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Effectiveness of different conservation measures for loggerhead sea turtle (*Caretta caretta*) nests at Zakynthos Island, Greece

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ABSTRACT

In this paper, we evaluate the effectiveness of different well-tested conservation techniques in an effort to successfully protect sea turtle nests. From an eight-year study on the island of Zakynthos, West Greece, we have experimentally investigated the effectiveness of two different conservation techniques applied to loggerhead sea turtle nests and provided statistical measures to evaluate their conservation value. The categories of nests evaluated include: (i) nests incubated in situ, (ii) translocated into a beach hatchery, or (iii) protected by metal cages. Results of the analysis showed significant interannual variations in hatching success as obtained for each one of the three groups of nests. Significant differences were also observed when comparing hatching success data among the three groups of nests during the eight-year period. Overall, our results indicate that relocation of nests laid at highly threatened locations and the placement of protective cages on nests in situ provide adequate conservation measures that could allow an increase in hatchling production; although their choice and application should be based on the specific conditions and threats of each nest.

1. Introduction

The ability to easily monitor, handle, and hence protect the early life stages of marine turtles in the terrestrial nesting habitat (i.e., eggs and hatchlings) has resulted in this becoming the primary focus for sea turtle conservation efforts worldwide (Witherington, 2003). Therefore, for several decades, the conservation focus of sea turtle projects has been on the early life stages (Balasingam, 1967). Hatcheries, protective cages and head-starting programs are three such conservation techniques that have been developed and which focus on the protection of eggs and hatchlings from natural and anthropogenic threats. However, the extent to which such techniques are beneficial for long-term population persistence is one of the most critical questions that sea turtle biologists and managers face. Theoretical and empirical studies (Frazer, 1994; Mortimer, 1988; Pritchard, 1979) result from experimental data (Irwin et al., 2004; Marcovaldi and Laurent, 1996), and modeling approaches (Crowder et al., 1994; Grand and Beissinger, 1997) have repeatedly contested the effectiveness of these commonly used strategies that focus on the protection of eggs and hatchlings alone.

Several studies have shown that hatching success rate was higher in nests that were translocated to beach hatcheries

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(Blanck and Sawyer, 1981; Eckert and Eckert, 1990; Limpus et al., 1979; Wyneken et al., 1998) or to a safer area of beach (Ilgas and Baran, 2001) than nests that were left in situ (i.e., naturally incubated nests). However, contrary results have been observed in other field studies (Frazier, 1993; Marcovaldi and Laurent, 1996; Pritchard et al., 1983). Furthermore, when trying to improve hatchling productivity using transplantation processes, serious problems for both developing embryos and hatchlings became also apparent. For instance, increased embryonic mortality has been widely observed during relocation procedures, (Limpus et al., 1979; Parmenter, 1980; Simon, 1975), while the robustness and genetic diversity of hatchlings has been questioned (Ackerman, 1980; Lutcavage et al., 1997; Mortimer, 1988). On the other hand, skewed sex ratios (Chan and Liew, 1995; Morreale et al., 1982; Mortimer, 1988; Mrosovsky and Yntema, 1980; Pritchard, 1979) have also generated critical questions on the benefits of these transplantation approaches with respect to long term population dynamics. One of the main goals for establishing hatcheries is to protect nests against natural predators (Baskale and Kaska, 2003). However, hatcheries at best significantly minimize predation rather than exclusively solve this problem (Frick, 1998). Special consideration should also be given to practical problems encountered in beach hatcheries, such as increased contamination produced by a/the previous seasons' eggshells or a possible rise in fresh water levels (Talbert et al., 1980). Increased infestation of eggs by insects has also been observed in transplanted nests in comparison to natural nests (Andrade et al., 1992). In contrast, recent findings from a long term experimental study suggests that concerns regarding the translocation of poorly located nests are unsubstantiated (Nordmoe et al., 2004).

Due to the many problems caused by nest relocation, researchers subsequently began developing and testing methods to minimize the potentially negative effects of this procedure. It was proposed that by emulating the physical characteristics of natural nests, or controlling thermal conditions during incubation, could effectively maintain natural sex ratios (Morreale et al., 1982). Delayed egg relocation (Parmenter, 1980) and artificial cooling (Harry and Limpus, 1989; Miller and Limpus, 1983) have been suggested as measures to prevent the reduced hatching success, resulting from embryonic fragility. Furthermore, Talbert et al. (1980) recommended that the hatchery area should be relocated annually to prevent the destructive effects of previous seasons' decaying eggshells. Regarding insect infestation, McGowan et al. (2001) showed that the determining factor for insect infestation is nest depth rather than nest translocation. Hence, they suggest that translocation methods might be beneficial in instances of elevated insect infestation at beach hatcheries (McGowan et al., 2001).

In this study, we investigated whether the establishment of a hatchery program, in the nesting area of Zakynthos, West Greece, would be a beneficial conservation tool in minimizing disturbance to nests. We analyze the hatching success data produced by nests incubated under natural conditions and nests subjected to additional protection measures within the framework of an organized pilot protection program on Laganas-Kalamaki beach, one of the six loggerhead nesting beaches on Zakynthos. We subsequently evaluate and apply our results to formulate conservation recommendations.

2. Methods

2.1. Study area

Our study area was located at Laganas Bay (37°38–37°56 N, 20°37–21°00 E), on Zakynthos Island, in Western Greece. Laganas Bay supports the main nesting area of the loggerhead sea turtle (*Caretta caretta*) in the Mediterranean, with a range of 857–2.018 nests annually (Margaritoulis et al., 1998). The total length of the six discreet nesting beaches is approximately 5.5 km. The current research focused on one of these beaches, the East Laganas beach. The East Laganas beach is a beach with fine sand and a gentle slope. It is the longest of the six nesting beaches extending approximately 2600 m. The beach length is rounded by an extensive sand dune system with a varying width ranging from 10 to 60 m comprising a mosaic of pebbles mixed with sand. The marine environment at the front of the beach is characterized by shallow waters and a sandy sea-bed. Long term research has shown that nests in Laganas Bay face the following threats: seawater inundation during southerly storms, human trampling, horse and vehicular trampling, penetration and shading by sun-umbrellas, root predation (Margaritoulis et al., 1997).

2.2. Field methods

The study site was divided into four sectors; from west to east: sector T, A, B, C with approximate lengths of 400, 875, 828, and 425 m, respectively. The division of the sectors was based on the intensity of the disturbances and the development of each site. Sectors T and A were characterized by an increased disturbance due to tourist development, while sectors B and C were less disturbed with the area that at the back of the beach being rather undeveloped. From 1988 to 1995, the beach was continuously studied during the nesting season. Extensive nest searches were performed by Archelon (the Greek Society for the Protection of Sea Turtles) personnel from May to September. In the Mediterranean, where tides are negligible due to the aspect of Laganas Beach, summer storms with southerly winds can result in nest inundation. In an attempt to minimize the risk of inundation caused by such wave action, nests located within 7 m of the sea were carefully translocated to a beach hatchery. From the 1988 nesting season onwards, nests laid close to specific plant species with invasive root systems that spread their roots into nests (exc *Tamarix sp. Phragmites communis, Pistacia lentiscus*) were also considered to be at threat and were translocated.

In 1988, a hatchery was established at the beginning of sector A at the top of the beach adjoining the sand dunes. This upper part of the beach was considered appropriate, since this area was used considerably for laying; hence, supporting our criteria for emulating natural conditions of the nesting environment. The hatchery was established within a fenced enclosure located about 25 m from the sea, in an area protected against the flooding effects of summer storm wave action. Every year the hatchery dimensions measured 7 m in length and 2.5 m in width, with the longest side running parallel to the sea. Within the hatchery, a 0.8×0.8 m area was available for each nest. In an attempt to approximate natural environmental conditions, the dimensions of each nest cavity were measured during each excavation and were duplicated in the artificial nests. Moreover, special care was taken to minimize disturbance of the eggs during translocation; i.e., by collecting and transferring eggs within 12 h of deposition, avoiding rotating the eggs when held, and placing the eggs in cylindrical styrofoam boxes prior to transfer to the hatchery area. The transferred eggs were then placed into the artificial nest, resembling their natural distribution within the eggchamber.

From 1990, the natural nests located in sectors T and A that were subject to increased disturbance from tourists and bathers were protected by metal cages. The cages had a diameter of 50 cm at their base and were surrounded by a metal mesh to further protect them from terrestrial predators. Each cage was placed over threatened nests, with the metal grid buried about 15 cm in the sand.

About 8 to 10 days after the last hatchling had emerged from each nest, it was opened and the contents were examined (Margaritoulis, 2005). The numbers of empty egg shells (hatched), unhatched eggs and dead hatchlings were counted. The total number of eggs per clutch was estimated by counting the unhatched eggs and assembling the shells produced by hatched eggs. The number of live hatchlings was estimated by subtracting the number of dead hatchlings found from the count of assembled egg shells. The hatching success rate was then calculated as the proportion of the total number of live hatchlings produced from the total number of eggs recorded.

2.3. *Data analysis*

We analyzed the eight-year data (from 1988 to 1995), recording hatching success in natural nests and nests transplanted to the hatchery. For caged nests, hatchling success data collected across six years (from 1990 to 1995) were assessed. The total number of nests included in the analysis is given in Fig. 1. We caution that hatching success for natural nests was estimated only from hatched nests. Notwithstanding the fact that the estimated values of hatching success for natural nests were an overestimation of the actual rates, the method allowed comparison among the nests hatched under the two protection techniques and the efficient natural nests (i.e., in situ hatched nests). Our analysis began by calculating the means of hatchling success for both nests left to hatch naturally in situ and those subjected to additional protection measures. We used two tailed Fisher's exact test (Zar, 1999) for each one of the three groups of nests to compare hatching success between successive years. Using the same test, we compared pairs of hatching success for the three categories of nests for each year. Furthermore, in order to detect significant differences between the three categories we compared aggregated hatching success data collected from 1990 to 1995 covering the period of application of all three methods. As the sample sizes were quite heterogeneous, not only among the different methods used, but also in successive years, we calculated the power of each test (Appendix). All calculations were performed using Mathematica code.

3. Results

Significant differences among hatching success data, obtained for each category of nests from one year to another, are given in Table 1. In natural nests, a significant variation in the hatching success rates was found for all consecutive years except between the 1990 and 1991 period. Similarly, during the eight-year period (1988–1995) in which the hatchery was operated, the hatching success varied significantly. As for nests protected by metal cages, hatching success fluctuated significantly except between 1993 and 1994.

Fig. 2 illustrates the comparative data obtained during the eight-year study. During 1988 and 1991, no significant differences were found between hatchery and natural nesting success rates (Table 2). Our analysis indicated that the hatching

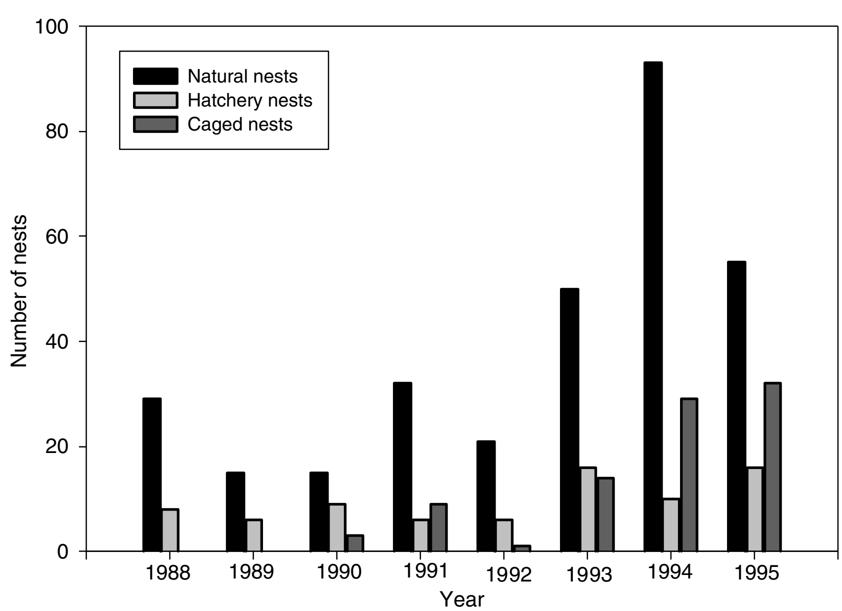

Fig. 1 – Total number of natural, hatchery and caged nests included in the analysis each year.

Table 1 – Hatching success for natural, translocated and caged nests and differences in hatching success obtained for each category of nests, from one year to another using the two-tailed Fisher's exact test

Years	Hatching success			Years	Comparisons					
	Natural nests (%)	Hatchery nests (%)	Caged nests (%)		Natural nests		Hatchery nests		Caged nests	
					p-Value	Power	*p*-Value	Power	*p*-Value	Power
1987	64.11			1987–88	0.263	0.208				
1988	65.53	62.87		1988–89	0.002	0.877	≪0.0001	0.999		
1989	61.25	51.03		1989–90	0.032	0.581	≪0.0001	0.999		
1990	57.71	68.70	47.26	1990–91	0.130	0.334	≪0.0001	0.999	0.045	0.539
1991	55.47	54.36	53.70	1991–92	0.0003	0.952	≪0.0001	0.999	0.05	0.505
1992	60.07	74.67	44.17	1992–93	≪0.0001	0.999	≪0.0001	0.999	≪0.0001	0.996
1993	67.58	57.55	65.49	1993–94	≪0.0001	0.999	≪0.0001	0.999	0.692	0.070
1994	60.74	67.03	66.06	1994–95	≪0.0001	0.999	0.012776	0.708	≪0.0001	0.999
1995	68.02	71.29	72.32							

The power of the test is also given.

success rate in natural nests was significantly different from that in the ones protected in cages, for all years, except for 1991. Comparative analysis of the hatching success rate between the two protective measures applied in our study area indicated no significant differences in 1991, 1994 and 1995 (Table 2).

Power calculation gave particularly high values in cases where significant differences were detected, indicating that considerable differences in sample sizes do not affect the significant differences between the two compared proportions.

The analysis of the aggregated hatching success data showed that the predominance of the two protective measures was significant as compared to natural nests (Table 3). On the other hand, no significant difference was observed between hatchery and caged nest groups.

4. Discussion

The results of our analysis revealed significant differences in hatching success rates between successive years for each one of the three groups of nests studied. However, it is clearly illustrated that no parallel fluctuations were found on an annual basis between different methods (Fig. 2). This is the case even for years when extreme weather events took place (such as extensive rainfalls in 1992 and 1995, see Margaritoulis, 2005). Therefore, it seems that such events had no overall impact on our experiment, since the reduction in hatching success was not apparent for all methods.

The analysis showed that hatching success differs significantly between consecutive years in hatchery nests. Considering the fact that hatchery nests were incubated under controlled conditions from 1990 to 1995 by emulating natural

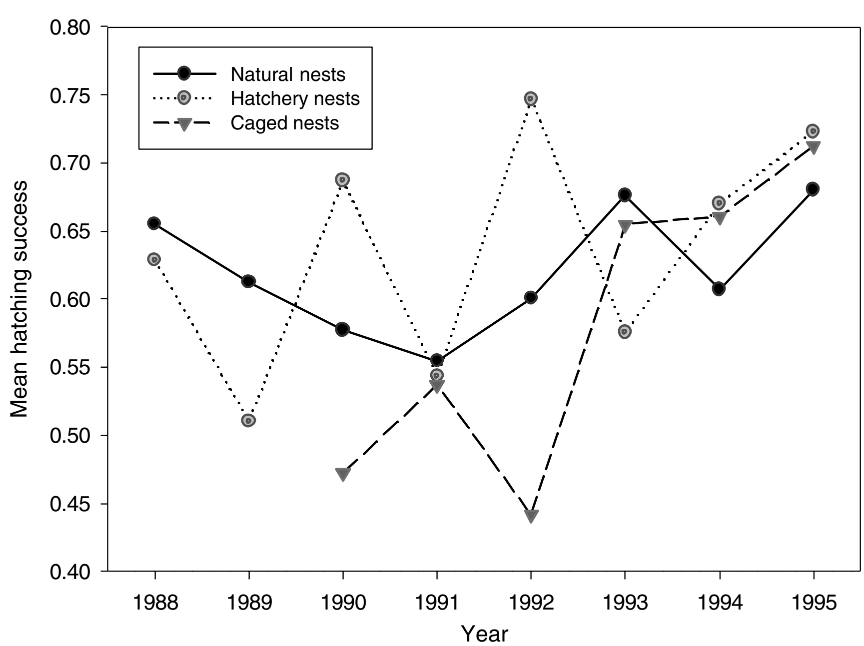

Fig. 2 – Hatching success as observed for nests hatched under natural conditions, those protected by cages and hatchery nests.

Table 2 – Two-tailed Fisher's exact test comparisons in hatching success among the three nest categories for each distinctive year and the power of each test

Years	Natural nests vs hatchery nests		Natural nests vs caged nests		Hatchery nests vs caged nests	
	p-Value	Power	*p*-Value	Power	*p*-Value	Power
1988	0.135	0.334				
1989	≪0.0001	0.998				
1990	≪0.0001	0.999	<0.0001	0.946	≪0.0001	1
1991	0.619	0.079	0.342	0.165	0.832	0.057
1992	≪0.0001	0.999	<0.0001	0.932	≪0.0001	0.999
1993	≪0.0001	0.999	0.089	0.399	≪0.0001	0.999
1994	≪0.0001	0.990	≪0.0001	0.999	0.567	0.093
1995	0.007	0.778	≪0.0001	0.996	0.421	0.132

Table 3 – Two-tailed Fisher's exact test comparisons in overall hatching success data between the three nest categories and the power of each test

Time period	Natural nests	Hatchery nests	Caged nests
1990–1995	62.81%	65.75%	66.31%

Natural nests vs hatchery nests		Natural nests vs caged nests		Hatchery nests vs caged nests	
p-Value	Power	*p*-Value	Power	*p*-Value	Power
≪0.0001	0.998	≪0.0001	0.999	0.436507	0.124

conditions of nests, it seems likely that micro-environmental conditions are those controlling the fate of nests. Regarding natural and caged nests, it should be noted that environmental conditions of each nest location (i.e., distance to the sea, distance to vegetation, etc.) is not possible to control on an experimental basis; therefore, it is likely that a series of environmental factors might have led to the observed interannual variability by controlling hatching success. However, the fact that once again extreme weather events did not have a similar effect in hatching success suggests that the specific micro-environmental conditions of each nest might be the key factors for incubation processes.

When comparing hatching success among the three different groups of nests over time, it is clearly illustrated that significant differences occurred for most of the given years. The results of the analysis showed that in some of the years, hatching success was lower for one group of nests in comparison to the other categories, while for other years hatching success for the same group was higher. Based on these results, it is clear that no significant evidence exists to support any negative effect of the two protection methods used upon hatching success. On the other hand, comparison of the overall hatching success data indicated that hatchery and caged nests predominated in comparison to natural nests. In this manner, we conclude that under the basic assumptions of our framework, the two protection measures could successfully be implemented to increase hatchling production. At this point, it should also be mentioned that this suggestion is further supported when considering the fact that hatching success of natural nests used in the analysis derived only from hatched nests; thus, the estimated values were an overestimation of the actual rates.

The conservation value of hatcheries as a tool for increasing hatchling recruitment has been questioned, since it may result in reduced hatching success but it could also bias the sex ratio of the hatchlings (Morreale et al., 1982; Mrosovsky and Yntema, 1980). Although we did not study the effect of hatcheries on hatchling sex ratios, in the present experiment, the close duplication and control of the environmental conditions of natural nests could significantly reduce any effect in natural sex ratios (García et al., 2003; Marcovaldi and Laurent, 1996). On the other hand, it has been suggested that the natural threats to a nesting site should be controlled only in the cases of highly threatened populations (Frazier, 1999; Witherington, 1999). However, since no clear evidence exists on the status of loggerhead sea turtles nesting in Zakynthos Island and no clear trend has been detected for this population (Margaritoulis, 2005), we invoke the argument that beach hatcheries should be used in the cases where natural and human threats are apparent (Ehrenfeld, 1995; García et al., 2003; Grand and Beissinger, 1997; Marcovaldi and Laurent, 1996; Parmenter, 1980).

Notwithstanding the fact that this experiment enabled us to evaluate the effectiveness of the different techniques applied, it is not possible to quantitatively determine the beneficial intervention of relocating nests considered to be greatly threatened. However, due to the fact that nest relocation and incubation at hatcheries on the beach and/or of artificial rearing of young turtles can reduce the immediate impact of the natural threats (i.e., predation, wave action, storms, hurricanes, and erosion) and human caused disturbances (i.e., coastal development, beach nourishment operations), the conservational value of such measures significantly increases. Though, at this point, it should be noted that such

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protection techniques should be applied in cases where the nesting sites suffer from severe nest losses due to natural or human induced threats. In the same manner, it has also been suggested that even under conditions of low reproductive success, the use of beach hatcheries and the maintenance of natural nests should be preferred, unless the location of natural nests or hatchery sites do not fulfill conditions of secure incubation, or laboratory incubation is not accomplishable (Bell et al., 2003).

Overall, the results of the analysis indicate that hatcheries and placement of protection cages could be critical conservation strategies that allow a significant increase in hatching production. However, the operation of such programs should be based on the evaluation of local characteristics including ecological and economic criteria. A clear need for monitoring and interpreting the observed patterns is also apparent, while heuristic models could provide some basic insights into direct actions.

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Appendix A.

A conservative estimate of the power of the test H_0: $p_1 = p_2$ vs. H_1: $p_1 \neq p_2$ is given by the following formula, proposed by Marscuilo and McSweenwey (1977):

$$P\left[Z \leqslant \frac{-Z_{\alpha(2)}\sqrt{\bar{p}\bar{q}/n_1 + \bar{p}\bar{q}/n_2} - (\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{p}_1\hat{q}_1/n_1 + \hat{p}_2\hat{q}_2/n_2}}\right] + P\left[Z \geqslant \frac{Z_{\alpha(2)}\sqrt{\bar{p}\bar{q}/n_1 + \bar{p}\bar{q}/n_2} - (\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{p}_1\hat{q}_1/n_1 + \hat{p}_2\hat{q}_2/n_2}}\right],$$

where

$$\bar{p} = \frac{n_1\hat{p}_1 + n_2\hat{p}_2}{n_1 + n_2}$$
$$\hat{q}_1 = 1 - \hat{p}_1$$
$$\hat{q}_2 = 1 - \hat{p}_2$$
$$\bar{q} = 1 - \bar{p}$$

where $\hat{p}_1, \hat{p}_2$ are the sample probabilities and n_1, n_2 the sample sizes. The calculations were made for $\alpha = 0.05$ (i.e., $Z_{0.05(2)} = 1.96$).

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