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ARTICLE

Breeding biology of the Atlantic Least Tern (*Sternula antillarum antillarum*) in a colony of the south of the Gulf of Mexico: new perspectives for its threat status

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ABSTRACT: Although the Atlantic Least Tern (*Sternula antillarum antillarum*) faces the same threats that caused the California Least Tern (*S. antillarum browni*) and the Interior Least Tern (*S. antillarum athalassos*) to be declared threatened, it is considered as "Least concern" globally, mainly because of its wide geographic distribution. However, many populations are threatened and the conservation status of several others is unknown. We evaluate, for the first time, the breeding biology of a colony of Atlantic Least Terns in the southern Gulf of Mexico. During the 2010 breeding season we censused and analyzed some relevant breeding variables for the stability of a colony at Terminos Lagoon. The starting date of egg laying and breeding peak, the clutch size variation during the breeding season and the main causes of eggs loss are similar to those reported for other colonies of this species. However, we found that the size of first (A) and single (S) eggs did not change throughout the season, but second (B) eggs size decreased. The number of breeding pairs and its higher hatching success in comparison with other colonies allow us to affirm that the colony of the Terminos Lagoon must be taken into account in conservation programs of this species. These results will settle the basis to reconsider its threat status globally and to compare breeding parameters with other colonies from the region.

KEY-WORDS: clutch size, conservation, egg size, hatching success.

INTRODUCTION

Seabirds are one of the most threatened taxon globally, with more than 25% of the species listed in risk categories by the International Union for Conservation of Nature and Natural Resources - IUCN (Croxall *et al.* 2012). This is mainly due to consequences of human activities like pollution, overfishing, egg collection for human consumption, loss of habitat, tourism, invasive species on the breeding grounds and global climate change (Croxall *et al.* 2012). These threats have raised the need of monitoring. However, because of its vulnerability, it is imperative that monitoring techniques to measure reproductive variables are noninvasive and low stress.

Laying start date, egg and clutch size are within the most important variables of reproductive success of birds. Females nesting early in the season mate with good quality males and choose the best nesting sites (Wendeln 1997), which is reflected in bigger eggs and larger clutches (Morris 1987). Larger chicks will hatch from larger eggs (Parsons 1970, Blomqvist *et al.* 1997, Krist 2011) which is beneficial because a greater size at hatching enhances survivorship (Parsons 1970, Amat *et al.* 2001) at least during the first days of life when chicks are more vulnerable to weather and predators (Galbraith 1988, Williams 1994, Dawson & Clark 1996). Thus, egg size influences fitness (Reviewed in Krist 2011) and clutch size is correlated with breeding success (the combination of hatching and fledging success; Langham 1974). At the present, in some seabird species, clutch and egg size are diminishing through time when compared with long term data sets: *i.e.* Glaucous-Winged Gulls (*Larus glaucescens*: Blight 2011) and Atlantic Puffins (*Fratercula arctica*: Barrett *et al.* 2012).

The Least Tern (*Sternula antillarum*) breeds throughout the coastal beaches and interior rivers of USA, Mexico, the Caribbean, and the coasts of Central and South America. Because of its large range, the Least Tern has been catalogued under the category of "Least concern" by the IUCN (IUCN 2016). However, its steep decline classifies two of its subspecies, the California Least Tern (*Sterna antillarum browni*) and the Interior Least Tern (*Sterna antillarum athalasso*), as "Endangered" in the USA (US Fish and Wildlife Service 2013), and under special protection in Mexico (Diario Oficial de la Federación 2010). These new categorizations are a consequence of the loss of their breeding and feeding grounds (Massey 1974), eggs and/or chick predation, tidal floods, human activities, and pollution (Hothem & Zador 1995). The third, nominate subspecies, the Atlantic Least Tern, is facing threats as well. Some states within United States have declared it under threat (*e.g.* Maine Department of Inland Fisheries and Wildlife 2009, Delaware Division of Fish and Wildlife 2013, New Hampshire Wildlife Action Plan 2015, New Jersey Department of Environmental Protection 2015). In addition, the colonies of St. Kitts, West Indies, and the main colony in the Caribbean in the Virgin Islands are also under threat (Lombard *et al.* 2010, Stimmelmayr *et al.* 2014). In Mexico, however, except for some published papers (Ornat *et al.* 1989, Winker *et al.* 1999, Navarro-Sigüenza & Peterson 2007) about their presence in some Mexican states and the sighting of a few nests in the Mexican state of Veracruz (Mellink *et al.* 2007), nothing is known about the conservation status of the Atlantic Least Tern. For this reason, the objective of the present study is to quantify variables related with reproductive success such as number of breeding pairs, number of eggs laid/hatched, eggs size and egg mortality of an Atlantic Least Tern colony in the Mexican part of the Gulf of Mexico. These results will provide a baseline data that will facilitate future research and a possible change of its threat status since it is clear that the Atlantic Least Terns in Mexico are facing the same threats that led the other two subspecies to become threatened.

METHODS

We carried out fieldwork during 2010 in one of the several small islets at the Terminos Lagoon located at the south of the Gulf of Mexico and west coast of the Yucatan Peninsula, Mexico (18°45'10.83''N; 91°29'14.23''W) in a population of Atlantic Least Terns which had not been reported previously. We counted, identified and marked the nests during the breeding season (from late April to the end of June). We started checking the nests on 26 April, when 30 nests were already present. During the first three weeks of our monitoring, nests were visited every 3 days. Because more than 10 new nests were found on each visit after the second week, we started to visit the colony daily from week 3 until 26 June, when the tropical storm Alex hit the islet and destroyed every active nest. We visited the islet again on the days 3 10 and 24 July, and no nest was found.

During each visit to the islet, we walked around the colony from 6:00 to 8:00 h in search of new nests. Every time we found a new nest, we identified it with a numbered flag and marked the first egg (A- or Single S-egg) with non-toxic red ink. After that, we marked any new eggs with blue (second or B-egg) and green (third or C-egg) to identify them according with their laying order. After marking each egg, we measured their breadth and length using a caliper with a 0.01 mm precision and calculated the egg volume with the equation $V = 0.4866 \times \text{length} \times \text{breadth}^2$ (Coulson 1963). Nest marking and egg checking was always done before 10:30 h or after 16:30 h to minimize heat stress. We divided the islet in a central zone of 1 m a.s.l. and a peripheral zone of less than 40 cm a.s.l.

The fate of each egg was defined as successful or failed: predated, overwashed (the main threat to Least Tern nests *sensu* Brooks *et al.* 2013) or abandoned. We considered a successful hatching if the egg completed their incubation time (counting 20–22 days after being laid) and inferred hatching by the presence of eggshells or a recently hatched chick within close proximity (25 cm) to the nest. We registered a nest as tidal flooded, by tidal marks on the beach and signs of the water to have reached or covered eggs (water on the nest cup, eggs moved on the beach). A nest was recorded as predated when eggs disappeared, eggshells were broken with yolk stains, and no evidence of tidal floods was found. We considered an egg as abandoned if it was cold and/or moisture was seen on the eggshell. Every day we conducted observations with binoculars from one of the ends of the islet for at least 3 h (90 min at 6:00 h and 90 min at 16:30 h) to identify the presence of potential predators and we registered the number of direct attacks (*sensu* Rounds *et al.* 2004).

We estimated the hatching date by counting 20–22 days after eggs were laid. We defined hatching success as (No. of eggs hatched/No. of eggs laid - Total No. of eggs lost) × 100. We excluded the eggs lost during the tropical storm because they did not complete incubation. We also calculated the hatching success along the breeding season and for each egg type (A, B or S). For this study we did not attempt to estimate fledging success because we did not have a way to individually marking chicks (*e.g.* colored rings) in order to recognize them, as they are cryptic and mobile since the first 1–2 days of hatching.

We performed statistical analyses using STATISTICA version 8.0 for Windows (StatSoft 2007).

RESULTS

We found 240 nests during the 2010 breeding season on the studied islet. However, 30 of them were not included in the analyses because we could not estimate the laying date and laying order. From the 210 marked nests, 120 had two eggs (57.1%), 83 had a single egg (39.5%)

and seven had 3 eggs (3.3%). Clutches were not laid in a continuous way, *i.e.* there were three laying waves: 1) from 6 May to 13 May; 2) from 23 May to 30 May; and 3) from 10 June to 18 June. During the first (55 nests) and second (118 nests) waves, all nests were located in the central area. On the contrary, during the third wave (37 nests), all of them were in the periphery. Because there were few three-egg clutches, they were not included on the analyses of egg size variation (Table 1).

Clutch size variation

Clutch size ranged from one to three eggs (Mode, Mo = 2, mean ± standard error SE = 1.64 ± 0.04 eggs per nest). Clutch size decreased along the breeding season ($\chi^2 = 24.37$, df = 4, n = 210, $P < 0.001$). First wave clutches were larger than clutches in the second ($\chi^2_1 = 15.36$, $P < 0.001$) and third waves ($\chi^2_1 = 12.42$, $P = 0.001$). Clutches in the second and third waves were similar in size ($\chi^2_1 = 0.11$, $P = 0.74$) (Table 1).

Egg size variation

Egg size varied accordingly with egg type (A-, B- and S-eggs), as well as the clutch size (GLM Factorial ANOVA: type of egg: $F_{1, 314} = 84.12$, $P < 0.001$ clutch size: $F_{1, 314} = 26.56$, $P < 0.001$). A significant interaction was found between wave number and egg type ($F_{2, 314} = 4.23$, $P = 0.015$). Tukey HSD *post hoc* tests showed that A- and S- eggs were similar in size along the breeding season, but B-eggs were smaller in the third wave than B-eggs in the first and second waves ($P < 0.001$; Figure 1). B eggs were smaller than both A- and S- eggs during the second and third waves ($P < 0.001$; Figure 1). Through the Wilcoxon's test we found that eggs in 2-egg clutches were smaller, in volume, than eggs from single egg clutches during the second ($W = 9996$, $P = 0.047$) and third waves ($W = 825$, $P = 0.025$), but we did not find significant differences in volume between eggs from different size clutches during the first wave ($W = 3858$, $P = 0.51$) (Table 1).

TABLE 1. Reproductive parameters of the three nesting waves of Atlantic Least Terns (*Sternula a. antillarum*), hatching success, and causes of egg loss at Terminos Lagoon, Mexico, during 2010.

Breeding parameters	Wave 1	Wave 2	Wave 3	Total
Number of nests	55	118	37	210
Clutch size (mean ± SE)	1.9 ± 0.04	1.7 ± 0.04	1.6 ± 0.06	1.64 ± 0.04
Clutch range	1–3	1–3	1–2	1–3
Nests with one egg	8	56	19	83
Nests with two eggs	42	60	18	120
Nests with three eggs[1]	5	2	0	7
S- Eggs (cm^3) (mean ± SE)	8.07 ± 0.13	7.99 ± 0.07	7.82 ± 0.10	7.96 ± 0.06
A- Eggs (cm^3) Mean ± SE)	8.3 ± 0.11	8.02 ± 0.07	7.71 ± 0.1	8.01 ± 0.06
B- Eggs (cm^3) (mean ± SE)	7.68 ± 0.09	7.48 ± 0.08	6.63 ± 0.10	7.43 ± 0.06
A+B Eggs (mean ± SE)	7.99 ± 0.08	7.75 ± 0.06	7.17 ± 0.12	7.75 ± 0.05
Total eggs laid	107	182	55	344
Total failed eggs (%)[2]	7 (6.5)	30 (16)	21 (38.2)	58 (20)
Total eggs hatched (%)[2]	100 (93.5)	122 (80.3)	10 (32.3)	232 (80)
S-Eggs (%)[2]	6/8 (75.0)	42/51 (82.3)	5/12 (41.7)	53/71 (74.6)
A-Eggs (%)[2]	42/42 (100)	43/48 (89.6)	4/11 (36.4)	89/101 (88.1)
B-Eggs (%)[2]	40/42 (95.0)	34/47 (66.0)	1/8 (12.5)	75/97 (77.3)
A+B Eggs (%)	82/84 (97.6)	77/95 (80.1)	5/19 (26.3)	164/198 (82.3)
Non-viable (%)[3]	0	7 (4.6)	0	7 (12.1)
Predated (%)[3]	7 (6.5)	23 (15.1)	10 (32.2)	40 (68.9)
Tidal flooded (%)[3]	0	0	11 (35.5)	11 (19.0)
Destroyed by tropical storm "Alex" (%)	0	30	24	54 (15.7)

[1] Eggs do not included in the eggs size variation.
[2] Eggs lost during the storm were not included to calculate these percentages.
[3] Percentage of the total of eggs lost.

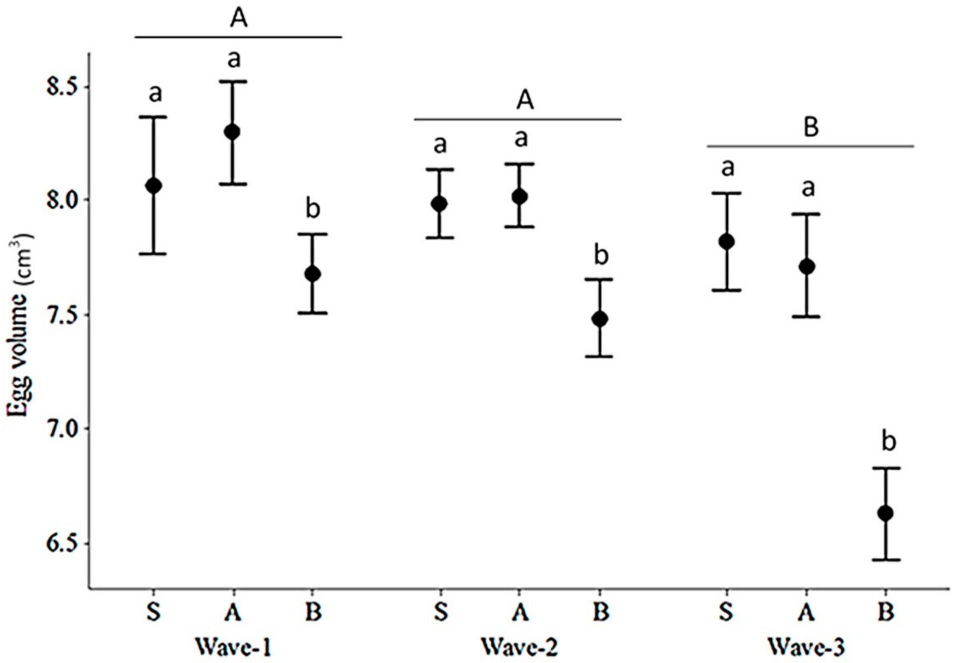

FIGURE 1. Variation in the egg size of Atlantic Least Terns (*Sternula a. antillarum*) between egg types and throughout the breeding season at the Terminos Lagoon, Campeche, Mexico. Points with different letters (a and b) indicate significant differences within waves (P < 0.05). Between waves differences were only found in B eggs from the third wave (B).

Predation and tidal floods threats

We observed several egg and chick predators: one Peregrine Falcon (*Falco peregrinus anatum*), two Great Blue Herons (*Ardea herodias*), two Great Egrets (*Ardea alba*), four Laughing Gull (*Leucophaeus atricilla*) pairs breeding on the same islet, and around 80 pairs breeding in nearby islets. Moreover, we registered attacks of Ghost Crabs (*Ocypode quadrata*), all of them repelled by incubating adults. We estimated that 69% of eggs lost before the tropical storm Alex hit the islet were due to predation, being the main cause of egg lost during the first and second waves. On the other hand, if we estimate the number of eggs lost as a percentage of the total eggs laid during each wave, the third wave was the period with the greatest percentage of predated eggs (Table 1). Tidal floods accounted for the loss of 19% of the eggs during the season. However, all eggs lost by tidal floods were from the third laying wave (Table 1) on peripheral nests. Moreover, 12% of the eggs lost were non-viable and occurred during the second wave (Table 1).

Of the seven non-viable eggs found, 4 were B eggs and a single S, A and C eggs each. Tidal floods accounted for the loss of 5 S, 3 A and 3 B-eggs. Predation was the cause of the loss of 40 eggs, 11 of which were S, 13 B and 5 C-eggs.

Hatching success

Until the arrival of the tropical storm Alex on 26 June, 290 eggs should have completed their incubation period, but only 232 of them hatched, the rest were lost due to predation, tidal floods or were non-viable, resulting in a pre-storm hatching success for the whole colony of 80%. At that time, all of the 107 eggs laid during the first wave, 152 out of the 182 eggs laid during the second, and 31 of the 55 eggs laid during the third wave should have completed their incubation time (Table 1). Hatching success in the first wave was higher than in the second (Fisher's exact test: P = 0.003), while success in the second wave was higher than in the third one (Fisher's exact test: P < 0.001). Hatching success was similar between A and S eggs along the season, but decreased in B eggs (Table 1). Two-egg nests were more successful early in the season (Fisher's exact test: P = 0.04), while single egg nests were not more successful than 2-eggs nests during the third wave (Fisher's exact test: P = 0.45).

DISCUSSION

While the distribution range of the Atlantic Least Tern is vast, there are numerous reports that cast doubt on the viability of populations throughout its range, as a result from reduced breeding success (Lombard *et al.* 2010), a reduction in the genetic diversity in populations (Draheim *et al.* 2012), the decrease of the number of colonies or the population size of the colonies in a given region (Alleng & Whyte-Alleng 1993) and the unknown status of several populations. The 344 nests found in this study are equivalent to the 4.7% of the breeding pairs from Maine to Virginia (between 7000 and 7500; Kress *et al*. 1983), 17% of the 2000 pairs nesting on natural beaches in coastal South Carolina, Georgia, and north Florida (Jodice *et al*. cited in Brooks *et al*. 2013) and 15% of the 2250 breeding pairs estimated for the Caribbean (McGowan *et al*. 2006). Therefore, based only on its size, we can affirm that the colony of the Atlantic Least Tern in the Terminos Lagoon, is large enough to be taken into account in the management plans for the species. The present work establishes a baseline for long-term monitoring of this subspecies in the Mexican Gulf of Mexico, taking into account its breeding biology, which will allow a more accurate assessment of the threat status of this subspecies along their geographical distribution.

Early nesting pairs (wave 1) have larger clutch sizes than those who laid on the second and third waves. In addition, adults nesting later (waves 2 and 3) tend to lay single egg clutches and when they lay 2 eggs in the third wave, the second egg is rather small (Figure 1). In the studied colony, early nesting pairs had higher hatching success than pairs nesting at the peak of the breeding season (second wave). Clutch size *per se* may not explain the hatching success variation because we found that clutch sizes were similar between waves two and three, but those from the second wave were more successful. Egg size alone did not explain hatching success: single eggs were as big as A eggs, and bigger than B eggs, but had overall lower hatching success than A eggs and were as successful as B eggs. Resource depletion as the season goes by may not explain our results because pairs nesting late (wave 3) had clutch sizes as large as in the peak of the breeding season, and cannot explain why S and A eggs remained as big as eggs laid early in the season.

However, results indicate that some of the interactions between those variables are important for hatching success. We suggest that those interactions depend on breeding time within a season. Nesting early in the season enable parents to choose the best nesting sites (Sergio & Newton 2003), face low competition for food and reduce the predation or tidal flood risk, so they can lay two or three egg clutches with a high probability of hatching. However, just pairs in good condition can do it (Eising *et al.* 2001). As the season goes on, the capacity of breeding pairs to produce two or more egg clutches decrease, thus, in order to maintain the size of A eggs without reducing the clutch size, they produce ever smaller B eggs or even stop producing them (single egg nests; Perrins 1996). Thus, we suggest that clutch size, egg size and the proportion of each type of egg in each wave during the breeding season should be included in the assessment of the conservation status of this species.

Results from the present study suggest that the Atlantic Least Tern population in the Terminos Lagoon is worthy to preserve. Nonetheless, given the great variation in reproductive success of this species between breeding seasons (*i.e.* Szell & Woodrey 2003) along with the loss of genetic diversity (Draheim *et al.* 2012), could put the survival of this population at risk. Thus, a periodic evaluation of the breeding biology of this colony is necessary. Reproductive parameters like clutch and egg size, as well as laying date, are altered by stressors such as tourists walking through the nesting sites (Erwin 1989), pollution (Rattner *et al*. 2013) and climate change (Jarvinen 1996, Ancona *et al*. 2011, Munroe *et al*. 2014). The value of this study is that it will serve as a baseline for long term studies on the Atlantic Least Terns breeding in the eastern coasts of Mexico and provide information in order to reevaluate its global status.

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