-generated heat of developing eggs and its potential effect on sex ratio of sea turtle hatchlings. Journal of Herpetology 31, 616–619.

Godley, B.J., Broderick, A.C., Downie, J.R., Glen, F., Houghton, J.D., Kirkwood, I., Reece, S., Hays, G.C., 2001. Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. Journal of Experimental Marine Biology and Ecology 263, 45–63.

Hanson, J., Wibbles, T., Martin, R.E., 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. Canadian Journal of Zoology 76, 1850–1861.

Houghton, J.D.R., Hays, G.C., 2001. Asynchronous emergence by loggerhead turtle (*Caretta caretta*) hatchlings. Naturwissenschaften 88, 133–136.

Kaska, Y., Downie, R., Tippett, R., Furness, R.W., 1998. Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. Canadian Journal of Zoology 76, 723–729.

Margaritoulis, D., Rees, A.F., 2003. Loggerhead nesting effort and conservation initiatives at the monitored beaches of Greece during 2002. Marine Turtle Newsletter 102, 11–13.

Margaritoulis, D., Argano, R., Baran, I., Bentivegna, F., Bradai, M.N., Camiñas, J.A., Casale, P., Metrio, G.D., Demetropoulos, A., Gerosa, G., Godley, B.J., Haddoud, D.A., Houghton, J., Laurent, L., Lazar, B., 2003. Loggerhead turtles in the Mediterranean Sea: present knowledge and conservation perspectives. In: Bolten, A.

B., Witherington, B.E. (Eds.), Loggerhead Sea Turtles. Smithsonian Institution, Washington, pp. 175–198.

Miller, J.D., 1985. Embryology of marine turtles. In: Gans, C., Billett, F., Maderson, P.F.A. (Eds.), Biology of Reptilia, Development A. John Wiley and Sons, New York, pp. 269–328.

Miller, J.D., 1999. Determining clutch size and hatching success. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group, Washington, DC, pp. 124–129.

Mrosovsky, N., Pieau, C., 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia 12, 169–179.

Mrosovsky, N., Baptistotte, C., Godfrey, M.H., 1999. Validation of incubation duration as an index of the sex ratio of hatchling sea turtles. Canadian Journal of Zoology 77, 831–835.

Mrosovsky, N., Kamel, S., Rees, A.F., Margaritoulis, D., 2002. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. Canadian Journal of Zoology 80, 2118–2124.

Speakman, J.R., Hays, G.C., Lindblad, E., 1998. Thermal conductivity of sand and its effect on the temperature of loggerhead sea turtle (*Caretta caretta*) nests. Journal of the Marine Biological Association of the United Kingdom 78, 1337–1352.

Tiwari, M., Bjorndal, K.A., 2000. Variation in morphology and reproduction in loggerheads, *Caretta caretta*, nesting in the United States, Brazil, and Greece. Herpetologica 56, 343–356.

Chapter three

High frequency of multiple paternity in the largest population of Mediterranean loggerhead sea turtles

Judith A. Zbinden, Carlo Largiadèr, Fabio Leippert, Dimitris Margaritoulis, and Raphaël Arlettaz

Manuscript

High frequency of multiple paternity in the largest population of Mediterranean loggerhead sea turtles

Judith A. Zbinden[1], Carlo Largiadèr[2], Fabio Leippert[1], Dimitris Margaritoulis[3], and Raphaël Arlettaz[1]

[1] Zoological Institute, Division of Conservation Biology, Baltzerstrasse 6, 3012 Bern, Switzerland
[2] Zoological Institute, Division of Population Genetics, Baltzerstrasse 6, 3012 Bern, Switzerland
[3] ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, 10432 Athens, Greece

Key words: mating system, multiple paternity, Mediterranean, Zakynthos, *Caretta caretta*

Corresponding author:
Judith Zbinden
Zoological Institute
Division of Conservation Biology
Baltzerstrasse 6
CH-3012 Bern
Tel.: +41 31 631 31 53
Fax: +41 31 631 45 35
judith.zbinden@nat.unibe.ch

Running head: Multiple paternity in loggerhead sea turtles

Abstract

Due to climate change, sex ratio is likely to become a key conservation issue in endangered species with temperature-dependent sex determination. Many species, for example sea turtles, will not be able to adapt fast enough to increasing temperatures. Because higher temperatures mean higher female proportions in this group, a lack of males will eventually become a problem. To anticipate possible effects, it is critical to collect information on current levels of female multiple matings and the relationship between individual female reproductive output and the number of sires in her clutches. We investigated patterns of multiple paternity in clutches of 15 female Mediterranean loggerhead sea turtles using four highly variable microsatellite loci. We tested for a relationship between clutch hatching success and the number of males discovered in a clutch. In clutches of 14 females more than one male was detected, with some females having mated with at least five males. Hatching success significantly increased with the number of males detected in a clutch. Moreover, the number of detected fathers was positively correlated with the body size of the mother. The high rate of multiple paternity might indicate that the population currently is not male-limited. We suspect that the correlation between hatching success and the number of fathers siring a clutch may not be causative. Assuming that it is and clutch hatching success is indeed affected by the number of sires, it could be predicted that individual female reproductive output will decrease with future climate change.

?

?

Introduction

Mating systems and the extent of multiple matings by female and male individuals are an important life history parameter of animal species. Within a species, these may vary for example according to population density or operational sex ratio (OSR, the ratio of reproductively active males to females at any one time). Sex ratios in species with environmental sex determination may be subject to considerable fluctuations. In the case of temperature-dependent sex determination, not only does primary sex ratio vary with respect to ambient conditions, but global warming is expected to lead to systematic shifts in sex ratios under the assumption that most species will not be able to react fast enough to increasing temperatures (ecological trap). In species where high temperatures lead to male differentiation (such as in crocodilians and many lizards (Janzen & Paukstis, 1991)), increasing temperatures are expected to lead to reduced population reproductive output. Where higher temperatures lead to females, as for example in sea turtles (Mrosovsky & Pieau, 1991), a rise in environmental temperatures may initially boost population reproductive output by increasing the number of females. At a certain point, however, a lack of males will lead to reduced fertilisation rates and thus reduced population reproductive output. That a low proportion of males might become an actual problem for populations is exemplified by a high rate of infertility (22% of clutches) among leatherback sea turtles (*Dermochelys coriacea*) in Malaysia. This observation has been attributed to an insufficient number of males in the population – a highly probable explanation given that in the past mainly clutches incubating during the warmer part of the nesting season had been protected (Chan, 1991).

At what ratio and by what mechanisms increasingly female-biased sex ratios will affect sea turtle populations is, however, largely a matter of speculation (Heppell *et al.*, 2003). Uncertainty exists for example as to the threshold value of adult sex ratio needed for fertilisation of all eggs. Moreover, the current extent of multiple matings by females and possible effects on their fitness remains unknown for most populations. Since mating often poses disadvantages to females such as increased risk of predation or disease transmission (Krebs & Davies, 1993), females are only expected to mate with several males if this renders benefits (assuming they are in control of matings). Indeed, evidence is growing that in a number of species females mating with more than one male increase their fitness through genetic benefits to their offspring (Madsen *et al.*, 1992; Olsson & Madsen, 2001; Osikowski & Rafinski, 2001; Fedorka & Mousseau, 2002). In sea turtles, the disadvantages of multiple matings for females may be considerable, the most obvious being physical damage (Miller, 1997). Females are thought to be in control of matings. Female sea turtles lay several clutches at intervals of about two weeks during one nesting season (Miller, 1997). Mating with one male is enough to fertilize all the eggs of a female turtle in a given reproductive season (FitzSimmons, 1998; Pearse *et al.*, 2001). Possible fitness benefits to females through good, diverse or compatible genes are however well conceivable. Sperm storage (Pearse & Avise, 2001) leaves ample opportunity for sperm competition and cryptic female choice. An important question concerning the effect of OSR on sea turtle populations is thus whether female multiple matings lead to increased reproductive output of individual females.

Sea turtles mate at sea, where they are hard to observe, and, due to their large adult size and complex life-history pattern, are not amenable to experimental studies. The development of appropriate molecular techniques has opened new opportunities to study aspects of mating behaviour indirectly. We used microsatellites, highly variable DNA-markers, to test the question of frequency of multiple paternity and its effect on hatching success in loggerhead sea turtles (*Caretta caretta*) of Zakynthos (Greece, Mediterranean). In our study population several observations of individual females mating with more than one male exist (Schofield, 2003). However, as the paternity study on Australian green sea turtles (*Chelonia mydas*) exemplifies, anecdotal observations on female mating behaviour may not be a good predictor of multiple paternity patterns in sea turtles (FitzSimmons, 1998). Our first goal was therefore to determine the frequency of multiple paternity in this largest Mediterranean sea turtle population (Margaritoulis *et al.*, 2003). We then investigated whether hatching success (as a measure of female reproductive output) was related to multiple paternity patterns. Lastly, we tested whether the number of males a female mated with was related to her morphological characteristics. The answers to these questions may help in predicting the effect of climate change on sea turtle~~s~~. pop. dynamics

Materials and methods

Study area

The nesting area of the Bay of Laganas on Zakynthos (hereafter referred to as Zakynthos) consists of six discrete but adjacent nesting beaches, spreading over approximately 5.5 km (Margaritoulis, 2005). These beaches are monitored comprehensively by ARCHELON (The Sea Turtle Protection Society of Greece). On average nearly 1300 clutches are laid here annually (Margaritoulis, 2005). Individual females frequently alternate between the beaches of the bay for successive egg layings within a season (ARCHELON internal reports, unpublished data). Loggerhead sea turtles nest on Zakynthos from the second half of May untill the first half of August (Margaritoulis, 2005).

Field methods

We collected tissue samples of females and their offspring on Gerakas beach (37°43`N, 20°53`E), one of the nesting beaches of the Bay of Laganas. We sampled adult females during nightly beach patrols conducted by ARCHELON. Samples consisted of skin-plugs resulting from flipper-tagging with plastic rototags, preserved in absolute ethanol. We measured female size (curved carapace length notch to tip), marked clutch locations during night patrol and located egg chambers the following morning. We triangulated the position of clutches to marker poles set up at the back of the beach. Clutches were either marked by a metal cage placed in the sand above the clutch (where this was required for nest protection) or with several labelled stones placed beneath the sand surface. We could identify study clutches during the entire incubation and unequivocally assign hatchlings to specific clutches. From 40 days of incubation onwards, a fence was built around study clutches during the night. We checked fences at intervals of <30 min for emerged hatchlings. Non-destructive blood samples (10-50 µl) of hatchlings were taken from the dorsal cervical sinus according to Bennett (1986). Either we pierced the blood vessel with a 27 gauge needle and collected the drop of blood with a micropipette, or a 30 gauge insulin syringe was used. Blood samples were stored in Lysis buffer (FitzSimmons *et al.*, 1999) at room temperature for up to several weeks in the field and later frozen. All hatchlings were released immediately after sampling. If a large number of hatchlings emerged together, a random part of them was released without being sampled, ensuring that no animal was trapped for longer than an hour. In keeping with ARCHELON monitoring protocols, we excavated clutches 14 days after first hatching and categorized contents according to Miller (1999). We preserved tissue of recently died embryos and hatchlings found during excavations in absolute ethanol. We collected samples from clutches laid from mid June to mid July in 2003 and 2004.

Skin-plugs from additional nesting females and tissue samples of recently died offspring found during clutch excavation of clutches with known mothers were collected in 2003 - 2005 on various beaches of the Bay of Laganas. They were used in addition to the genotypes of the mothers sampled for paternity analysis to determine population allele frequencies. We assured that all samples stemmed from different females that were not identical to the females sampled for paternity.

Microsatellite genotyping and characterization of microsatellite loci

DNA was extracted using magnetic beads (Promega, Wallisellen, Switzerland), after standard digestion and ethanol purification. All samples were typed at the four microsatellite loci Cc7 (FitzSimmons, 1998), Cc117 (FitzSimmons *et al.*, 1995), Cc141 (FitzSimmons *et al.*, 1999), and CCM2 (Moore & Ball, 2002). All loci were amplified simultaneously with a Multiplex PCR kit (QUIAGEN, Basel, Switzerland), according to the recommendations of the manufacturer. The amplified fragments were resolved on an automated DNA sequencer (ABI 3100). A negative control was included in every PCR reaction. Alleles were scored with Genemapper® version 3.0 (ABI, Rotkreuz, Switzerland) and checked by eye. Samples with unclear results were repeated, where necessary in single-locus reactions.

Independent genotypes were tested for deviations from Hardy-Weinberg equilibrium per locus using a Markov chain method in GENEPOP (web version, (Raymond & Rousset, 1995). The same method was used to test for linkage disequilibrium between all pairs of loci. The frequencies of null alleles [alleles that fail to amplify due to mutations in primer binding sites, (Pemberton *et al.*, 1995)] were estimated with the program CERVUS (Marshall *et al.*, 1998). Mean expected paternal exclusion probabilities (probabilities of excluding a single randomly-chosen unrelated individual from parentage) were calculated across loci in GERUD (Jones, 2001). Statistical power of detecting multiple paternity (probability of detecting multiple paternity, PrDM) was assessed for each clutch by the model of Neff and Pitcher (Neff & Pitcher, 2002). This model applies a Monte Carlo simulation and takes into account the number of loci analysed and their allele frequencies in the population, the number of offspring sampled, the genotype of the mother and the expected skews in the contribution of fathers. For the latter, we examined two situations: equal and skewed (10:90%) contributions by two fathers (The model determines the probability to detect multiple paternity, irrespective of the number of fathers involved in a clutch).

Paternity analysis

To keep PrDM reasonably high and similar between clutches, offspring arrays of less than 15 samples were excluded from paternity analysis. Based on extensive evidence (Miller, 1997; FitzSimmons, 1998; Kichler *et al.*, 1999) we assumed that females would not mate again after having laid their first clutch. We therefore pooled all offspring of given mothers for analysis, after comparing paternal alleles between the clutches of individual females. For simplicity, 'clutch' hereafter refers to both single clutches and consecutive clutches from the same female. Maternal genotypes were determined directly from sampled females and observed in the offspring genotypes. Paternal alleles were inferred from offspring genotypes once maternal alleles were accounted for. If an extra paternal allele indicating an additional father appeared in only one hatchling at one locus among the offspring of a mother, this allele was classified as a mutation. We assessed the minimum number of fathers by using the program GERUD (Jones, 2001). This program reconstructs all possible multi-locus genotypes of fathers and searches for the minimum number of males (due to technical limitations, up to five males) that can explain the progeny array. If multiple solutions of father genotypes are obtained for a given minimum number of fathers, GERUD can rank them based on the Mendelian segregation of alleles and the allele frequencies in the population.

Correlates of paternity patterns

For cases where offspring were derived from two clutches from the same mother, parameters were determined from the pooled clutches. We tested whether hatching success was related to the number of fathers detected amongst the offspring of a female (logistic regression). To control for possible artefacts caused by differences between clutches in the probability to detect fathers, we included the number of offspring sampled (the main factor determining the probability to detect fathers) and the interaction between female size and number of offspring sampled as factors in this test. We further tested for a relationship between number of detected fathers and the size of mothers (ordinal logistic regression), and included the number of offspring sampled and the interaction between number of fathers detected and number of offspring sampled as factors in this test. The first logistic regression was performed in the program R (R Developmental Core Team 2004) and the second test in the program JMP IN © (version 4.04, SAS Institute).

Results

Population allele frequencies were based on 53 independent genotypes (38 samples where offspring is not included in the data set for paternity analysis plus 15 mothers of the paternity analysis clutches). Allele frequencies at all loci were within expectations of Hardy-Weinberg equilibrium ($p > 0.05$), and no evidence of linkage disequilibrium was found ($p > 0.05$). The number of alleles varied from eight to 11 (Fig. 1). Observed heterozygosity values ranged from 0.64 to 0.93 (Fig. 1). The frequency of null alleles was 6.2% at maximum (Fig. 1). Paternity exclusion probability for all four loci combined was 0.956. PrDM were very high (above 98%) for all clutches when assuming equal paternal contributions of two fathers, and above 77% when assuming a considerable skew in paternal contributions (Table 1).

Samples had to be excluded from analyses for several reasons. In one of the study nests, none of the alleles of the mother was present in most offspring at one locus (Cc7) and therefore this clutch was excluded from all further analysis. Only one mutation at a maternal allele was detected in one offspring and the respective multilocus genotype excluded. We further excluded 20 offspring from 11 mothers because of apparent mutations of paternal alleles. These putative mutations concerned all loci.

Except that in some cases one clutch contained additional alleles in low frequencies, there was no difference in paternal alleles between the clutches of one mother. We therefore pooled offspring from identical mothers. Microsatellite data for all four loci were obtained for 571 offspring (excluding the above-mentioned samples) from 15 females, including five mothers from which we sampled consecutive clutches (Table 1). Proportions of offspring sampled from total clutch sizes ranged from 14 to 57% (average 32%, SD 12%, Table 1).

Multiple paternity was found in all but one clutch and several clutches had up to a minimum of five different fathers (Table 1). The confidence of ranking different solutions of male genotypes was in most cases low, especially when more than two males were represented in a clutch, as predicted by Jones (2001).

Clutch hatching success significantly increased with the number of males detected in a clutch (X^2 number of fathers 62.67, $p < 0.001$, X^2 number of offspring sampled 0.32, $p = 0.570$, X^2 interaction 3.70, $p = 0.17$, Fig. 2). Graphic presentation of predicted hatching success for clutches of different number of fathers showed that there was a difference in hatching success between clutches sired by one or two males vs. clutches sired by more than three males.

There was a significant correlation between the number of fathers detected in a clutch and the size of the mother (X^2 size of mother 5.11, $p = 0.024$, X^2 number of offspring sampled 7.76, $p = 0.0053$, X^2 interaction 0.32, $p = 0.57$, Fig. 3). Clutches of larger females were sired on average by more males than clutches of smaller females.

Discussion

This first thorough paternity study in Mediterranean sea turtles revealed multiple paternity in 14 out of 15 clutches. This rate is to our knowledge the highest found in a marine turtle so far. Given that we found no deviations from Hardy-Weinberg equilibrium, a low estimated frequency of null alleles and a very low apparent mutation rate, the finding that multiple paternity is widespread in the loggerhead sea turtle population of Zakynthos seems undisputable. Moreover, some of the alleles considered to be mutations from paternal alleles might actually stem from extra fathers and their exclusion might have led to underestimating the minimum number of fathers detected. More importantly, the fact that the probability of detecting multiple paternity (PrDM, skewed contributions) varies considerably among our study clutches indicates that a number of male contributions might have gone undetected mainly due to the low numbers of offspring sampled in some clutches (Table 1).

This is to our knowledge the second loggerhead sea turtle population with reliable estimates of multiple paternity. Moore & Ball (2002) found evidence of multiple paternity in nearly a third of 70 *Caretta caretta* clutches from Florida. Taking into account that they genotyped only around ten offspring per clutch, the frequency of multiply fathered clutches in this population is likely to be much higher than what their data suggests. Considering that in another sea turtle species (the green turtle, *Chelonia mydas*), striking differences in the rate of multiple paternity between populations apparently exist (FitzSimmons, 1998; Lee & Hays, 2004), it would however be premature to conclude that multiple paternity is generally common in loggerhead sea turtles. The fact that both the Florida and Zakynthos population are large strengthens this statement.

Male-female encounter rates are likely to be a key determinant of the frequency of multiple paternity. In individual bird species, for example, the rate of extra-pair copulations appears to increase with density (Westneat & Sherman, 1997). Encounter rates in our case probably depend on population size, density of turtles in the mating area, mating opportunities along migration paths and operational sex ratio. Under these considerations, the very high rate of multiple paternity in Zakynthian loggerhead sea turtles is not surprising. The Zakynthos population is relatively large (Margaritoulis, 2005) and the main mating area may be confined to the Bay of Laganas, where turtle densities are high (personal observation). OSR may be subjected to considerable year-to-year fluctuations due to the fact that most female loggerhead sea turtles do not reproduce annually. The time period between reproductive seasons is likely to be dependent on food availability (Hays, 2000), leading to large year-to-year fluctuations in the number of reproducing females. Males are thought to be less affected by foraging conditions and therefore thought to migrate annually (Miller, 1997). However, clutch numbers in the two seasons we collected samples were average (ARCHELON internal reports, unpublished data), and therefore the estimate of the frequency of multiple paternity can be considered representative for the population. The high rate of multiple paternity suggests that the population currently is not male-limited.

The relationship between hatching success and the number of fathers siring a clutch may not have any biological significance, but simply reflect choice of better nest-sites (leading to higher clutch hatching success) or more viable eggs of females mating with higher numbers of males than those mating with one or two males. Assuming this is not the case and clutch hatching success indeed is affected by the number of sires, individual female reproductive output may decrease with future climate change. A positive correlation between female reproductive output and the number of mates has been found in a range of taxa (Madsen *et al.*, 1992; Kempenaers *et al.*, 1999; Newcomer *et al.*, 1999) and attributed to genetic benefits to offspring, for example through post-copulatory choice of sperm with good or compatible genes. On the other hand, Lee & Hays (2004) and Pearse et al. (2002) could not detect a difference in various parameters of clutch success in singly vs. multiply fathered clutches in green sea turtles and painted turtles (*Chrysemys* *picta*), respectively. More research is therefore needed to conclusively reveal whether female sea turtles can gain benefits from multiple matings. While experimentally studying the sea turtle mating system is not feasible, a solution to minimize the effects of at least some potentially confounding factors would be to move clutches immediately after deposition to a designated area of the beach, so as to manipulate thermal conditions

We found a positive correlation between paternity patterns and female body size. In a sample of 18 clutches of Ascension Island green sea turtles, the mothers of multiply sired clutches tended to be, on average, larger than those laying clutches with only one father detected, but the difference was not significant (Lee & Hays, 2004). Pearse *et al.* (2002) found clutches of painted turtles with multiple paternity containing significantly more eggs than clutches with only one male detected. Although paternity patterns were not significantly related to carapace length, they speculated that the relation may be caused by the higher fecundity of larger females and male preference for larger females. We further hypothesise that the higher rate of multiple paternity in larger females could be the outcome of more accumulated sperm from previous seasons rather than a higher mating rate in a given season. It is conceivable that sea turtles can store viable sperm over several years, as has been shown for painted turtles (Pearse *et al.*, 2001). Because size is an indicator of age in sea turtles, larger females have on average reproduced for more seasons than smaller females. Understanding the importance of long-term sperm storage in sea turtles seems crucial for an improved understanding of their mating system.

→D broader conclusion

Acknowledgements

The major part of this research was funded by a grant from the MAVA Foundation, to which we are extremely grateful. This study would not have been possible without the tireless support of R. Egli, C. Nienhuis and L. Southern during fieldwork. We would also like to thank D. Dimopoulos,

K. Grimanis, H. Strantzalos and A. Rees (ARCHELON) for support. C. Carreras as well as N. FitzSimmons generously shared unpublished information on primers. We likewise thank H. Freeman and ARCHELON volunteers who helped with the fieldwork. A. Aebischer and C. Rutte (Zoological Institute, University of Bern) contributed to the manuscript. We gratefully acknowledge the comments of H. Murray (Applied Linguistics, University of Bern) on an earlier version of the text and the help of M. Schaub (Zoological Institute, University of Bern) in statistics.

References

Bennett JM (1986) A Method for Sampling Blood from Hatchling Loggehead Turtles. *Herpetological Review*, **17**,

Chan EH (1991) Sea turtlles. In: *The state of nature conservation in Malysia* (eds Kiew R), pp. 120-134. Malayan Nature Society, Kuala Lumpur.

Fedorka KM, Mousseau TA (2002) Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour*, **64**, 361-367.

FitzSimmons NN (1998) Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). *Molecular Ecology*, **7**, 575-584.

FitzSimmons NN, Moritz C, Bowen BW (1999) Population Identification. In: *Research and Management Techniques for the Conservation of Sea Turtles* (eds Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M), pp. 72-79. IUCN/SSC Marine Turtle Specialist Group,

FitzSimmons NN, Moritz C, Moore SS (1995) Conservation and Dynamics of Microsatellite Loci over 300 Million Years of Marine Turtle Evolution. *Molecular Biolology and Evolution*, **12**, 432-440.

Hays GC (2000) The Implications of Variable Remigration Intervals for the Assessment of Population Size in Marine Turtles. *Journal of Theoretical Biology*, **206**, 221-227.

Heppell SS, Snover ML, Crowder LB (2003) Sea turtle population ecology. In: *The biology of sea turtles* (eds Lutz PL, Musick JA, Wyneken J), pp. 275-298. CRC Press, Boca Raton.

Janzen FJ, Paukstis GL (1991) Environmental sex determination in reptiles: Ecology, evolution, and experimental design. *The Quarterly Review of Biology*, **66**, 149-179.

Jones AG (2001) GERUD1.0: a computer program for the reconstruction of parental genotypes from progeny arrays using multilocus DNA data. *Molecular Ecology Notes*, **1**, 215-218.

Kempenaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? *Behavioral Ecology*, **10**, 304-311.

Kichler K, Holder MT, Davis SK, Marquez R, Owens DW (1999) Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. *Molecular Ecology*, **8**, 819-830.

Krebs JR, Davies NB (1993) An Introduction to Behavioural Ecology. Blackwell Science, Oxford.

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: Females make the best of a bad job. *PNAS*, **101**, 6530-6535.

Madsen T, Shine R, Loman J, Hakansson T (1992) Why do female adders copulate so frequently? *Nature*, **355**, 440-441.

Margaritoulis D (2005) Nesting activity and reproductive output of loggerhead sea turtles, *Caretta caretta*, over 19 seasons (1984-2002) at Laganas Bay, Zakynthos, Greece: The largest rookery in the Mediterranean. *Chelonian Conservation and Biology*, **4**, 916-929.

Margaritoulis D, Argano R, Baran I *et al.* (2003) Loggerhead turtles in the Mediterranean Sea: Present knowledge and conservation perspectives. In: *Loggerhead sea turtles* (eds Bolten AB, Witherington BE), pp. 175-198. Smithsonian Institution, Washington.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639-655.

Miller JD (1997) Reproduction in sea turtles. In: *The biology of sea turtles* (eds Lutz PL, Musick JA), pp. 51-81. CRC Press, Boca Raton.

Miller JD (1999) Determining clutch size and hatching success. In: *Research and management techniques for the conservation of sea turtles* (eds Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M), pp. 124-129. IUCN/SSC Marine Turtle Specialist Group, Washington DC.

Moore MK, Ball RM (2002) Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. *Molecular Ecology*, **11**, 281-288.

Mrosovsky N, Pieau C (1991) Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia*, **12**, 169-179.

Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology*, **61**, 739-750.

Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of polyandrous females. *Proc. Natl. Acad. Sci. USA*, **96**, 10236-10241.

Olsson M, Madsen T (2001) Promiscuity in sand lizards (*Lacerta agilis*) and adder snakes (*Vipera berus*): causes and consequences. *The Journal of Heredity*, **92**, 190-197.

Osikowski A, Rafinski J (2001) Multiple insemination increases reproductive success of female Montandon's newt (*Triturus montandoni*, Caudata, Salamandridae). *Behavioral Ecology and Sociobiology*, **49**, 145-149.

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage and genetic paternity. *The Journal of Heredity*, **92**, 206-211.

Pearse DE, Janzen FJ, Avise JC (2001) Genetic markers substantiate long-term storage and utilization of sperm by female painted turtles. *Heredity*, **86**, 378-384.

Pearse DE, Janzen FJ, Avise JC (2002) Multiple paternity, sperm storage, and resproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioural Ecology and Sociobiology*, **51**, 164-171.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249-252.

Raymond M, Rousset F (1995) GENEPOP: a population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.

Westneat DF, Sherman PW (1997) Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioural Ecology and Sociobiology*, **41**, 205-215.

Table 1: Sampling design and number of inferred fathers.

Clutches consisting of two 'C'-codes indicate that samples originate from two consecutive clutches of the same mother. The probabilities of detecting multiple paternity (PrDM) with respect to the number of offspring genotyped (N) were estimated for equal and skewed (10:90%) paternal contributions.

Year	**Clutch code**	**Carapace length of mother (cm)**	**N**	**% clutch genotyped**	**PrDM**		**Minimum number of fathers**
					Equal contribution	**Skewed contribution**	
2003	2003 C1 C6	81	63	50	0.99	0.98	**4**
2003	2003 C3	90	32	26	0.99	0.91	**4**
2003	2003 C7 C12	82	71	57	0.99	0.98	**5**
2003	2003 C8	75	18	21	0.98	0.78	**1**
2003	2003 C13 C14	91	17	14	0.99	0.77	**4**
2004	2004 C1 C14	91	55	43	0.99	0.97	**5**
2004	2004 C3	87.5	31	45	0.99	0.91	**2**
2004	2004 C4	84	21	21	0.98	0.83	**4**
2004	2004 C6	89	36	24	0.99	0.93	**4**
2004	2004 C9	91	38	33	0.99	0.95	**4**
2004	2004 C10	84	40	43	0.99	0.94	**5**
2004	2004 C13 C18	86	62	27	0.99	0.98	**5**
2004	2004 C16	87	22	21	0.99	0.84	**3**
2004	2004 C17	68	41	29	0.99	0.95	**2**
2004	2004 C20	82	24	24	0.99	0.87	**2**
total			571				

Figure legends

Fig. 1: Allele frequencies, observed (H_o) and expected (H_e) heterozygosity and estimated frequency of null alleles (f_N) based on the genotypes of 50 adult females and three hatchlings for each of the four microsatellite loci analysed.

Fig. 2: Hatching success (%) as a function of the number of fathers detected in a clutch through genetic paternity analysis.

Fig. 3: Number of fathers plotted against female size (CCL: curved carapace length).

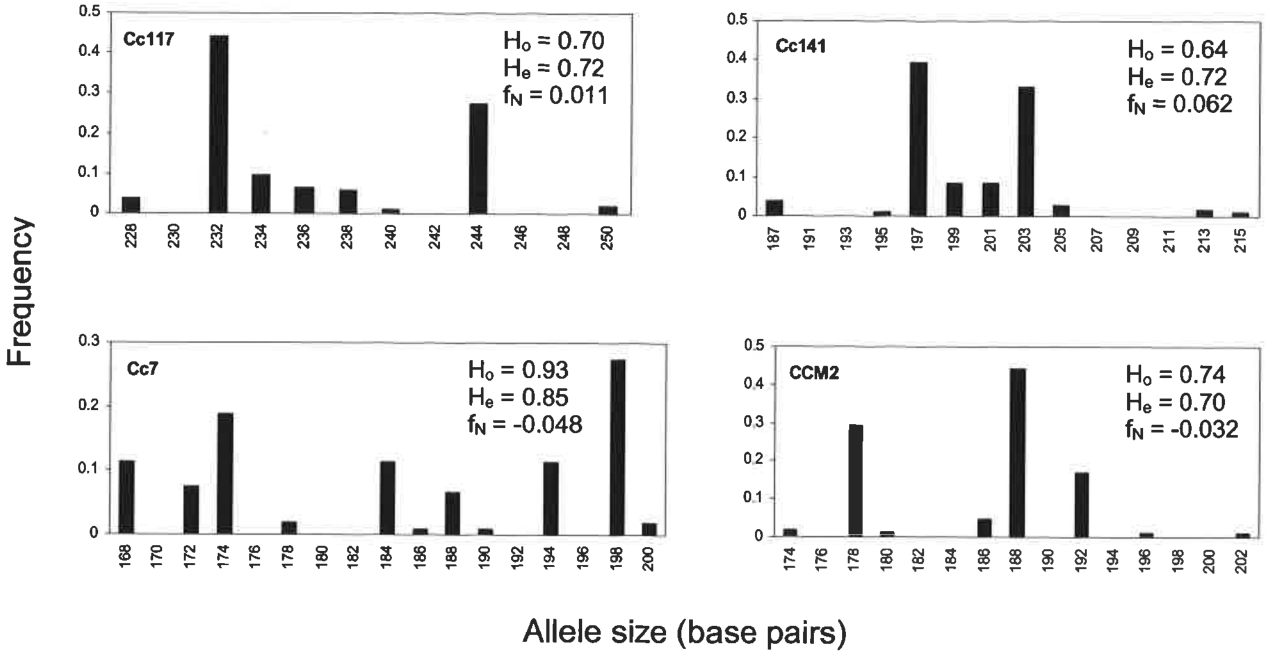


Fig. 1, Zbinden et al.

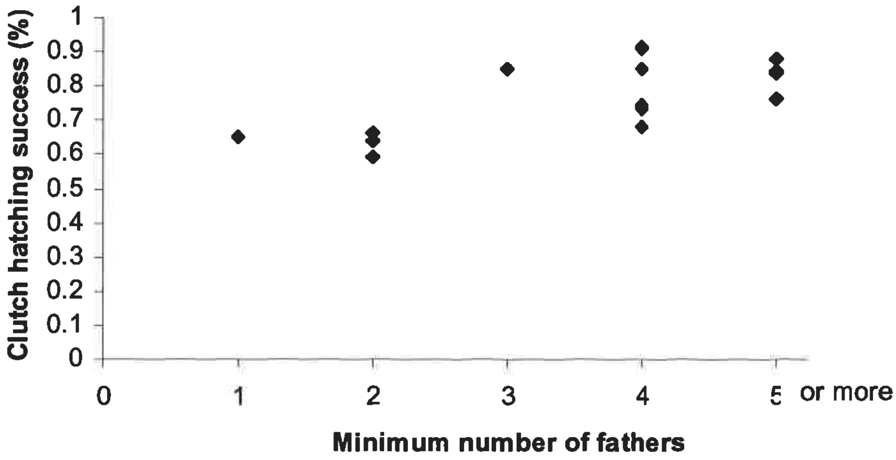


Fig. 2, Zbinden et al.

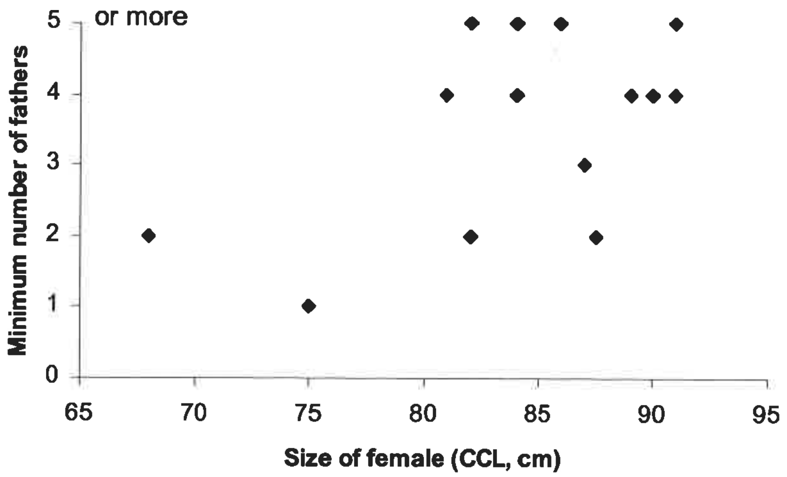


Fig. 3, Zbinden et al.

Chapter four

Post-nesting migrations and foraging areas of loggerhead sea turtles from a major Mediterranean nesting area

Judith A. Zbinden, Adrian Aebischer, Dimitris Margaritoulis, and Raphaël Arlettaz

Manuscript

Post-nesting migrations and foraging areas of loggerhead sea turtles from a major Mediterranean nesting area

Judith A. Zbinden[1], Adrian Aebischer[1], Dimitris Margaritoulis[2], and Raphaël Arlettaz[1]

[1] Zoological Institute, Division of Conservation Biology, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

[2] ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, GR-10432 Athens, Greece

Key words: post-nesting migration, seasonal migration, Mediterranean, Zakynthos, *Caretta caretta*

Corresponding author:
Judith Zbinden
Zoological Institute
Division of Conservation Biology
Baltzerstrasse 6
CH-3012 Bern
Tel.: +41 31 631 31 53
Fax: +41 31 631 45 35
judith.zbinden@nat.unibe.ch

Running head: Migrations of adult Mediterranean loggerheads

Abstract

Sea turtle populations worldwide suffer from reduced survival of immatures and adults due to fishery bycatch. Unfortunately, information about the dispersal of turtles outside the breeding habitat is scarce in most areas, hampering the development of spatially explicit conservation action plans. The metapopulation of loggerhead sea turtles in the Mediterranean suffers from a high fishery bycatch rate, which is especially critical because of its relatively small size. Recaptures of adults tagged on nesting beaches as well as bycatch reports suggest that the Adriatic Sea and Gulf of Gabès are important foraging areas, but such information could be heavily biased. In order to obtain unbiased information, we satellite tracked seven post-nesting loggerhead sea turtles from the largest Mediterranean population (Bay of Laganas, Zakynthos, Greece). Four females settled in the Adriatic Sea and the main individual foraging habitats of two turtles were located in the Gulf of Gabès, confirming previous anecdotal findings. One individual did not occupy a distinct home range at sea. We further witnessed major seasonal migrations in two individuals which left the northern Adriatic Sea at the onset of winter. The Adriatic Sea and the Gulf of Gabès thus represent key areas for the implementation of conservation measures aimed at mitigating fishery bycatch.

Introduction

Habitat use of marine animal species is generally poorly documented compared to that of terrestrial ones. This lack of information often impedes the design of efficient conservation strategies for endangered species. This is the case in sea turtles, where conservation and research activities have traditionally focused on the terrestrial habitat (beaches). Population dynamics models have however unequivocally revealed the prominent importance of immature and adult survival rates for the fate of sea turtle populations (Crouse et al., 1987; Heppell et al., 2003). These life stages are clearly marine. Indeed, changes in mortality rates of juvenile and adult turtles are the most likely explanation for both rapidly decreasing and recovering sea turtle populations (Spotila *et al.*, 2000; Hays, 2004). In most if not all extant sea turtle populations, survival rates are much reduced due to fishery-related mortality (bycatch). There is thus an urgent need to extend conservation activities beyond the traditional protection of nesting habitat (Crouse *et al.*, 1987). However, for the majority of sea turtle populations, the dispersal and ecology of individuals at sea is conjectural at best, hampering an efficient, spatially explicit implementation of mitigation measures.

Traditionally, information on adult dispersal was gained through recovery of individuals flipper-tagged on nesting beaches (Limpus *et al.*, 1992; Margaritoulis *et al.*, 2003; Troëng *et al.*, 2005) and fishery bycatch reports. However, these recapture data only provide point-to-point movement information with no details on the exact migration route or whether the point of recovery is the animal's destination or a point along the route. Moreover, recaptures may be heavily biased by differences in observing effort and/or tag reporting amongst actual destinations (Schroeder *et al.*, 2003). Such biases are even more pronounced for turtle bycatch reports. Although these two sources can provide valuable information on adult turtle distribution at sea, they might not mirror the actual importance of at-sea habitats for populations. Monitoring the whereabouts of individual turtles seems the only feasible way to gain unbiased data on a population's habitat use at sea. Such information can verify hypotheses based on recoveries of tagged turtles and bycatch rates. Satellite tracking of marine turtles has been used to identify migratory pathways, foraging habitats and behaviour in the foraging habitat (Godley *et al.*, 2002a; Godley *et al.*, 2002b; Troëng *et al.*, 2005).

The Mediterranean loggerhead sea turtle (*Caretta caretta*) metapopulation is of relatively small size (ca. 5000 clutches annually on average, (Margaritoulis *et al.*, 2003) and demographically independent from Atlantic populations (Bowen *et al.*, 1993; Laurent *et al.*, 1993; Laurent *et al.*, 1998). Moreover, Mediterranean loggerheads have evolved specific adaptations to their environment (Tiwari & Bjorndal, 2000). For these reasons they represent a separate conservation unit. Although fishing has a long tradition in the Mediterranean, the recent intensification of fisheries has led to turtle bycatch numbers that are thought to be incompatible with long-term population persistence (Laurent *et al.*, 1998; Gerosa & Casale, 1999). Recovery of tagged turtles (Margaritoulis *et al.*, 2003) and bycatch statistics (Gerosa & Casale, 1999; Casale *et al.*, 2004) suggest that the Adriatic Sea and the Gulf of Gabès area are hotspots for adult loggerhead sea turtles.

We studied the at-sea spatial behaviour of female loggerhead sea turtles nesting in the Bay of Laganas on the island of Zakynthos (Greece), which harbours the largest loggerhead sea turtle population in the Mediterranean, accounting for roughly a fourth of all documented nests (Margaritoulis *et al.*, 2003). The Zakynthos population is likely to play a key role within the Mediterranean metapopulation not only for its size, but also because it likely produces a less female-skewed hatchling sex ratio than most other Mediterranean populations (chapter one). Considering the proposed male-mediated gene flow amongst Mediterranean loggerhead sea turtle populations (Carreras et al., in press), Zakynthos male progeny may become central for the metapopulation under scenarios of global warming, where male production is decreased. This study is a first direct assessment of migration paths and foraging areas of adult loggerhead sea turtles originating from a major Mediterranean nesting area.

Material and methods

A total of seven adult female turtles (three in 2004 and four 2005; see Table 1) were equipped with satellite transmitters after egg laying on beaches of the Bay of Laganas, Zakynthos, Greece (37°43′ N, 20°53′ E; Fig. 1). Satellite transmitters (KiwiSat 101; 630 g; Sirtrack Ltd., New Zealand) were attached on the second central carapace scute, which was freed from barnacles and cleaned with a pot-scrubber, sandpaper and acetone. We used two-part epoxy resin (Durostick ®, Durostick, Greece in 2004 and Araldite AW2101, Vantico, Basel, Switzerland in 2005) as a fixative. Turtles were restrained in a wooden portable corral with their head covered by a piece of fabric during the attachment procedure, which lasted one to two hours, depending on the state of the carapace.

Transmitters were programmed for a signal emission every 36 s with an output power of 1 W. Transmitters purchased in 2004 had duty cycles of 10:10 h (on/off) for 100 days, followed by 10:78 h for the rest of battery life. Transmitters purchased in 2005 were programmed to emit continuously for 60 days, followed by a 10:10 h duty cycle for the rest of their life. Transmitters had a salt-water switch to suppress transmissions when submerged. Except for one individual, turtles were tracked during at least part of the internesting period (Table 1, see chapter five). All turtles were tracked at least until they reached their individual main foraging areas and for several weeks thereafter.

Turtles were located through the Argos system (www.argosinc.com) with fixes categorized into seven location classes (LCs). According to Argos and confirmed by Hays et al. (2001), the location errors follow a normal distribution with the standard deviation of the location error for the three most accurate location classes (LCs 3, 2 and 1 in decreasing accuracy) being less than 1 km. Argos does not indicate the accuracy of classes 0, A, B and Z, but Hays et al. (2001) showed that of these, LC A is generally most accurate (comparable to LC 1). We therefore disregarded only fixes of LCs O, B and Z unless otherwise stated. Fixes requiring an unrealistic travel speed of more than 5 km/h (Luschi *et al.*, 1998) were consistently eliminated. To minimize differences due to variation in the frequency of fixes, the location with the highest accuracy per day was used for calculation of distances. If more than one fix fulfilled this criterion, the one closest to midday was used. Migration was divided into oceanic and neritic phases. An individual was considered to have reached a foraging or over-wintering area when movement was no longer directed for at least three consecutive days. We regarded the area where an individual completed its post-nesting migration as the main foraging area, although we cannot actually prove foraging.

Data were downloaded and analysed in STAT (Coyne & Godley, 2005), which provides information about sea surface temperatures and depth of the seafloor. Migration routes were plotted using the MAPTOOL program (www.seaturtle.org).

Results

Overview

All individuals left Zakynthos in late July or early August (Table 1). The duration of tracking ranged from 130 to 633 days at the time of writing (24.3.2006; Table 1). Six turtles showed a clear post-nesting destination: four migrated to the Adriatic Sea (three to the northern and one to the southern part) and two to the area of the Gulf of Gabès in North Africa (Fig. 1a). One turtle did not end her migration once she reached the very northern part of the Adriatic Sea, but resumed her route heading south and was constantly on the move (Fig. 1b).

Post-nesting migrations

The six turtles covered between 697 and 1238 km to their initial foraging areas. These migrations took them between 19 and 34 days (Table 2). All routes were remarkably straight and directed towards the initial foraging area (Fig. 1a). Travelling speeds for all individuals on both phases of the journey (i.e. oceanic vs. neritic) ranged from 1.4 - 1.9 km/h with no distinctive pattern between the oceanic and neritic phases (Table 2).

Individual main foraging areas

Six individuals occupied spatially well-defined home ranges upon the completion of their post-nesting migrations. Individual main foraging areas were all situated above the continental shelf, at a maximum average sea floor depth of 32 m and within a maximum of 15 km from the coast, except for that of turtle G (Fig. 1a, Table 3). She occupied a main foraging area about 120 km from the coast at a water depth of nearly 100 m (Fig. 1a). Turtles stayed in their main foraging areas for a minimum of 38 days (Table 3).

Individual over-wintering areas

The data from four individuals clearly reveal that they left their individual main foraging areas during the months of October and November to travel to an over-wintering area further south, at a distance of 100 to nearly 1'000 km from the individual main foraging area (Table 3, Fig. 2). We obtained detailed location information during the cold season for two turtles (E and F). Despite rather directional routes (Fig. 2), these journeys were carried out at a lower cruising speed than the post-nesting migration (on average 0.9 km/h and 0.55 km/h for turtle E and F, respectively). The over-wintering home-ranges of these two turtles were comparable in size to their individual main foraging areas (Fig. 2a). They laid a few kilometres offshore at water depths only slightly deeper than in the main foraging areas (Table 3). Turtle B spent the cold season off the Greek Island of Corfu. Turtle C moved some 250 km south during the winter (Fig. 2b). The following warm season she was back in the Gulf of Tunis and migrated eastwards along the north African coast past her previous over-wintering area during the next winter (Fig. 2b).

Movements of the turtle not occupying a distinct foraging area

Turtle D never occupied a foraging home range, but was constantly on the move. However, she travelled at a much reduced average speed (0.34 km/h) during the 25 days she spent in the northernmost part of the Adriatic Sea compared to the rest of her migration (Fig. 1b). She was tracked for an additional 84 days after having left the north Adriatic Sea and migrating into oceanic waters, until her transmitter stopped for unknown reasons on 22 December 2005. During this time, she crossed 2093 km of ocean at an average speed of 1.0 km/h, covering in total 3338 km.

Discussion

Six out of the seven loggerhead sea turtles tracked in this study migrated along relatively direct routes to neritic foraging areas situated 700-1200 km from their nesting area. Such behaviour seems to be typical for post-nesting loggerhead sea turtles worldwide (Schroeder *et al.*, 2003). The speed sustained by the loggerhead sea turtles from Zakynthos is within the range of cruising speeds reported in comparable studies (average 1.3 km/h and 1.5 km/h, (Papi *et al.*, 1997; Godley *et al.*, 2002a). The fact that their travelling speeds did not differ between the coastal and oceanic phase suggests that our study animals did not rely on foraging opportunities en route. Such biphasal migratory behaviour is well-documented in green turtles (Godley et al., 2002b; Hays et al., 2002). It has also been documented for a loggerhead sea turtle, which travelled through coastal waters at only about 50% of the speed maintained during pelagic crossings (Godley *et al.*, 2002a).

The most frequented areas, namely the north Adriatic Sea and the Gulf of Gabès, and are by far the largest areas of continental shelf in the entire Mediterranean. These two areas are regions of exceptionally high primary productivity (Agostini & Bakun, 2002). They are also areas of high fishery intensity (Gerosa & Casale, 1999), which may make them even more attractive for marine turtles, as several authors report marine turtles scavenging on discarded fish (Shoop & Ruckdeschel, 1982; Houghton *et al.*, 2000; Tomas *et al.*, 2001). The Adriatic Sea and Gulf of Gabès are the two regions where the majority (70%) of flipper-tagged loggerhead sea turtles originating from Zakynthos and the nearby Peloponnesus were reported from (Margaritoulis *et al.*, 2003). Together with our satellite tracking data, this suggests that these two areas represent essential habitats for a substantial number of Mediterranean loggerhead sea turtles.

The migratory behaviour of female D contrasted with the dispersal patterns of the other tracked turtles. The relatively slow movements in the extreme north of the Adriatic Sea suggest intensive foraging in this region. Extended wanderings in the pelagic area of the Ionian Sea indicate feeding on pelagic prey. There is strong evidence that a considerable proportion of mainly small adult loggerhead sea turtles of Japan feed in oceanic waters (Hatase et al., 2002). With our small sample size, we cannot speculate how widespread oceanic wandering behaviour is in adult females from Zakynthos. It should be mentioned that turtle D had laid 29 small, yolkless eggs together with only 59 normal-sized eggs, a clutch composition that is extremely abnormal in Greek loggerheads (ARCHELON internal reports, unpublished data) and suggesting that this female may was not yet fully mature.

Turtles left their main foraging areas for more southerly regions when water temperature dropped. These seasonal migratory routes of the individuals initially foraging in the north Adriatic Sea were far longer than those of turtles which had settled at lower latitudes (Gulf of Gabès or south Adriatic Sea). Seasonal migrations in the Adriatic Sea were previously suggested based on the observation that turtle bycatch rates decrease in the north-eastern Adriatic Sea in the cold season, matching an increase farther south (Lazar *et al.*, 2002). The southward movement at the onset of the cold season observed in individuals E and F supports the hypothesis.

The fact that the turtle which was tracked over a time period of two winter seasons spent the cold seasons in two different over-wintering areas indicates that the fidelity shown by loggerhead sea turtles towards their nesting area and (based on growing evidence) their initial foraging area, might not apply for the over-wintering area. Perhaps turtles chose the over-wintering area according to prevalent environmental conditions and/or their status within the breeding cycle rather than previous experience.

Implications for conservation

Our results add further weight to recommendations for concerted conservation efforts in the Adriatic Sea and Gulf of Gabès region. There are reasons to consider both these areas to be imminently important ecosystems for sea turtles, and not merely as key habitats for adult Zakynthian females. The north Adriatic Sea is used by large numbers of subadult loggerhead sea turtles (Casale *et al.*, 2004), while the north African coastline was identified as a key foraging area for critically endangered Mediterranean green turtles (Godley *et al.*, 2002b).

Adult loggerhead sea turtles suffer from mortality caused by trawls and gillnets (Gerosa & Casale, 1999). Most countries bordering the Mediterranean have adopted laws which protect waters within 5.6 km from shore and of less than 50 m sea floor depth from trawling (Gerosa & Casale, 1999). The main foraging and over-wintering areas of all but one of the turtles tracked in this study are situated within these limits. While proper implementation of the existing law might therefore protect the majority of adults from trawling, subadults are likely not adequately protected by the existing legislation, as suggested by the high bycatch rates of subadults in offshore trawling in the north Adriatic Sea (Casale *et al.*, 2004). Gillnets are used in areas not otherwise exploited by fisheries (Gerosa & Casale, 1999) and because they are heavily used by recreational fishermen, bycatch reduction in this fishing practice might be especially hard to tackle.

Adequate protection of the loggerhead sea turtle nesting habitat in Zakynthos was a most tedious process (Dimopoulos, 2001), which is still in progress. We recently showed that the marine protected zones of the Bay of Laganas well protect turtles in the inter-nesting habitat from direct mortality (chapter five). Considering the immense efforts to protect the reproductive habitat and intense monitoring of turtles therein for over two decades (Margaritoulis, 2005), it would be ironic not to take concerted action toward better conservation of these same adults at sea.

Acknowledgements

The major part of this research was funded by a grant from the MAVA Foundation, to which we are extremely grateful. We thank the Karl Mayer Foundation and the Ocean Science and Research Foundation for additional funding provided. We are most grateful to A. Rees for generously sharing practical experience with attaching transmitters. Thanks go to C. Davy, C. Dean and E. Ransome as well as several ARCHELON volunteers for helping with transmitter attachment. K. Grimanis (ARCHELON) is thanked for dealing with logistic issues in the study area. Valuable discussions with F. Bontadina (Conservation Biology, University of Bern) helped in the interpretation of results, and comments by C. Davy improved the manuscript. We thank F. Bentivegna (Stazione Zoologica Anton Dohrn, Naples) for returning a transmitter and K. Lay (Sirtrack) for excellent cooperation. The authors wish to acknowledge use of the Maptool program for analysis and graphics in this paper. Maptool is a product of SEATURTLE.ORG.

Literature

Agostini VN, Bakun A (2002) 'Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). *Fisheries Oceanography*, **11**, 129-142.

Artegiani A, Bregant D, Paschini E, Pinardi N, Raicich F, Russo A (1997) The Adriatic Sea general circulation. Part I: Air-sea interactions and water mass structure. *Journal of physical oceanography*, **27**, 1492-1514.

Bowen BW, Avise JC, Richardson JI, Meylan AB, Margaritoulis D, Hopkins-Murphy SR (1993) Population structure of loggerhead turtles (*Caretta caretta*) in the northwestern Atlantic Ocean and Mediterranean Sea. *Conservation Biology*, **7**, 834-844.

Carreras C, Pascual M, Cardona L *et al.* (in press) Genetic structure of the loggerhead turtle (*Caretta caretta*) Mediterranean nesting populations. In: *Second Mediterranean Conference on Marine Turtles* (eds pp. Kemer, Turkey.

Casale P, Laurent L, Metrio GD (2004) Incidental capture of marine turtles by the Italian trawl fishery in the north Adriatic Sea. *Biological Conservation*, **119**, 287-295.

Coyne MS, Godley BJ (2005) Satellite tracking and analysis tool (STAT): an integrated system for archiving, analyzing and mapping animal tracking data. *Marine Ecology Progress Series*, **301**, 1-7.

Crouse DT, Crowder LB, Caswell H (1987) A stage-based population model for loggerhead sea turtles and implications for conservation. *Ecology*, **68**, 1412-1423.

Dimopoulos D (2001) The National Marine Park of Zakynthos: A Refuge for the Loggerhead Turtles in the Mediterranean. *Marine Turtle Newletter*, **93**, 5-9.

Godley BJ, Broderick AC, Glen F, Hays GC (2002a) Post-nesting movements and submergence patterns of loggerhead marine turtles in the Mediterranean assessed by satellite tracking. *Journal of Experimental Marine Biology and Ecology*, **4075**, 1-16.

Godley BJ, Richardson S, Broderick AC, Coyne MS, Glen F, Hays GC (2002b) Long-term satellite telemetry of the movements and habitat utilisation by green turtles in the Mediterranean. *Ecography*, **25**, 352-363.

Hatase H, Takai N, Matsuzawa Y *et al.* (2002) Size-related differences in feeding hatitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analysis and satellite telemetry. *Marine Ecology Progress Series*, **233**, 273-281.

Hays GC (2004) Good news for sea turtles. *Trends in Ecology and Evolution*, **19**, 349-351.

Hays GC, Akesson S, Godley BJ, Luschi P, Santidrian P (2001) The implications of location accuracy for the interpretation of satellite-tracking data. *Animal Behaviour*, **61**, 1035-1040.

Hays GC, Broderick AC, Godley BJ, Lovell P, Martin C, McConnell BJ, Richardson S (2002) Biphasal long-distance migration in green turtles. *Animal Behaviour*, **65**, 895-898.

Heppell SS, Snover ML, Crowder LB (2003) Sea turtle population ecology. In: *The biology of sea turtles* (eds Lutz PL, Musick JA, Wyneken J), pp. 275-298. CRC Press, Boca Raton.

Houghton JDR, Woolmer A, Hays GC (2000) Sea turtle diving and foraging behaviour aroud the Greek Island of Kefalonia. *Journal of the Marine Biology Association of the United Kingdom*, **80**, 761-762.

Laurent L, Casale P, Bradai MN *et al.* (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. *Molecular Ecology*, **7**, 1529-1542.

Laurent L, Lescure J, Excoffier L *et al.* (1993) Etude Genetique des relations entre les populations mediterraneenne et atlantique d'une tortue marine (*Caretta caretta*) à l'aide d'un marqueur mitochondrial. *Compte Rendu de l'Académie des Sciences, Paris*, **316**, 1233-1239.

Lazar B, Borboroglu PG, Tvrtkovic N, Ziza V (2002) Temporal and spatial distribution of the loggerhead sea turtle Caretta caretta in the eastern Adriatic sea: a seasonal migration pathway? In: *22nd Annual Symposium on Sea Turtle Biology and Conservation* (eds pp. NOAA Technical Memorandum, Miami.

Limpus CL, Miller JD, Parmenter CJ, Reimer D, McLachlan N, Webb R (1992) Migration of Green (*Chelonia mydas*) and Loggerhead (*Caretta caretta*) Turtles to and from Eastern Australian Rookeries. *Wildlife Research*, **19**, 347-358.

Luschi P, Hays GC, Seppia CD, Marsh R, Papi F (1998) The navigational feats of green sea turtles migrating from Ascension Island investigated by satellite telemetry. *Proceeding of the Royal Society London B*, **265**, 2279-2284.

Margaritoulis D (2005) Nesting activity and reproductive output of loggerhead sea turtles, *Caretta caretta*, over 19 seasons (1984-2002) at Laganas Bay, Zakynthos, Greece: The largest rookery in the Mediterranean. *Chelonian Conservation and Biology*, **4**, 916-929.

Margaritoulis D, Argano R, Baran I *et al.* (2003) Loggerhead turtles in the Mediterranean Sea: Present knowledge and conservation perspectives. In: *Loggerhead sea turtles* (eds Bolten AB, Witherington BE), pp. 175-198. Smithsonian Institution, Washington.

Papi F, Luschi P, Crosio E, Hughes GH (1997) Satellite tracking experiments on the navigational ability and migratory behaviour of the loggerhead turtle *Caretta caretta*. *Marine Biology*, **129**, 215-220.

Schroeder BA, Foley AM, Bagley DA (2003) Nesting patterns, reproductive migrations, and adult foraging areas of loggerhead turtles. In: *Loggerhead Sea Turtles* (eds Bolten AB, Witherington BE), pp. 114-124. Smithsonian Books, Washington.

Shoop CR, Ruckdeschel C (1982) Increasing turtle strandings in the southeast United States: A complicating factor. *Biological Conservation*, **23**, 213-215.

Spotila JR, Reina RD, Steyermark AC, Plotkin PT, Paladino FV (2000) Pacific leatherback turtles face extinction. *Nature*, **405**, 529-530.

Tiwari M, Bjorndal KA (2000) Variation in morphology and reproduction in loggerheads, *Caretta caretta*, nesting in the United States, Brazil, and Greece. *Herpetologica*, **56**, 343-356.

Tomas J, Aznar FJ, Raga JA (2001) Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean. *J. Zool. Lond.*, **255**, 525-532.

Troëng S, Evans DR, Harrison E, Lagueux CJ (2005) Migration of green turtles *Chelonia mydas* from Tortuguero, Costa Rica. *Marine Biology*,

Table 1: Summary data for seven tracked loggerhead sea turtles originating from Zakynthos.
CCL: curved carapace length (notch to tip).

Turtle	CCL (cm)	Transmitter attachment date	Start of postnesting migration	Duration of tracking (days)
A	85	27.06.2004	38210	130
B	86	28.06.2004	38192	279[1]
C	84	29.06.2004	38209	633[2]
D	79	16.06.2005	38562	189
E	87	19.06.2005	38560	278[2]
F	89	21.06.2005	38572	276[2]
G[3]	76	10.08.2005	38574	226[2]

[1] Turtle B found stranded on 2.4.2005 with transmitter still attached. Symptoms indicated she had been trapped in a fishing net (F. Bentivegna, pers. comm.).

[2] Transmitters still emitting at time of writing (24.3.2006)

[3] Refurbished transmitter of individual B

Table 2: Post-nesting migrations of the six satellite-tracked loggerhead sea turtles that occupied individual main foraging areas with respect to phase of migration (oceanic vs. neritic).

Indicated are distances covered, durations and travelling speeds.

Individual		**A**	**B**	**C**	**E**	**F**	**G**
Oceanic	Distance (km)	367	475	967	419	–	855
	Duration (days)	9	12	22	12	–	22
	Speed (km h^{-1})	1.8	1.7	1.9	1.4	–	1.7
Neritic	Distance (km)	792	222	75	819	1072	–
	Duration (days)	28	7	4	22	32	–
	Speed (km h^{-1})	1.4	1.4		1.5	1.4	–
Total	Distance (km)	**1'159**	**697**	**1042**	**1238**	**1072**	**855**
	Duration (days)	**37**	**19**	**26**	**34**	**32**	**22**

Table 3: Description of individual main foraging areas and over-wintering areas for six satellite-tracked loggerhead sea turtles.
Because of a thermally homogenize water column in the Adriatic Sea during winter (Artegiani et al., 1997), sea surface temperature data is a good approximation of the temperature experienced by the turtles in this region.

	Individual	**A**	**B**	**C**	**E**	**F**	**G**
Individual main foraging area	Water depth (m)	16	25	3	32	29	99
	Distance from shore (km)	12.2	1.1	0.9	6.7	14.4	118.7
	Time period	20.9.-at least 3.11.2004[1]	12.9.-at least 31.10.2004[2]	6.9.-22.11.2004	31.8.-8.10.2005	5.9.-20.10.2005	7.9.-18.11.2005[5]
	Minimum water temperature (°C)	–	–	22.8	19.8	18.5	23.1
Over-Wintering area	Distance to individual main foraging area (km)	–	ca. 100[3]	ca. 250[3]	404	932	–
	Water depth (m)	–	–	–	40	38	–
	Distance from shore (km)	–	–	–	4	3	–
	Time period	–	–	–	27.10.2005-[4]	31.12.2005-[4]	–
	Minimum water temperature (°C)	–	–	–	12.8	13.7	–

[1] Transmissions stopped for unknown reasons on 3.11.2004

[2] Exact date of departure unknown

[3] Data doesn't allow exact determination of over-wintering home range

[4] Turtles haven't left over-wintering area at the time of writing (24.3.2006)

[5] Turtle left foraging area, but localizations thereafter unreliable

Figure legends

Fig. 1: Migration pathways and individual main foraging areas (where applicable) of seven loggerhead sea turtles originating from the Bay of Laganas, Zakynthos (Greece). Individual main foraging areas are circled.
A) Tracks from the six turtles with individual main foraging areas.
B) Migration of the turtle not occupying a foraging area.

Fig. 2: Seasonal migrations from individual main foraging to over-wintering areas. Individual main foraging areas are circled, over-wintering areas are marked with hatched circles.
A) Turtles in the Adriatic Sea.
B) Turtle with main foraging area in the Gulf of Tunis.

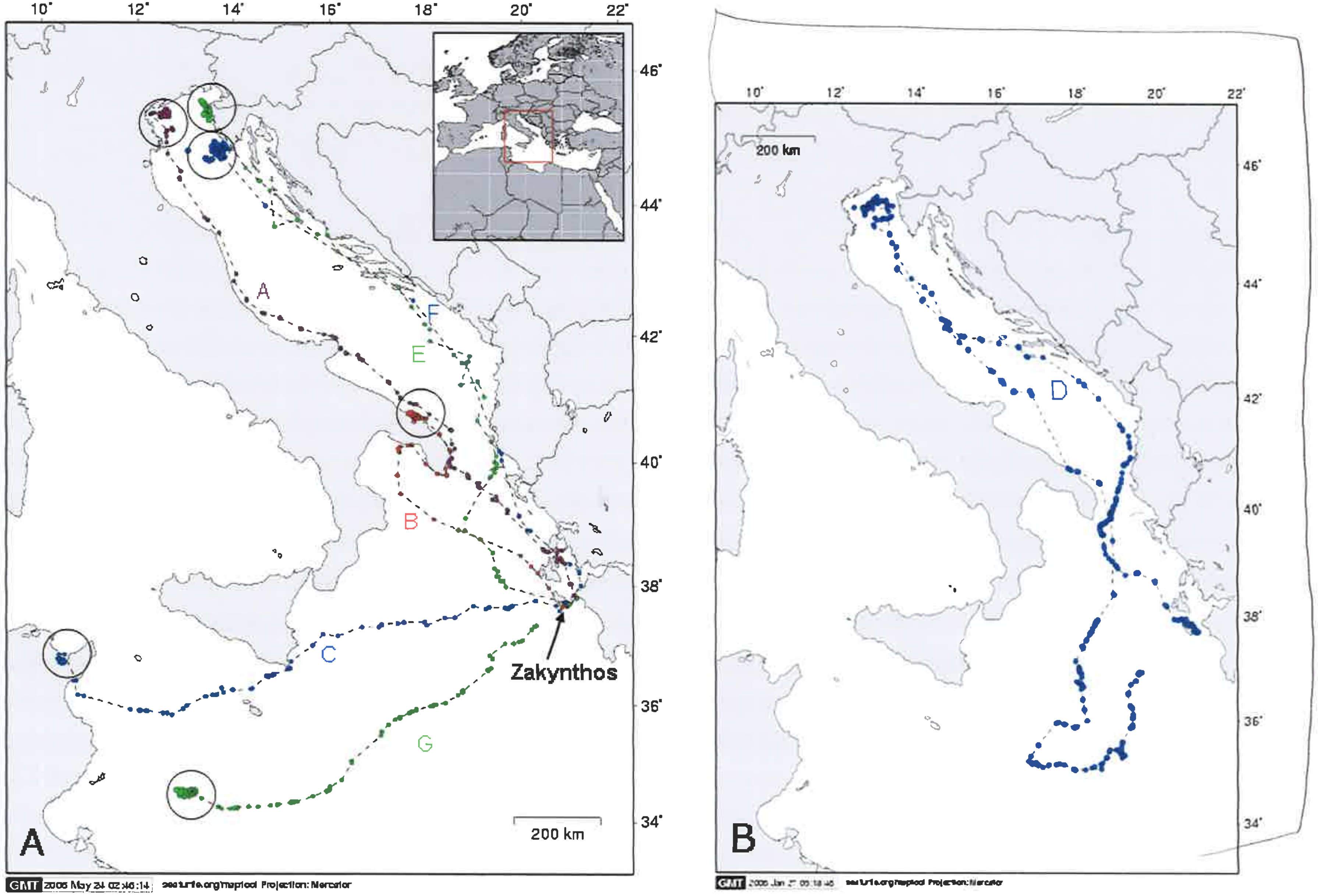


Fig. 1, Zbinden et al.

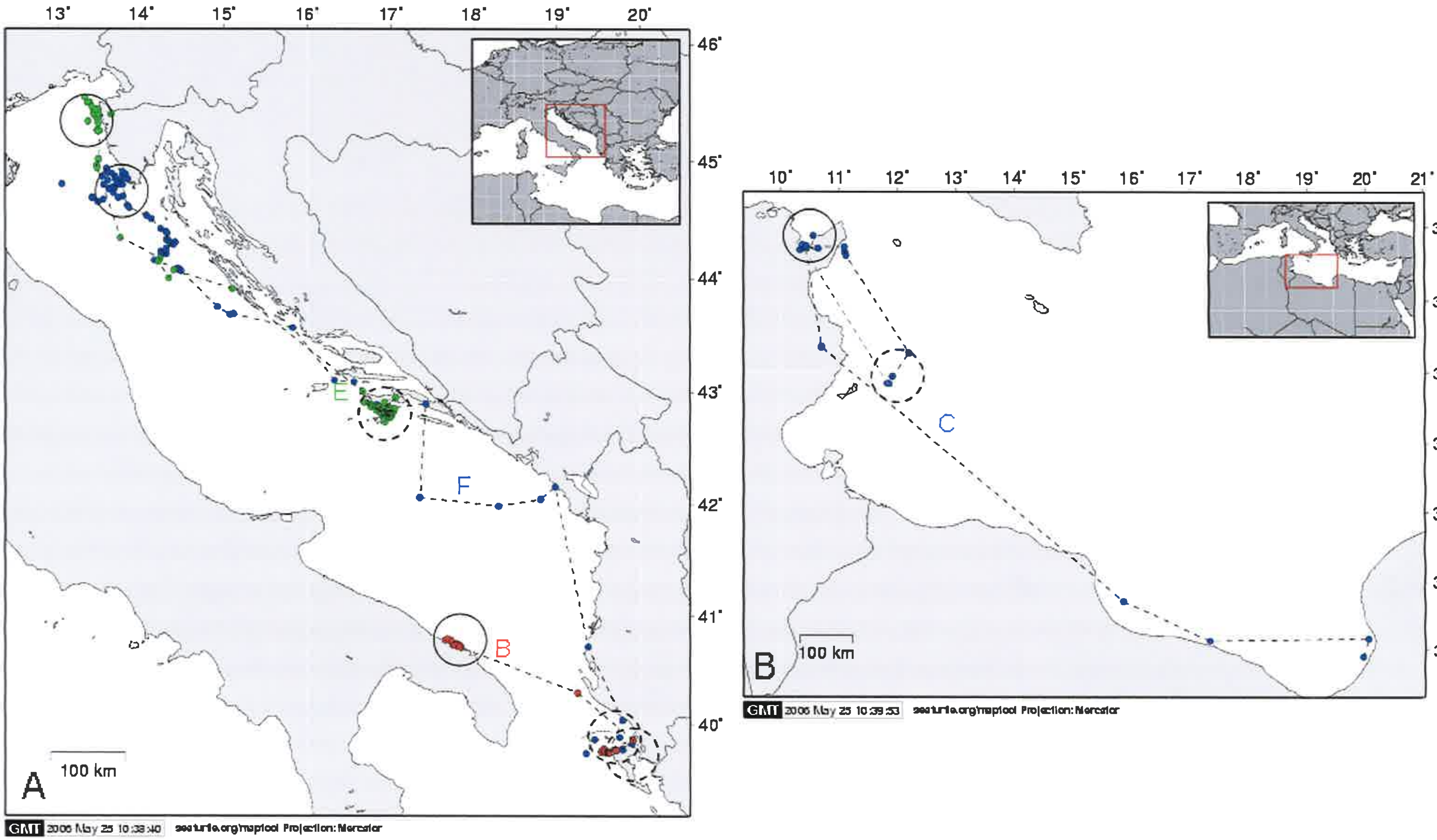


Fig. 2, Zbinden et al.

Chapter five

Spatial behaviour of satellite-tracked loggerhead sea turtles during the internesting period at the main Mediterranean breeding area

Judith A. Zbinden, Adrian Aebischer, Dimitris Margaritoulis, and Raphaël Arlettaz

Manuscript

Spatial behaviour of satellite-tracked loggerhead sea turtles during the internesting period at the main Mediterranean breeding area

Judith A. Zbinden[1], Adrian Aebischer[1], Dimitris Margaritoulis[2], and Raphaël Arlettaz[1]

[1] Zoological Institute, Division of Conservation Biology, Baltzerstrasse 6, 3012 Bern, Switzerland

[2] ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, 10432 Athens, Greece

Key words: internesting habitat, human disturbance, long-distance migration, Mediterranean, Zakynthos, *Caretta caretta*

Corresponding author:
Judith Zbinden
Zoological Institute
Division of Conservation Biology
Baltzerstrasse 6
CH-3012 Bern
Tel.: +41 31 631 31 53
Fax: +41 31 631 45 35
judith.zbinden@nat.unibe.ch

Running head: Spatial behaviour of internesting loggerhead sea turtles

Abstract

Long-distance migrations with no or little food resources both along the route and in the reproductive area pose considerable challenges to energy budgets. Female sea turtles can optimize energy allocation by minimizing energy expenditures during the internesting period (the time over which they lay their successive clutches). Satellite telemetry data allowed us to scrutinize internesting habitat use of loggerhead sea turtles nesting in the Bay of Laganas (Zakynthos, Greece). We also estimated the efficacy of two marine reserves with distinct levels of protection which have been created to reduce the impact of tourism (mainly boats) on adult turtles at sea. Four out of six turtles equipped with satellite transmitters rendered usable information. Three spent the majority of time within the bay, a result consistent with a strategy to minimize energy expenditure. The turtles' whereabouts in the bay were not obviously linked to the specific location a turtle nested. Turtles showed no obvious preference for the highly protected area of the bay. The availability of warmer water in the less protected area may be more crucial than avoidance of boat disturbance. A possible habituation to boats may however prove detrimental outside protected areas, where vessel speed is not limited to avoid collisions.

Introduction

Long-distance migratory behaviour is widespread in numerous taxa although it implies sophisticated mechanisms for orientation and navigation, as well as physiological adaptations for energy deposition and metabolism (Alerstam *et al.*, 2003). In most species, migration takes place between two areas of seasonally good food supplies. In others, migration is directed to breeding grounds which offer marginal or no foraging opportunities. For females of the latter pattern, energy storage, metabolism and allocation strategies need to be particularly sophisticated to cover migration, production of eggs or offspring, and the stay in the area of reproduction. Adult sea turtles are a paradigmatic group of such long-distance dispersers with no or little food resources available along migration routes and in the reproductive area (Plotkin, 2003). Moreover, female sea turtles lay several large clutches of nutrient-rich eggs during the so called internesting period, which may last up to several months (Miller, 1997). Given that they are constrained to stay in the generally nutrient-poor vicinity of the nesting beaches, any strategy for minimizing metabolic costs during the internesting period would be highly beneficial (Hays *et al.*, 1999).

Methodological limitations have to date prevented thorough investigations of individual spatial behaviour during the internesting period. Data collected through visual tracking of marked individuals (Mortimer & Portier, 1989; Meylan, 1995), for instance, may be systematically biased. Although the advent of satellite telemetry for wildlife tracking has revolutionised the study of long-distance migrations of sea turtles (Luschi *et al.*, 1998; Godley *et al.*, 2002; Troëng *et al.*, 2005), the frequency and accuracy of locations have been insufficient for investigating small-scale spatial behaviour. The generally long submergence durations in the internesting period (Plotkin, 1998; Hays *et al.*, 1999; Godley *et al.*, 2002) restricts the quality and quantity of locations, because the number of signals emitted depends on the time a turtle spends on the surface. Submergence durations reflect activity levels in sea turtles (Hays *et al.*, 1999). Indeed, data from time-depth recorders have indicated that animals are rather inactive and rest predominantly on the seafloor during the internesting period (Hays *et al.*, 2000; Houghton *et al.*, 2002). More active dive patterns were however also recorded in a green sea turtle (*Chelonia mydas*) population (Hochscheid *et al.*, 1999). They were interpreted to be associated with foraging, indicating behavioural plasticity in green turtle internesting behaviour where turtles would be more active and feed in areas where food is available and be inactive in areas where no or little food is found (Hays *et al.*, 2002b). Variation in individual internesting activity patterns was also reported in loggerhead sea turtles of Cyprus, and tentatively proposed to be associated with foraging activity (Houghton *et al.*, 2002).

Well-performing satellite transmitters allowed us to investigate individual spatial behaviour of loggerhead sea turtles (*Caretta caretta*) in the Bay of Laganas (Zakynthos, Greece, Fig. 1) during the internesting period. We tested first to which extent turtles stayed within the confined area of the Bay of Laganas. We then compared habitat use in relation to existing protection zones within the bay to find out whether turtles showed a preference for the highly protected zone. Unfortunately, changes in habitat use following the installation of protection zones in 1999 (Dimopoulos, 2001) could not be monitored, preventing a rigorous evaluation of turtle's reactions to offered protection levels.

Materials and methods

The Bay of Laganas (Zakynthos, Greece, ca. 37°43'N 20°53'E) hosts the largest population of loggerhead sea turtles in the Mediterranean with an average of nearly 1300 clutches per season on approximately 5.5 km of beach (Margaritoulis, 2005). This makes it one of the densest nesting areas of the species worldwide. The beaches are monitored comprehensively by ARCHELON (The Sea Turtle Protection Society of Greece). The nesting beaches as well as the bay were declared a national marine park in 1999, with the main aim to protect loggerhead sea turtles from mass tourism (Dimopoulos, 2001). Henceforth, Bay of Laganas refers to the marine protected zones, although the actual bay is slightly larger (Fig. 1). The nesting area consists of six discrete beaches (Fig. 1) with individual females frequently switching between the various beaches for successive egg layings (ARCHELON internal reports, unpublished data). Sea turtles nest on Zakynthos from the second half of May till the first half of August (Margaritoulis, 2005).

We stopped turtles on their way back to the sea after they had successfully nested and been measured (CCL: curved carapace length, notch to tip) to attach satellite transmitters (Kiwisat101; 630 g; Sirtrack Ltd., New Zealand) on the second central carapace scute. The respective area of the carapace was freed from barnacles and cleaned with a pot-scrubber, sand paper and acetone. We used two-part epoxy resin (Durostick ®, Durostick, Greece in 2004 and Araldite AW2101, Vantico, Basel, Switzerland in 2005) as a fixative. Turtles were restrained in a wooden portable corral with their head covered by a piece of fabric during the attachment procedure, which lasted one to two hours, depending on the state of the carapace.

A total of six turtles (N = 5 on Gerakas beach, N = 1 on Kalamaki beach, Fig. 1) were equipped with transmitters, three in 2004 and three in 2005. Transmitters were programmed for a 36 seconds repetition rate and an output power of 1 W. For transmitters attached in 2004 (individuals A, B, and C), on/off duty cycles during the internesting period were 10:10 h. The transmitters attached in 2005 (individuals D, E, and F) were programmed for emitting continuously during the internesting period. Transmitters had a salt water switch to suppress transmissions when submerged.

Data were transmitted through the Argos satellite system (www.argos-system.org) with fixes being categorized into six location classes (LCs). We only used fixes of the three most accurate location classes (1, 2 and 3). According to Argos and confirmed by Hays et al.

(2001), the location errors follow a normal distribution with the standard deviation for the three most accurate location classes being less than 1 km. For the assignment of fixes to protection zones, we considered fixes falling on land as belonging to the closest point off the coast. Individuals from which fewer than 12 data points were obtained were excluded. We managed and filtered data with the STAT program of seaturtle.org (Coyne & Godley, 2005), from where we also derived sea floor depth values. For the calculation of median water depths, we excluded locations on land. Data points were exported into the Maptool program of seaturtle.org to draw maps.

Results

All six turtles provided data throughout the internesting period. Transmission success rate (the average number of fixes of LCs 1, 2, and 3 per day) was, on average, 0.48, but varied hugely amongst individuals (Table 1). We excluded two individuals (B and F) (see methods, Table 1). The location points of individuals A and C-E are plotted in Figure 2. Based on an internesting interval of 10 to 20 days on Zakynthos (Margaritoulis, 1983) and the fact that loggerhead sea turtles leave the nesting area immediately following deposition of the last clutch (Schroeder *et al.*, 2003), the four turtles laid each three or four clutches after transmitter attachment. In six instances (additional to the nesting events when transmitters were attached), an ARCHELON team observed turtles with transmitters during beach night patrols (Fig. 2).

For three of the four turtles (individuals A, C and E), over 80% of locations were situated within the bay with median sea floor depth of only 6.5 - 15.5 m, whereas for turtle D only about half the locations were situated within the bay (Table 1). The spatial distribution of satellite tracking locations indicates that movements differed greatly amongst individuals. For instance, turtle D apparently swam around very actively, whereas turtle E stayed within a rather restricted home range in the middle part of the bay close to shore with all the locations in the eastern part associated with nesting (Fig. 2). The whereabouts were seemingly independent of the specific beach a turtle nested (Fig. 2).

The three individuals that spent the majority of time in the bay (turtle A, C and E) showed similar patterns of habitat use in relation to the protection zones. Of the data points within the bay, 79-85% (range of individuals) were situated within the less protected area (LPZ) and only 15-21% within the highly protected area (HPZ, Table 1). Considering that the less protected zone accounts for roughly 87% and the highly protected zone for roughly 13% of the area within the bay, turtles apparently did not show any preference for the highly protected zone.

Discussion

This study shows that current satellite telemetry technology can render accurate information on internesting behaviour. Tracking individual turtles during the internesting period allows investigating individual patterns of space utilisation that cannot easily be assessed by other methods. However, satellite telemetry studies suffer from the notorious small sample sizes due to the high costs per individual. Recently developed GPS-based transmitters for marine turtles (Yasuda & Arai, 2005) are a promising tool to study internesting displacements.

A positive correlation has been found between dive duration and depth of resting in marine turtles (Hays *et al.*, 2000; Minamikawa *et al.*, 2000; Houghton *et al.*, 2002), suggesting that transmission success rate may be dependent on bathymetry. In our case, the locations from the relatively deep waters outside the bay may therefore underestimate the time spent in this area compared to time spent in the shallow waters in the protected zones. However, this potential bias would not have affected the distribution of locations among the two protected areas within the bay, given that they are both in similarly shallow waters (Fig. 1).

This

Our data show that loggerhead sea turtles predominantly use the shallow, sheltered bay as internesting habitat. The residence in a restricted area supports the hypothesis of minimizing energy expenditure during the internesting period. Individual D departs from the general pattern, but tracking of this turtle during the post-nesting dispersal showed a peculiar pattern (chapter four). Individual D probably was a young, the inexperienced adult, which is supported by her relatively small body size (Table 1).

Our finding that turtles did not apparently prefer to stay in the highly protected area implies that sea turtles may not adapt behaviour in response to offered protection zones at sea, at least not during the internesting period. In order to optimize protection of turtles during the internesting period, we recommend local habitat suitability to be assessed before enforcing management zones. Location of the beach where clutches are laid is clearly not a good indication of at sea habitat use. Anecdotal observations of turtles resting off East Laganas beach in the evening before nesting on Sekania beach (Fig. 1) the same night (ARCHELON internal report, unpublished data) support the satellite tracking data in this regard.

One could expect presence of boats to be a main factor responsible for the distribution of turtles in the bay. But our data do not support this, although the density of boats intending to watch turtles in the less protected area is often considerable. Despite the lack of a comprehensive analysis of all potential factors determining the distribution of loggerhead sea turtles in the bay, we suggest that water temperature, for instance, might be a key factor in small-scale internesting habitat choice. The length of the internesting interval is largely determined by body temperature, which in terms is highly correlated to water temperature (Sato *et al.*, 1998; Hays *et al.*, 2002a). Temperature varies amongst areas of the bay with generally warmest temperatures in the northernmost shore-region (belonging to the less protected zone, ~~pers. obs.~~). An increase in temperature by just 1 °C is predicted to decrease the length of the internesting interval by about one day (Hays *et al.*, 2002a). From an energetic point of view it would therefore be advantageous for turtles to stay in the northern, warm zone, as long as the energy loss from reaction to disturbances is compensated by the thermal metabolic gain. The fact that turtles spent considerable time in the less protected area might indicate that this physiological strategy operates with turtles possibly getting habituated to boat and human presence. Although habituation to humans would be adaptive in the context of energy saving in the internesting habitat, a possible habituation to boats may have detrimental effects outside the bay, where boat speed is not restricted, potentially leading to casualties. In Hawaiian green sea turtles, Balazs (1995) observed a trend towards greater habituation to humans with ongoing protection of marine habitats, but evidence about an increase number of casualties lacks.

Acknowledgements

The major part of this research was funded by a grant from the MAVA Foundation, to which we are extremely grateful. We thank the Karl Mayer Foundation for additional funding provided. We are grateful to Alan Rees (ARCHELON) for generously sharing practical experience with attaching transmitters. Thanks go to Christina Davy, Christopher Dean and Emma Ransome as well as several ARCHELON volunteers for helping with transmitter attachment. Kostis Grimanis (ARCHELON) is thanked for dealing with logistic issues in the study area. We acknowledge use of the Maptool program (www.seaturtle.org) for graphics.

References

Alerstam T, Hedenström A, Akesson S (2003) Long-distance migration: evolution and determinants. *OIKOS*, **103**, 247-260.

Balazs GH (1995) Behavioral changes within the recovering Hawaiian green turtle population. In: *15th Annual Symposium on Sea Turtle Biology* *and Conservation* (eds Keinath JA, Barnard DE, Musick JA, Bell BA), pp. 16-20, NOAA Technical Memorandum,

Coyne MS, Godley BJ (2005) Satellite tracking and analysis tool (STAT): an integrated system for archiving, analyzing and mapping animal tracking data. *Marine Ecology Progress Series*, **301**, 1-7.

Dimopoulos D (2001) The National Marine Park of Zakynthos: A Refuge for the Loggerhead Turtles in the Mediterranean. *Marine Turtle Newletter*, **93**, 5-9.

Godley BJ, Richardson S, Broderick AC, Coyne MS, Glen F, Hays GC (2002) Long-term satellite telemetry of the movements and habitat utilisation by green turtles in the Mediterranean. *Ecography*, **25**, 352-363.

Hays GC, Adams CR, Broderick AC, Godley BJ, Lucas DJ, Metcalfe JD, Prior AA (2000) The diving behaviour of green turtles at Ascension Island. *Animal Behaviour*, **59**, 577-586.

Hays GC, Akesson S, Godley BJ, Luschi P, Santidrian P (2001) The implications of location accuracy for the interpretation of satellite-tracking data. *Animal Behaviour*, **61**, 1035-1040.

Hays GC, Broderick AC, Glen F, Godley BJ, Houghton JDR, Metcalfe JD (2002a) Water temperature and internesting intervals for loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. *Journal of Thermal Biology*, **27**, 429-432.

Hays GC, Glen F, Broderick AC, Godley BJ, Metcalfe JD (2002b) Behavioural plasticity in a large marine herbivore: contrasting patterns of depth utilisation between two green turtle (*Chelonia mydas*) populations. *Marine Biology*, **141**, 985-990.

Hays GC, Luschi P, Papi F, Seppia Cd, Marsh R (1999) Changes in behaviour during the inter-nesting period and post-nesting migration for Ascension Island green turtles. *Marine Ecology Progress Series*, **189**, 263-273.

Hochscheid S, Godley BJ, Broderick AC, Wilson RP (1999) Reptilian diving: highly variable dive patterns in the green turtle *Chelonia mydas*. *Marine Ecology Progress Series*, **185**, 101-112.

Houghton JDR, Broderick AC, Godley BJ, Metcalfe JD, Hays GC (2002) Diving behaviour during the internesting interval for loggerhead turtles *Caretta caretta* nesting in Cyprus. *Marine Ecology Progress Series*, **227**, 63-70.

Luschi P, Hays GC, Seppia CD, Marsh R, Papi F (1998) The navigational feats of green sea turtles migrating from Ascension Island investigated by satellite telemetry. *Proceeding of the Royal Society London B*, **265**, 2279-2284.

Margaritoulis D (1983) The inter-nesting interval of Zakynthos loggerheads. In: *Adaptations to Terrestial Environments* (eds Margaris NS, Arianoutsou-Faraggitaki M, Reiter RJ), pp. 135-144. Plenum Press, New York.

Margaritoulis D (2005) Nesting activity and reproductive output of loggerhead sea turtles, *Caretta caretta*, over 19 seasons (1984-2002) at Laganas Bay, Zakynthos, Greece: The largest rookery in the Mediterranean. *Chelonian Conservation and Biology*, **4**, 916-929.

Meylan A (1995) Behavioral ecology of the west Caribbean green turtle (*Chelonia mydas*) in the internesting habitat. In: *Biology and Conservatoin of Sea Turtles* (eds Bjorndal KA), pp. 67-80. Smithsonian Institution Press, Washington.

Miller JD (1997) Reproduction in sea turtles. In: *The biology of sea turtles* (eds Lutz PL, Musick JA), pp. 51-81. CRC Press, Boca Raton.

Minamikawa S, Naito Y, Sato K, Matsuzawa Y, Bando T, Sakamoto W (2000) Maintenance of neutral buoyancy by depth selection in the loggerhead turtle *Caretta caretta*. *The Journal of Experimental Biology*, **203**, 2967-2975.

Mortimer JA, Portier KM (1989) Reproductive homing and internesting behavior of the Green turtle (*Chelonia mydas*) at Ascension Island, south Atlantic Ocean. *Copeia*, **4**, 962-977.

Plotkin PT (1998) Interaction between behavior of marine organisms and the performance of satellite transmitters: A marine turtle case study. *Marine Technical Society Journal*, **32**, 5-10.

Plotkin PT (2003) Adult migrations and habitat use. In: *The Biology of sea turtles* (eds Lutz PL, Musick JA, Wyneken J), pp. 225-241. CRC Press,

Sato K, Matsuzawa Y, Tanaka H, Bando T, Minamikawa S, Sakamoto W, Naito Y (1998) Internesting intervals for loggerhead turtles, *Caretta caretta*, and green turtles, *Chelonia mydas*, are affected by temperature. *Canadian Journal of Zoology*, **76**, 1651-1662.

Schroeder BA, Foley AM, Bagley DA (2003) Nesting patterns, reproductive migrations, and adult foraging areas of loggerhead turtles. In: *Loggerhead Sea Turtles* (eds Bolten AB, Witherington BE), pp. 114-124. Smithsonian Books, Washington.

Troëng S, Dutton PH, Evans D (2005) Migration of hawksbill turtles *Eretmochelys imbricata* from Tortuguero, Costa Rica. *Ecography*, **28**, 394-402.

Yasuda T, Arai N (2005) Fine-scale tracking of marine turtles using GPS-Argos PTTs. *Zoological Science*, **22**, 547-553.

Table 1: Summary of satellite tracking six loggerhead sea turtles in the Bay of Laganas (Zakynthos, Greece) during the internesting period: Body size (CCL: curved carapace length), transmitter performance (TSR: transmission success rate), median water depth and number/percentages of locations within the bay with respect to protection zone (see Fig. 1). HPZ: highly protected zone, LPZ: less protected zone.

Turtle	CCL (cm)	Attachment date	Internesting tracking duration (days)	No. Locations	TSR	Median water depth (m)	Number (%) of locations		
							In bay	HPZ	LPZ
A	85	27.06.2004	44	18	0.42	15.5	15 (84 %)	3 (17%)	12 (67 %)
B	86	28.06.2004	25	3	0.12	–	–	–	–
C	84	29.06.2004	41	19	0.45	6.5	19 (100 %)	4 (21 %)	15 (79 %)
D	79	16.06.2005	42	43	1.00	54.6	21 (49 %)	13 (30 %)	8 (19 %)
E	87	19.06.2005	38	20	0.51	6.7	20 (100 %)	3 (15 %)	17 (85 %)
F	89	21.06.2005	43	11	0.25	–	–	–	–

Figure legends

Fig. 1: Location of the Bay of Laganas (Zakynthos, Greece) showing the marine protected zones and the six nesting beaches (MAR: Marathonissi, LAG: East Laganas, KAL: Kalamaki, SEK: Sekania, DAP: Daphni, GER: Gerakas). In the highly protected zone (HPZ), no boat traffic at all is allowed. In the less protected zone (LPZ), boat speed is limited to six knots.

Figs. 2: Locations of four satellite tracked loggerhead sea turtles during the internesting period. Locations of observed nestings are indicated with hatched circles.

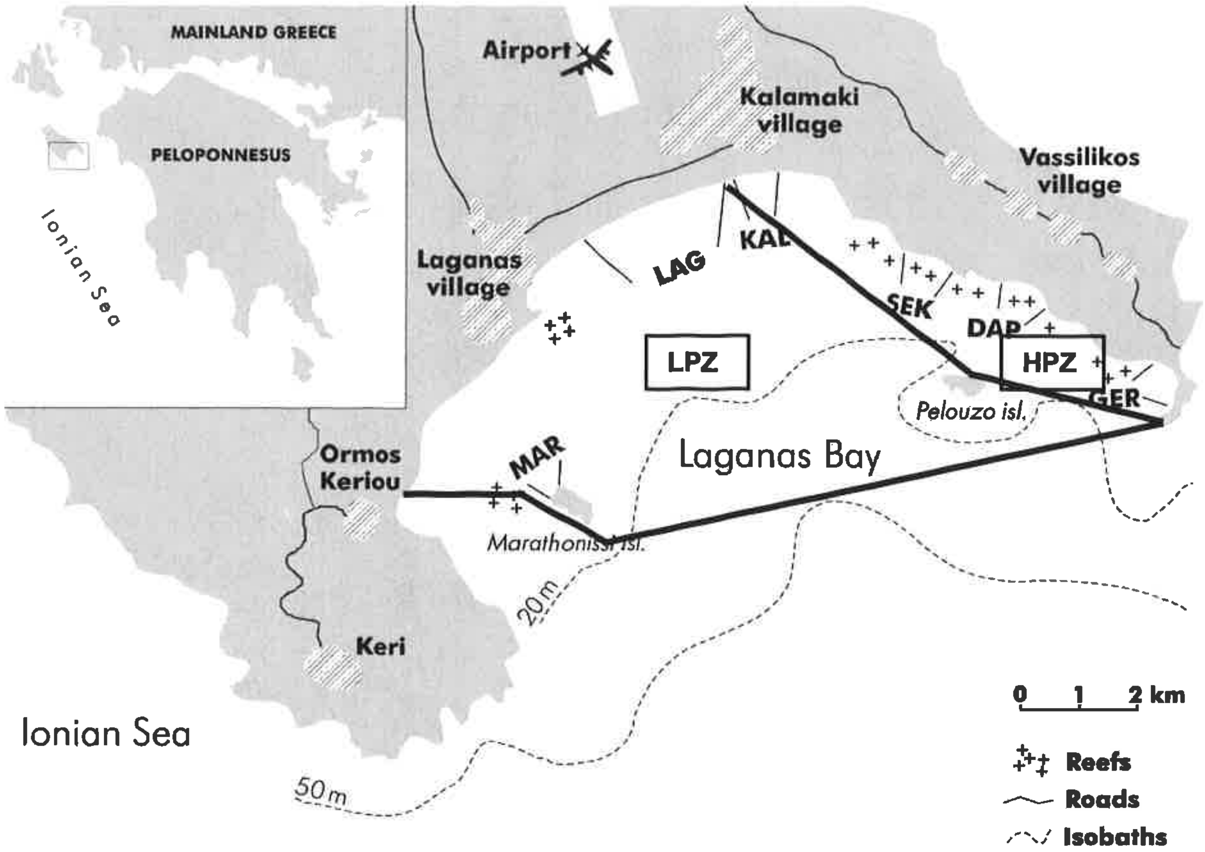


Fig. 1, Zbinden et al.

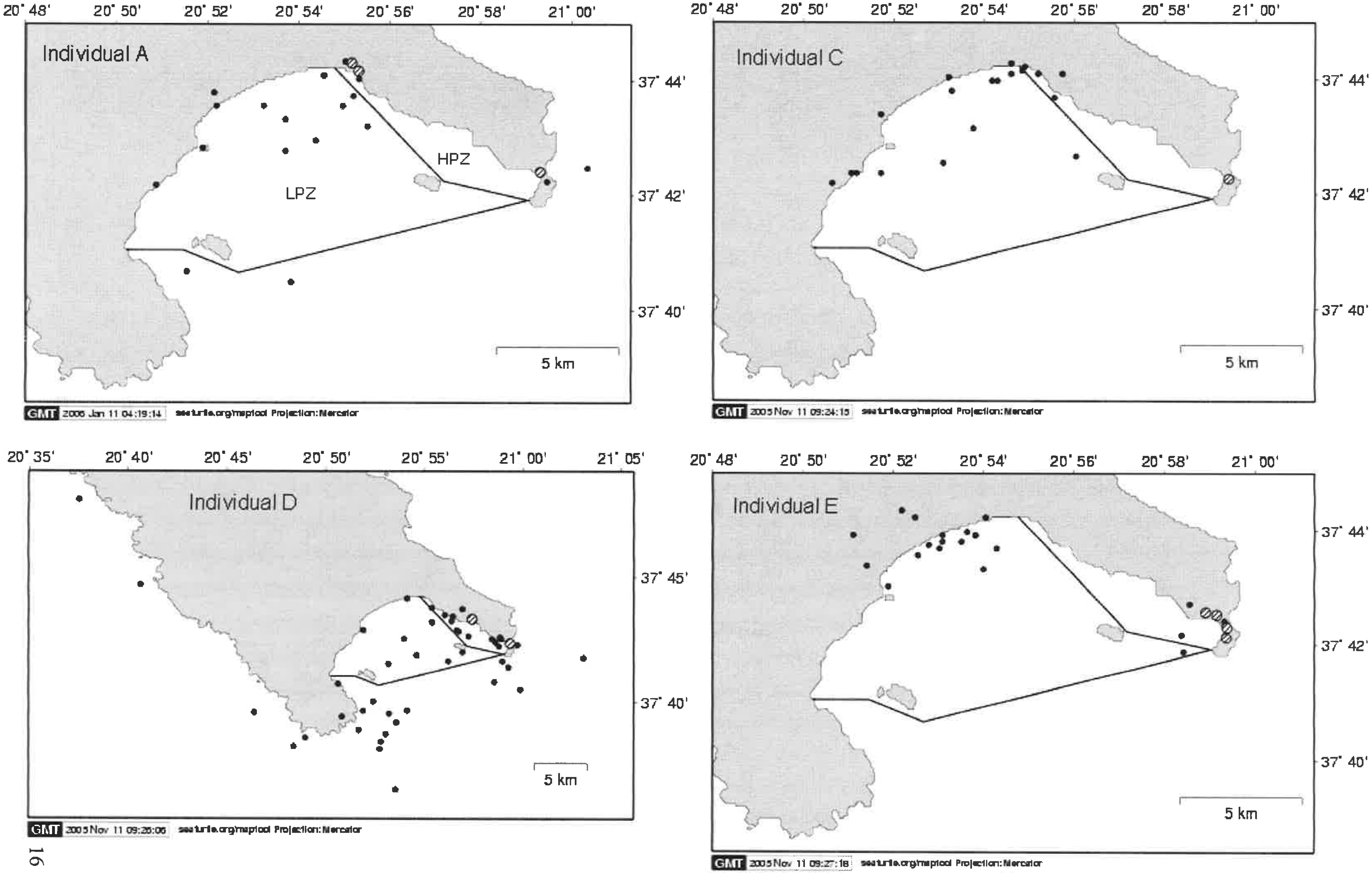


Fig. 2, Zbinden et al.

Acknowledgements

The major part of this research was funded by the MAVA Foundation for the Protection of Nature. Literally the foundation made this PhD possible and I feel deeply indebted to them. The Karl-Mayer Foundation as well as the Ocean Science and Research Foundation both made valuable financial contributions.

I'd like to thank Prof. Dr R. Arlettaz for trusting me to conduct this somehow 'exotic' project, for his supervision of the scientific aspect of the project as well as his commitment in fundraising.

I am grateful to ARCHELON (The Sea Turtle Protection Society of Greece) for allowing me to carry out fieldwork. D. Margaritoulis of ARCHELON is thanked for co-supervision.

Dr B. Godley (Marine Turtle Research Group, University of Exeter) provided valuable comments during the planning of the study. I am grateful for constructive discussions concerning data analysis and interpretation to A. Aebischer, F. Bontadina, C. Rutte, M. Schaub (Conservation Biology, University of Bern) and C. Davy.

The assistance of K. Grimanis and A. Rees (both ARCHELON) in various practical matters was greatly appreciated.

Special thanks go to the people that were involved with fieldwork: A. Bishop, P. Bradshaw, L. Coe, C. Davy, C. Dean, R. Egli, L. Emo, H. Freeman, K. Heglund, A. Lazou, C. Nienhuis, E. Ransome, A. Reed, L. Southern, M. Sturt and many additional ARCHELON volunteers. Without their commitment, this project could not have been conducted. I further wish to thank them for being such great company under often adverse circumstances.

Likewise, I would also like to thank the members of the division of Conservation Biology for their company and support.

I would like to thank H. Strantzalos for fruitful discussions on the sea turtle conservation context in Zakynthos and for his encouragement and support during the early stages of this PhD.

I thanks D. Bennett, A. Bishop, M. Michalopoulos, K. Reid, K. Reyes, A. Rees and various members of the 'Glasgow University Turtle Conservation Expedition to Northern Cyprus' 1997, who taught me about sea turtle field work during the seasons before I started this research.

Participation in conferences was instrumental in the realisation of this project and I acknowledge the organisers and funding organisations of various International Sea Turtle Symposia as well as the 2nd Mediterranean Sea Turtle Conference.

Last but not least I would like to extend my thanks to my friends and family for their continuous support.

Curriculum Vitae

Personal data

Name	Judith Zbinden
Birth date	21 January 1973 in Bern, Switzerland
Citizen of	Rüschegg (BE)

Education

[illegible]

2002 – 2006	University of Bern, PhD
1994 – 1999	University of Bern, MS in Biology. Diploma study at the Zoological Institute, Division of Population Biology under the supervision of Dr C. Largiadèr and Prof. Dr A. Scholl. Title: Analysis of the genetic population structure and post-glacial recolonisation of the bullhead *Cottus gobio* in Switzerland (in german)
1998	Field course in Tropical Ecology and Conservation of the 'Tropical Biology Association' in Uganda
1999	Course in 'Conservation Biology and Wildlife Management' of the Smithsonian Institution in Assam, India

Working experience

2001 and 2002	Monitoring leader of the Zakynthos project of the NGO ARCHELON (The Sea Turtle Protection Society of Greece). Coordination of beach monitoring work, training and supervision of assistants and volunteers, data processing and reporting
2001	Research assistant in the leatherback sea turtle research program of the 'Caribbean Conservation Cooperation', Costa Rica.
2000	Assistant at the University of Bern, Division of Ethology and Behavioural Ecology
2000	Assistant leader of the Kyparissia Bay project of ARCHELON. Monitoring, coordination and training of volunteers.
1997	Volunteer in the 'Glasgow University Turtle Conservation Expedition to Northern Cyprus'