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Reproductive success of the South American Tern, *Sterna hirundinacea* Lesson, 1831 (Aves: Laridae), at an artificial site in the coast of São Paulo state, Brazil

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ABSTRACT

Sterna hirundinacea, a seabird species, has been extensively studied in natural habitats; however, information on its reproduction in artificial environments is still limited. This study investigates the nesting behavior and reproductive success of *S. hirundinacea* at the Terminal Aquaviário da Petrobrás (TEBAR), an artificial site located on the north coast of São Paulo state, Brazil. From April to September 2021, 57 nests were selected for monitoring at 48-72-hour intervals from a total of 159 nests and 318 mature individuals reproducing within the colony. The breeding season lasted 145 days, with an average clutch size of 1.21 ± 0.41 eggs per nest, including single-egg nests (n = 45) and double-egg nests (n = 12). Nine instances of egg replacement were recorded. A total of 78 eggs were laid, with a mean length of 4.56 ± 0.22 cm, a mean width of 3.26 ± 0.09 cm, and a mean volume of 25.45 ± 2.01 cm^3. The study documented a mean egg mass reduction of 0.39 ± 0.02 g per interval. Egg losses (n = 63) were attributed to predation (60.3%), inviability (34.9%), and stillbirth (4.8%). The mean incubation period was 23.73 ± 2.34 days. Of the remaining 15 healthy fledglings, 11 (73.3%) did not survive; causes of mortality included predation or falls into the sea (54.5%), collisions (36.4%), and natural causes (9.1%). Mortality peaked during the first nine days after hatching, further increasing at 21-30 days of age. Only four fledglings made it to flight, resulting in a reproductive success rate of 5.1%. TEBAR showed both similarities and differences in *S. hirundinacea* reproduction at the artificial site compared with natural nesting sites. Human activities at the site likely contributed to higher egg and fledgling losses due to predation and disturbance. Regular monitoring and further research are essential to assess the impact on *S. hirundinacea* and to develop conservation strategies if necessary.

Keywords: Artificial environment, Colony, Seabirds, Terns, Reproduction

INTRODUCTION

Sterna hirundinacea Lesson, 1831, commonly known as the South American Tern, is a seabird of the order Charadriiformes and family Laridae that can reach up to 41 cm in length (Sick, 1997; Pacheco et al., 2021). This species has a wide distribution that extends across South America from Tierra del Fuego (Argentina) to Bahia (Brazil) (Sick, 1997), along the Atlantic coast, and from the southern part of the continent to Peru, along the Pacific coast (Gochfeld and Burger, 1996). Classified as a seasonal resident in the state

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of São Paulo, this migratory bird travels from southern South America to the Brazilian coast in late autumn for winter breeding and leaves the country in early spring for summer breeding (Figueiredo, 2002; Campos et al., 2004; Barbieri and Mendonça, 2008; Portflitt-Toro et al., 2018; Pacheco et al., 2021). Nationally, it is considered vulnerable (MMA, 2018; 2022), and in the state of São Paulo, it is almost threatened (Bressan et al., 2009; São Paulo, 2014).

Colonies of these birds have been recorded in natural areas on the coast of São Paulo (excluding the Alcatrazes Archipelago), in the municipalities of São Sebastião (Itaçucê and Apara islands) and Ilhabela (Prainha, Codó, and Figueira islands) (Campos et al., 2004). The islands of Itaçucê and Apara are included in the Marine Environmental Protection Area of the North Coast (APAMLN), while the islands of Prainha, Codó and Figueira are part of the Ilhabela State Park (São Paulo, 2015; 2020). In addition, unlike natural island environments, the Terminal Aquaviário da Petrobrás (TEBAR), also known as Transpetro Pier and located in São Sebastião, has been found to serve as a breeding area for the species (Campos et al., 2004).

Studies investigating the reproductive behavior of *S. hirundinacea* are notably scarce and mostly focus on natural nesting habitats, typically characterized by rocky island environments with no vegetation or areas featuring grasses and small shrubs (Scolaro et al., 1996; Branco, 2003; Fracasso et al., 2010; Hogan et al., 2010; Fracasso and Branco, 2012; Fracasso et al., 2014; Roseki and Barbieri, 2022). Only one study, conducted in Coquimbo (Chile), included artificial environments, documenting the reproduction of these birds on three boats in La Herradura Bay (Portflitt-Toro et al., 2018).

As this species' reproduction in artificial environments has never been studied in Brazil, this study aimed to test the hypothesis that *S. hirundinacea* shows differences in its reproductive ecology in artificial areas. In addition to the lack of information on the species' reproduction in southeastern Brazil, none of the existing studies on this topic focused on obtaining data in artificial environments with ongoing anthropogenic activities.

This study aimed to evaluate the differences in the reproductive ecology of *S. hirundinacea* in an artificial environment on the coast of the state of São Paulo, Brazil. To date, there are no records of studies specifically focused on the reproduction of this species in artificial environments with anthropogenic activities in the country. Coastal seabirds, such as terns, may experience lower reproductive success and greater vulnerability in terms of reproduction and foraging (Fracasso and Branco, 2012) compared to other species, such as seagulls, which can easily adapt to adverse conditions in areas modified by human activities (Giaccardi et al., 1997; Yorio et al., 1998). This study investigates the reproduction of *S. hirundinacea* at the Admiral Barroso Terminal (TEBAR), encompassing a description of the species' reproduction in an artificial environment, an analysis of different types of eggs (based on the order of their posture), and an evaluation of reproductive success at the artificial nesting site.

METHODS

The study was conducted in the municipality of São Sebastião (23°21'20''S 45°21'00''W, SIRGAS 2000) (Figure 1), located on the northern coast of the state of São Paulo, Brazil. This area is 520 km^2 wide, with approximately 300 km^2 covered by the Atlantic Forest biome (Lefèvre et al., 2007). The municipality is located at an elevation of 2 m above sea level and characterized by a tropical climate, classified as Af according to Köppen and Geiger, with an average annual precipitation of 1.973 mm (Climate-Data, 2021).

Data collection took place during the reproductive season of 2021 at one of the sites with previous records of use as a breeding site by *S. hirundinacea* (Campos et al., 2004) (Figure 2): the Petrobras Aquatic Terminal (Admiral Barroso Terminal – TEBAR) (23°48'08''S 45°23'22''W, SIRGAS 2000), located on the São Sebastião Canal (Figure 1). This terminal represents the largest operational unit of Transpetro in terms of product movement, receiving both national and imported petroleum via oil tankers and supplying the four refineries of the state of São Paulo via pipelines (Transpetro, 2021).

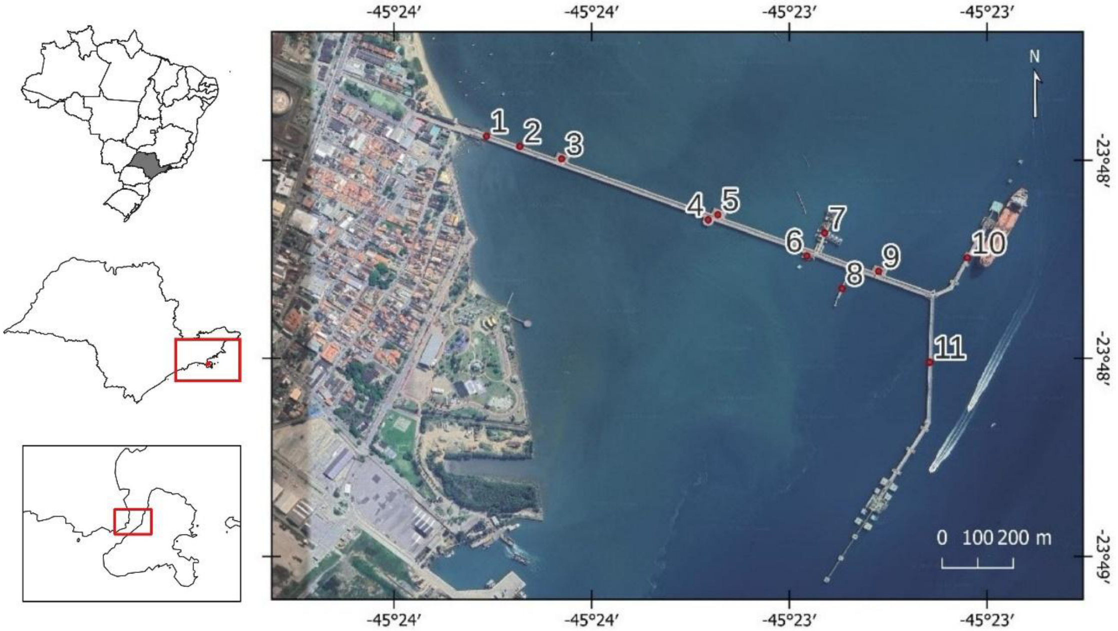

Figure 1. Study area and view of the Pier at TEBAR, in detail. Structures: (1) Access Bridge; (2) Pipeline; (3) Platform 1; (4) Meteorological Station; (5) Platform 2; (6) Pump Platform; (7) North Tug Pier; (8) South Tug Pier; (9) Platform 3; (10) North Pier; (11); South Pier. Source: Main Image - Google Earth (2024).

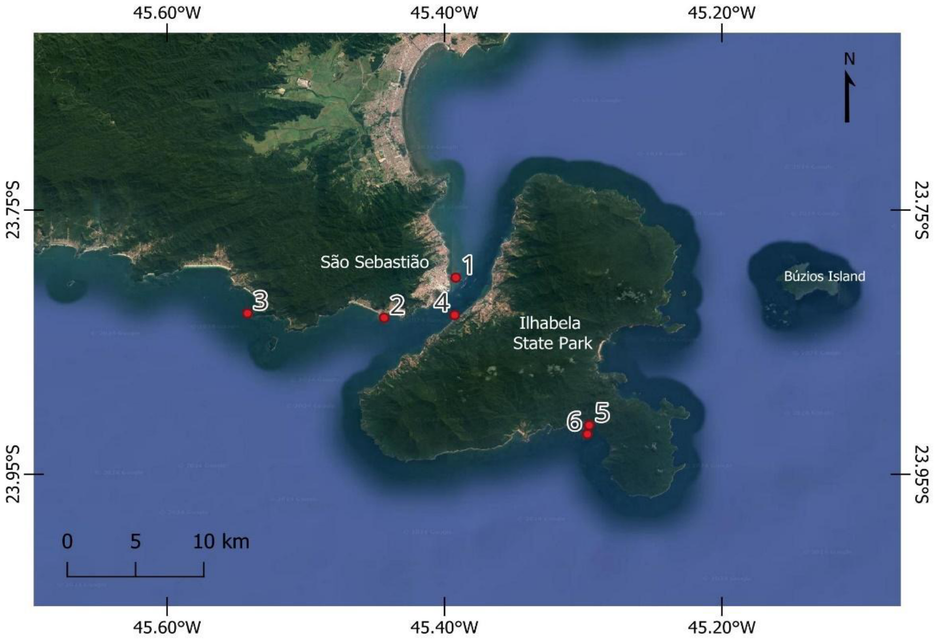

Figure 2. Locations with previous records of use as breeding sites by *S. hirundinacea* in the municipalities of São Sebastião (excluding Alcatrazes Archipelago) and Ilhabela, state of São Paulo. Locations: (1) TEBAR; (2) Itaçucê Island; (3) Apara Island; (4) Prainha Island; (5) Figueira Island; (6) Codó Island. Source: Google Earth (2024).

The Pier itself (Figure 1) was constructed from concrete and feature pipelines responsible for fluid transport (known as the Tubovia), facilitating the loading and unloading of materials from ships. In essence, it consists of an extensive access bridge that culminates in a "Y"-shaped bifurcation, dividing into the North Pier and the South Pier. In addition to the Tubovia, the access bridge includes a meteorological station (where sympatric breeding of *S. hirundinacea* and *Thalasseus acuflavidus* has previously been observed [AvesAmar, field observation, unpublished]) and a small garden with vegetation consisting of grasses, small shrubs, and cacti. Platforms 1 through 3 (referred to "loopings"), a pump platform, and two tugboat piers (North and South) are also part of the structure (Figure 1). As a whole, the Pier consists primarily of concrete and iron surfaces, with minimal vegetation only in the area of the meteorological station.

Monitoring of the species' presence at the site was conducted from April to September 2021, with field monitoring occurring from May to September at 48-72-hour intervals, totaling 200 hours of sampling effort. The field methods used in this study followed those used by Scolaro et al. (1996), incorporating certain aspects from Branco (2003), Branco et al. (2010), Fracasso et al. (2010), Fracasso and Branco (2012), and Fracasso et al. (2014).

Due to the large number of nests, the limited access to some of them, and the limited availability of access to TEBAR, a fraction of the colony (57 nests) was selected for continuous monitoring to ensure systematic sampling, based on the study by Scolaro et al. (1996), which was conducted in a natural breeding area in Punta Loma, Argentina. Nests were randomly selected starting from the first sighting record and following a sequence of new records until reaching 57 nests.

The selected nests were enclosed with wooden structures (Figure 3a) and assigned a numerical identification, and the number of eggs laid in each of them was recorded, with each egg appropriately marked using a highlighter pen (Branco et al., 2010) (Figure 3b) according to the laying interval. This identification by interval facilitated the classification of egg types, with the first egg designated as "A," the second as "B," and any replacement eggs resulting from re-laying events as "A2". The interval was also documented for subsequent determination of the time taken for each new egg to be laid after the first. The classification of the eggs into different types (A, B and A2) was done to analyze potential patterns and reproductive behaviors of the species. In addition, it was attempted to calculate the mean interval between the hatching of healthy nestlings from type A and B eggs in broods with more than one egg laid. However, this calculation was not possible because siblings were recorded on the same day in all cases.

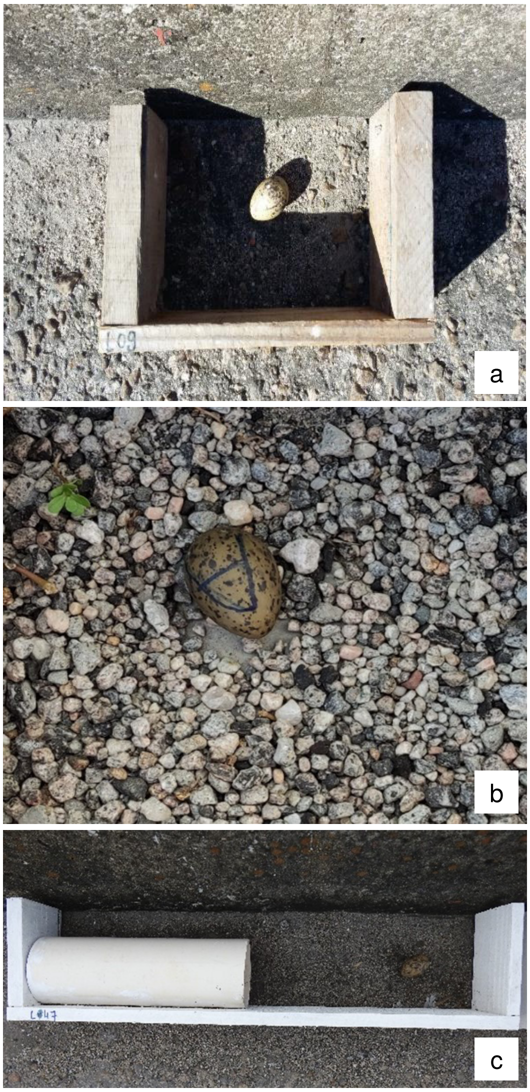

Figure 3. (a) Example of a wooden enclosure used for nest protection (30 x 25 cm). (b) Egg marked with a highlighter pen. (c) Example of a wooden enclosure with a hiding device for nestlings (90 x 20 cm).

During colony visits, eggs showing signs of unviability (determined by the presence of thick liquid oozing from the shell, increased mass, and foul odor), the presence of stillborn individuals, or signs of predation were identified and removed from the colony, making it possible to calculate the number of losses due to predation or other factors (Branco et al., 2010). Incubation success was calculated using the following equation: number of healthy hatchlings born/number of eggs laid (x 100) (Scolaro et al., 1996).

For biometrics, values of total length (Lt) and width (Wid) (both in cm) and mass (Wt) (in grams) were obtained for each individual egg (Branco, 2003). All length and width measurements were made using a 0.02 mm caliper, and mass was assessed using a digital scale with a precision of 1 g. Due to the decrease in egg mass caused by water loss to the environment during incubation, this measurement was taken during each visit when monitoring the 57 nests (Fracasso and Branco, 2012). Egg volume (Vol) was estimated following Hoyt (1979): Vol (cm^3) = $Kv.Lt.Wid^2$, in which $Kv = V/Lt.Wid^2$, and Kv represents the volumetric coefficient; Lt represents the total length (cm); and Wid represents the width of the largest egg axis (cm).

For the nestlings, which are nidifugous, the nests at TEBAR were enclosed and numbered using a pen directly on the enclosure or on the cemented area around it. Some of the enclosures had structures where the nestlings could hide (Figure 3c). In addition to these structures, the nestlings were banded with J-sized leg bands, enabling individual identification. The banding technique followed the instructions provided by Centro Nacional de Pesquisa e Conservação de Aves Silvestres/Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (CEMAVE/IBAMA) (1994).

Nestlings that perished due to natural causes (any circumstance in which the individual was found dead that was not the result of predation or vehicle collision), predation or falls into the sea (individuals that disappeared without a confirmed cause), and vehicle collision (individuals were considered to have died from this cause when found run over on the bridge) were documented and removed from the colony during each visit (Branco, 2003). The cause of death was determined when the deceased bird was found or when its disappearance was noted. Falls into the sea were observed at TEBAR in 2021, when nestlings were startled by human approach and fell off the bridge. This type of event is consistent with a factor previously reported for tern species in the literature (e.g., Sick and Leão, 1965). This approach prevented recounting, made it possible to estimate survival, and facilitated the calculation of reproductive success, which was determined by the number of healthy nestlings/number of eggs laid (x 100) (Scolaro et al., 1996).

The results obtained were organized in spreadsheets and then plotted in graphs using statistical software R (version 4.1.2) (R Core Team, 2014) for better visualization in subsequent analyses. Graphs depicting the overall nesting chronology and laying chronology were constructed cumulatively.

Regarding nestling survival, a graph depicting the frequency of death in relation to age (in days of life) was created. The following estimates were made: (1) highest probability of nestling mortality; (2) period (in days) during which the risk of nestling mortality decreased along the rearing (considering time after birth); (3) critical age (in days) for survival; and (4) age (in days) at which 50% of nestling survival occurred.

For all measures of central tendency, the standard deviation was subsequently presented. When comparing egg biometrics data of different types (length, width, and volume), statistical tests were performed using R software (R Core Team, 2014), with the significance level set at $p < 0.05$. First, the Shapiro-Wilk test was used to check the normality of the data, followed by the Bartlett test to check the homoscedasticity of the data. A parametric analysis of variance (ANOVA) test was then performed to identify possible significant differences between values. Mean contrasts were examined using the Tukey-Kramer test to confirm the presence or absence of sources of variation (Branco, 2003). Student's t-test was used for pairwise comparisons of values.

Because egg mass is reduced daily due to water loss (Fracasso and Branco, 2012), 67 eggs were selected for analysis of this reduction, as they were present in the colony long enough to provide at least three reduction measurements each. The mean value within intervals of 48-72 hours was calculated for each egg and type, resulting in a mean total reduction value and a mean reduction value per egg type. These means were summed by type, and the sum was divided by three $(\bar{X}A + \bar{X}B + \bar{X}A2/3)$ for eggs in general, yielding the overall mean reduction for eggs in this study. Linear regression analysis was used to verify the reduction values by interval and egg type. In addition, using the mean mass values obtained by type and interval throughout the sampling period, the normality of the data was checked using the Shapiro-Wilk test, followed by the Bartlett homoscedasticity test. The ANOVA test was then used to determine if there were significant differences in mean mass values between intervals based on egg type, followed by the Tukey-Kramer test to identify specific differences between egg types. All of the tests mentioned above were performed using R statistical software (R Core Team, 2014).

The rate of egg loss by egg type (Figure S1) was generally calculated as the number of lost eggs in a certain category (A, B, or A2)/number of laid eggs in the same category (A, B, or A2) (x 100). For rates based on the cause of egg loss (Figure S1), this was calculated separately as the number of eggs in a certain category (A, B, or A2) lost due to a particular cause/number of laid eggs in the same category (A, B, or A2) (x 100).

For nestlings, the mortality rate based on the type of egg from which they hatched (Figure S1) was also generally calculated as the number of lost nestlings in that category (A, B, or A2)/number of healthy nestlings in the same category (A, B, or A2) (x 100). For rates based on the cause of nestling loss (Figure S1), this was calculated separately as the number of nestlings in a certain category (A, B, or A2) lost due to a particular cause/number of healthy nestlings in the same category (A, B, or A2) (x 100).

RESULTS

The timeline of events throughout the reproductive season of *S. hirundinacea*, considering the entire colony in 2021, was: Arrival at the Canal (April 8, 2021); First landing at TEBAR (April 12, 2021); First visible nest (April 28, 2021); First visible posture (April 28, 2021); Last visible posture (August 16, 2021); Last record of juveniles (September 20, 2021). The egg-laying period, total reproductive season, and total stay of the species in the Canal lasted for 110, 145 and 165 days, respectively. A total of 159 nests were counted (Figure 4a), containing 318 mature individuals. However, this number is even higher, as a certain number of birds settled in lower or inaccessible parts of the area, making it impossible to calculate the total number of adults in the colony.

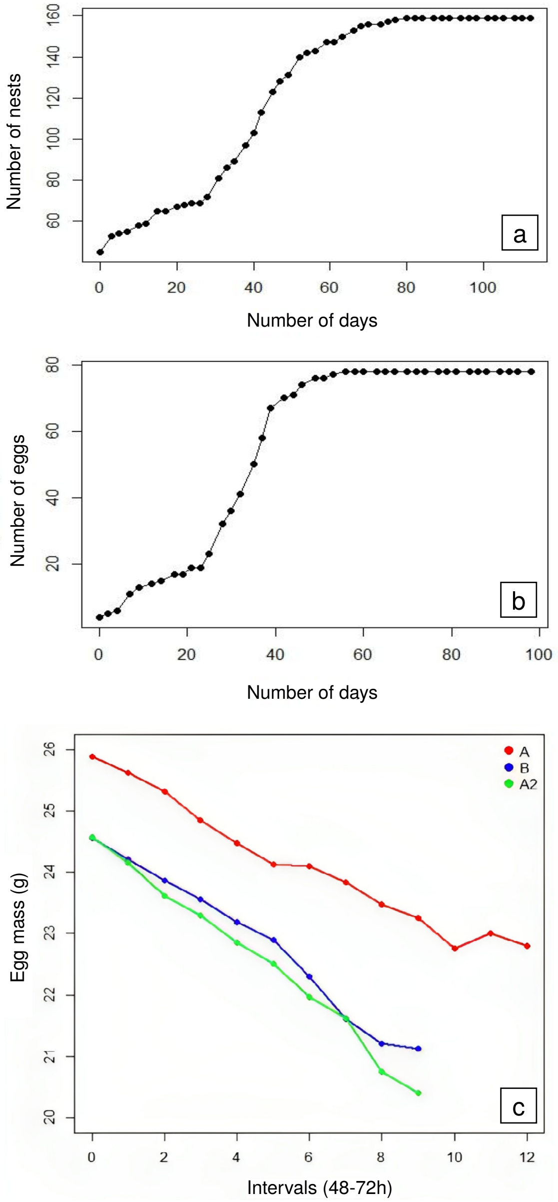

Figure 4. (a) Cumulative nesting timeline during the reproductive season of 2021 at TEBAR, from May 28, 2021 to September 17, 2021. (b) Cumulative laying timeline during the monitoring of 57 nests in the 2021 reproductive season at TEBAR. (c) Reduction in mean mass values for A, B and A2 eggs at 48-72-hour intervals.

Among the 57 nests selected from the colony, the first egg was laid on May 31, 2021, and the last egg was laid on July 26, 2021. Two different events were observed: regular laying (first laying of one or two eggs) and re-laying (second laying after the pair lost the egg(s) from the first laying), which consisted of only one egg. For regular laying, the mean clutch size was calculated to be 1.21 ± 0.41 eggs per nest, and the mean interval between first and second laying in nests with two eggs was 94.00 ± 49.50 hours. Egg laying occurred recurrently within the first 20 days of fieldwork and continued at longer intervals until it ceased after about 60 days, as shown by the asymptote in Figure 4b.

A total of 78 eggs were laid, with 69 (88.5%) being laid during regular laying events, of which 57 (82.6%) were A eggs and 12 (17.4%) were B eggs. Among the regular laying events, 45 nests (78.9%) had only one egg, while only 12 (21.1%) had two eggs. Of the total 57 nests, nine (15.8%) had re-laying events, of which seven (77.8%) contained only one egg lost from the previous regular laying and two (22.2%) contained two eggs lost from the previous laying, resulting in a total of nine A2 eggs (11.5%) laid during re-laying events. Figure S1 shows the relationship between the number of eggs and their type.

The eggs had a mean length of 4.56 ± 0.22 cm, a mean width of 3.26 ± 0.09 cm, and a mean volume of 25.45 ± 2.01 cm^3. Table 1 shows the mean values of length, width, and volume by egg type. Using parametric tests, ANOVA followed by Tukey-Kramer tests revealed that there was no significant difference in length, width, and volume between the three egg types analyzed. However, in the pairwise comparison of the length and volume of egg types A and B (corresponding to regular laying), Student's t-test indicated a significant difference in both length (t = -2.5661, df = 18.314, p-value = 0.01926) and volume (t = 2.2882, df = 21.491, p-value = 0.03235) based on egg type.

Table 1. Mean values of length, width, and volume of eggs by egg type. A (n = 57); B (n = 12); A2 (n = 9).

Egg	Length (cm)	Width (cm)	Volume (cm^3)
A	4.60 ± 0.21	3.27 ± 0.09	25.78 ± 2.03
B	4.45 ± 0.18	3.26 ± 0.07	24.65 ± 1.43
A2	4.50 ± 0.26	3.22 ± 0.09	24.41 ± 2.07

Mass reduction was recorded for 67 eggs at 48-72-hour intervals, including 49 type A eggs, 10 type B eggs, and eight type A2 eggs (Figure 4c), representing a mean water loss of 0.37 ± 0.32 g, 0.39 ± 0.12 g, and 0.41 ± 0.15 g, respectively. The overall mean mass reduction for eggs in general was 0.39 ± 0.02 g. However, by simple linear regression, the mass reduction value per interval was determined to be 0.29 g, 0.39 g and 0.42 g for eggs A, B and A2, respectively.

From the data of mean masses by type and interval, the ANOVA test indicated a significant difference in this factor based on egg type (Df = 2, Sum Sq = 15.88, Mean Sq = 7.939, F value = 5.297, Pr(> F) = 0.0107). The Tukey-Kramer test revealed that the difference was between type A and A2 eggs (diff = -1.916, lwr = -3.243434, upr = -0.5885658, p adj = 0.0036929), with the A-B comparison being nearly significant using this test (diff = -1.266385, lwr = -2.535877, upr = -0.003107538, p adj = 0.0506633). When comparing values in pairs, the result of Student's t-test was: A-B (t = 2.5758, df = 17.742, p-value = 0.01919); A-A2 (t = 2.9037, df = 16.393, p-value = 0.01017); and B-A2 (t = 0.46209, df = 17.774, p-value = 0.6496).

The overall mean incubation period until the hatching of healthy nestlings (excluding stillbirths) was 23.73 ± 2.34 days. Figure S1 shows the mean incubation period by egg type. Overall, 63 of the total eggs laid did not result in the hatching of healthy nestlings, resulting in an overall egg loss rate of 80.8% (Figure 5a). In terms of egg type, 49 (77.8%) type A eggs, seven (11.1%) type B eggs, and seven (11.1%) type A2 eggs were lost (Figure S1). Figure S1 shows the number of eggs lost by cause and their respective rates, both overall and by egg type.

A total of 15 healthy nestlings hatched, eight (53.3%) from type A eggs, five (33.4%) from type B eggs, and only two (13.3%) from type A2 eggs (Figure S1). Based on the hatching of healthy nestlings, the overall incubation success for this study was calculated to be 19.2%. Figure S1 shows the incubation success by egg type.

In general, of the total number of healthy nestlings born, 11 did not survive, resulting in an overall nestling mortality rate of 73.3%. Based on the egg type from which they hatched, five (45.4%)

nestlings from type A eggs, four (36.4%) nestlings from type B eggs, and two (18.2%) nestlings from type A2 eggs were lost (that is, all nestlings born from type A2 eggs died), as shown in Figure 5b. Figure S1 shows the number of nestlings lost by cause and their respective rates, both overall and by egg type. The mean time for a nestling to die was calculated to be 14.27 ± 11.42 days.

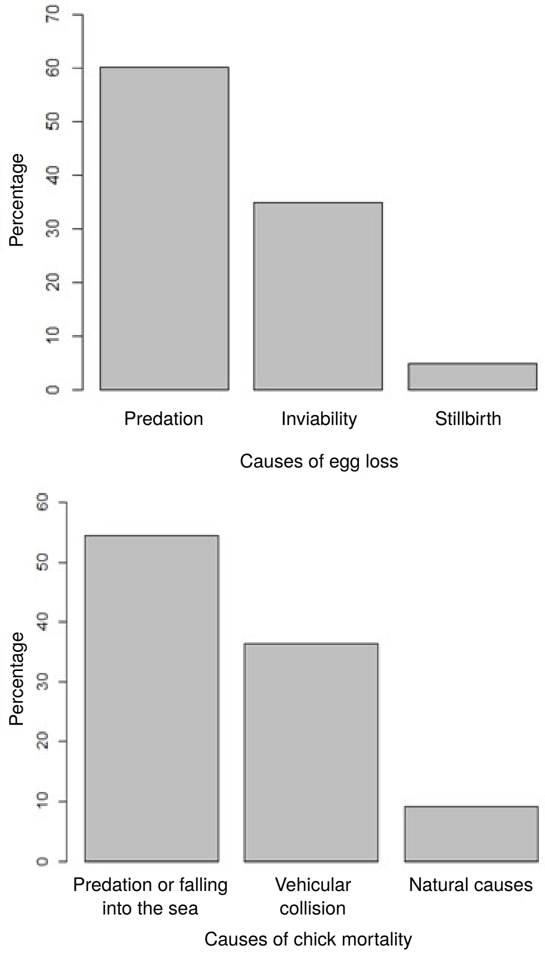

Figure 5. (a) Causes and respective percentages of egg loss. (b) Causes and respective percentages of nestling loss.

Nestling mortality data (Figure 6) revealed that the highest number of deaths (54.5% of all deaths) occurred during the first nine days after hatching. During this period, mortality was primarily caused by predation or fall into the sea (83.3%), characterized by the disappearance of the individual from the colony, and by vehicle collision with cars crossing the bridge (16.7%), characterized by the encounter of the individual run over on the bridge. The risk of nestling death decreased after 10-20 days of life. However, individuals aged 2-4 days were at the highest risk of nestling death (that is, the probability of death was the highest for them), as more than one nestling died on the second and fourth days of life. The risk of mortality increased again when the animals reached 21-30 days of life, at the end of the parental care period. Mortality during this period was caused by vehicle collision (60.0%), predation or falls into the sea (20.0%), and natural causes (20.0%), characterized by the encounter of dead individuals with no signs of predation or vehicle collision. Lastly, the critical age for survival (when at least 75% of the nestlings were still alive) was calculated to be three days, and the median, indicating the survival of at least 50% of the nestlings, was calculated to be 21 days.

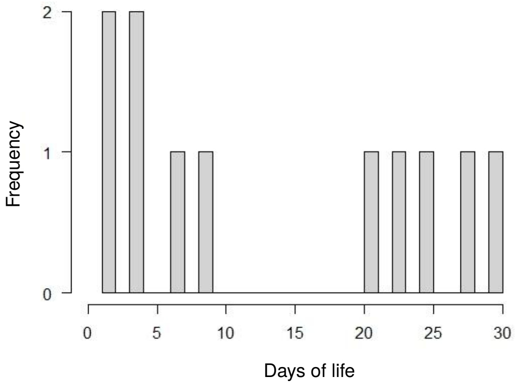

Figure 6. Frequency of nestling death by age (in days of life).

Only four (26.7%) of the 15 nestlings that hatched managed to fly, of which three (75.0%) were born from type A eggs and only one (25.0%) was born from a type B egg (none [0.0%] of the type A2 nestlings managed to fly) (Figure S1). Based on the number of healthy nestlings that successfully fledged, the overall reproductive success for this study was calculated to be 5.1%. Figure S1 shows the reproductive success by egg type. No pair successfully reared two nestlings. The mean breeding season duration for successful pairs was 58.25 ± 4.19 days. Lastly, the mean time for a nestling to fledge was calculated to be 31.75 ± 2.87 days.

DISCUSSION

TEBAR was confirmed to be an artificial site with a substantial population of *S. hirundinacea* individuals using it as a breeding site. Several aspects of the species' reproductive biology observed at this site mirrored findings from previous studies, conducted mainly in natural habitats. Notably, reproductive pairs showed a preference for laying a single egg, and both incubation and reproductive success were observed to be low. In addition, the investigation of the causes of egg and nestling loss highlighted human intervention (e.g. vehicle collision) as a contributing factor to the reduced reproductive success of the species in this artificial environment.

However, TEBAR was found to have much greater establishment potential compared to that observed in Chile by Portflitt-Toro et al. (2018). While only a few pairs were observed on the boats in Coquimbo (no more than 10, according to the number of active nests in the study), 159 breeding pairs were counted at TEBAR, and this number could be even higher due to the presence of inaccessible areas. These data provide an overview of the reproductive dynamics of *S. hirundinacea* in the study area and highlight the importance of considering additional factors when calculating the total population of the species, especially in locations that are hard to reach.

The arrival time of the birds to the São Sebastião Canal and the duration of the breeding season and the stay on the São Paulo coast are in line with what has been described for the species in the literature (Campos et al., 2004; Campos et al., 2007). The laying period occurred throughout the breeding season, from May 31, 2021 to July 26, 2021, with a higher number of type A eggs and few type B eggs, indicating that for breeding pairs, investing in a second egg and rearing a second nestling could be a significant energetic effort, although some studies have observed a higher number of nests with two eggs compared to one egg (Scolaro et al., 1996; Hogan et al., 2010). Reproductive events characterized as re-laying attempts were the last effort of pairs to achieve success in the breeding season after losing the eggs from the first clutch. However, the limited number of pairs that did so suggests that parents do not necessarily choose to make this effort, perhaps to conserve energy for migration at the end of the breeding season. These results provide detailed information on the timing of *S. hirundinacea* reproduction in the study area, showing distinct temporal patterns for different reproductive events. Identifying the intervals between clutches and analyzing clutch distribution over time are essential for understanding the reproductive patterns of this species. Moreover, the presence of an asymptote suggests a saturation point in reproduction, indicating the end of the breeding season and the beginning of the parental care phase (Figure 4b). These findings contribute to the understanding of the reproductive ecology of *S. hirundinacea* and are relevant for the development of effective conservation and management strategies for this species. However, it is important to note that additional analyses and in-depth studies are needed to confirm and refine these conclusions.

Regarding egg type, the higher number of type A eggs can be considered normal, as it has been documented that parents invest more in a single egg than in two, as shown in the study by Fracasso and Branco (2012). Even though there were couples that did not initially lay two eggs, but ended up investing in a second egg via re-laying, the number of type A eggs was higher. These detailed insights into the distribution of eggs in different clutches (original and re-laying) are crucial for understanding the reproductive dynamics of this species. These results are valuable for understanding the reproductive ecology of *S. hirundinacea*, but it is important to note that the data were collected in a single breeding season.

The mean length, width, and volume values found for type A and B eggs were consistent with those described in the literature (Branco, 2003; Fracasso et al., 2010; Hogan et al., 2010). However, in terms of water loss to the environment per interval, values differed between the present study and the study by Fracasso and Branco (2012), possibly due to the fact that daily samplings were performed in the other studies, whereas in this study sampling was performed at 48-72-hour intervals. Since the other studies did not include separate measurements for re-laying eggs, a direct comparison with the values obtained in the present study was not possible.

There were some differences in measurements (length and volume of type A and B eggs, mean mass per interval of type A and B eggs, and type A and A2 eggs) based on egg type. The findings about the length and volume of type A and B eggs are relevant for understanding the variation in *S. hirundinacea* egg characteristics during regular laying. In addition, the lack of significant differences between the three egg types (A, B and A2) indicates an overall homogeneity of the eggs laid by the species. On the other hand, identifying these differences when comparing values between type A and B eggs, which make up regular laying, helps to identify possible factors influencing egg formation and characteristics during the reproductive process.

Although there is previous information on significant differences in *S. hirundinacea* egg biometrics, it mainly refers to the comparison of values at different times during the breeding season(s) and at different nesting sites. The literature provides little detail on the comparison of measurements by egg type, focusing more on comparisons between different periods of the same breeding season or between different nesting sites. However, Fracasso et al. (2010) found that there was no significant difference in the size of type A and B eggs. Furthermore, the difference in mean mass per interval between type A and B eggs in the present study may be due to the greater number of type A eggs. It has been observed that the mean mass per interval is higher for A eggs than for B eggs (Fracasso and Branco, 2012).

The higher total loss of type A eggs may be related to their greater abundance in the colony. However, the total loss was also high for type A2 eggs, suggesting that solitary eggs in nests may be more susceptible to the causes of loss observed in this study. When the causes of egg loss are examined individually, it appears that predation may not be directly associated with egg type, but rather with the presence of predators in the vicinity. This conclusion is supported by the frequent sightings of numerous individuals of *Larus dominicanus* (approximately 30 individuals per visit) at TEBAR. This species is a natural predator of *S. hirundinacea* (Branco, 2003; Branco et al., 2009; Hogan et al., 2010; Fracasso et al., 2011a, b; Fracasso and Branco, 2012; Fracasso et al., 2014).

Type B eggs did not fail to hatch, while type A and A2 eggs did. Although type A2 eggs also failed to hatch, the fact that type B eggs did not fail could be due to their lower number (n = 12), as there is no prior information on different egg types in a colony showing resistance to this phenomenon. The occurrence of stillborn individuals is common in the reproductive colonies of *S. hirundinacea* (Fracasso and Branco, 2012), and most of them occurred in A-type eggs, possibly due to the greater number of these eggs in the monitored nests.

Although more nestlings hatched from type A eggs (possibly due to their greater abundance in the monitored nests), incubation success was higher for type B eggs. However, this may have been due to the lower number of type B eggs, from which a significant proportion hatched; and this event appears to be unrelated to egg type.

The mean incubation period observed for eggs in general is consistent with the results of studies by Branco (2003), Fracasso and Branco (2012), and Hogan et al. (2010); although these authors did not assess different egg types. When comparing the incubation success documented in the 2021 breeding season in this study (19.2%) with other studies conducted in Brazil, it appears to be lower in contrast with the work of Fracasso and Branco (2012) on Cardos Island, Santa Catarina. In that study, significantly higher values were observed in three different breeding seasons (62.7%, 41.1%, and 76.4%, respectively, for each breeding season studied). It is important to note that this research was conducted by the authors in a natural area where factors such as habitat, climate, and prey availability are likely significantly different, and a much larger number of nests and eggs were studied.

Conversely, the incubation success observed for TEBAR was more similar to that reported by Hogan et al. (2010) on Deserta Island, Santa Catarina, where the authors reported 15 nests and 21 eggs. Incubation failure is considered normal and is present in the cited studies. However, exposure to weather conditions and predators at TEBAR may have influenced the low incubation success, as reflected in the different causes of egg loss.

Nestling survival is an important indicator of the reproductive success of a species and can directly impact population conservation. Identifying the main causes of mortality is crucial for developing appropriate conservation and management strategies aimed at protecting nestlings and increasing their survival and, consequently, reproductive success. The higher number of type A nestlings that died could be explained by their greater number compared to other types, as well as the high mortality rate of type A2 nestlings, a rate that refers to the death of the only two individuals born from this type of egg. The causes of death may have been unrelated to the type of egg from which nestlings hatched. However, the natural death of a type B nestling could indicate food scarcity or lack of parental care, as it has been described in the literature that type A nestlings (from the first egg laid in nests with more than one egg) receive more care, attention, and food from parents compared to the second nestling (at least at the beginning of parental care) (Scolaro et al., 1996).

Predation is a consistent factor in nestling mortality (Fracasso and Branco, 2012; Fracasso et al., 2010), often falling into the category of "natural causes" in previous studies. However, it is noteworthy that there is a lack of information in the literature regarding the cause of "vehicle collision," as there are no studies reporting this factor for *S. hirundinacea*. This knowledge gap likely stems from the fact that all previous research was conducted in natural breeding habitats or locations where vehicular collisions are unlikely, as exemplified by the study by Portflitt-Toro et al. (2018). As this was the second most common cause of nestling mortality at TEBAR (due to the proximity of nests and individuals to vehicles at the terminal), it is important to highlight that this factor needs further study to determine its long-term impact on species reproduction at the site.

The increased risk of nestling death at the onset of parental care may be related to the vulnerability of individuals at that moment, which is a common trait in birds and therefore requires extreme parental attention (Barbieri and Delchiaro, 2009; Hickman et al., 2016), making them susceptible to predation or death due to adult neglect (e.g., Scolaro et al., 1996).

The increased risk of death observed at the end of the parental care period may be related to the higher activity level of the nestlings as they moved around the site and began their attempts to fly. This increased their susceptibility to vehicle collisions as they ventured near the vehicles crossing the area and also resulted in some of them falling into the sea (some nestlings from the colony that were not part of the 57 monitored nests were observed to be startled by human movement and fall towards the water).

As some of the nests were surrounded by tubular structures to help keep the nestlings inside and facilitate their identification, it was observed that the presence of these structures may not have affected the results for survival or mortality of the individuals, since among those that died, five belonged to nests with tubes and six belonged to nests without tubes, and among those that survived until fledging, only one belonged to a nest with a tube and three belonged to nests without tubes. In addition, it is not possible to affirm that the presence of these structures protected the nestlings from the causes of death observed in this study or that they were an effective safety measure, and more studies on their use are needed to determine whether or not they can serve as an effective measure.

Although a greater number of type A nestlings successfully fledged than type B nestlings, the greater reproductive success of the second type could be related to the fact that many more type A eggs were laid and a very low number of nestlings hatched and fledged from them, which was not observed for nestlings from type B eggs, for which the opposite occurred.

The reproductive success obtained at TEBAR (5.1%) was lower than that observed in studies conducted in different locations, and some values were placed in contrast with those recorded by Scolaro et al. (1996) in Figure S2. However, the closest value—6.0%—was reported by Hogan et al. (2010), who observed 15 nests and 17 fledged nestlings. The reproductive success values presented by Fracasso and Branco (2012) were higher (35.9%, 50.9%, and 53.5%, depending on the reproductive season studied). It is important to emphasize that the monitoring in the mentioned study included a much larger number of nests.

Anthropogenic factors leading to habitat loss, as evidenced by the human disturbance observed at the colony, were particularly prevalent in the present study. Strategies need to be developed to mitigate and prevent such impacts in *S. hirundinacea* breeding areas. Management and conservation plans should incorporate these considerations to ensure the long-term viability of the species in the region. Although our results provide valuable insights into the reproductive ecology of *S. hirundinacea*, further studies are urgently needed to explore the differences observed in the artificial site more comprehensively.

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AUTHOR CONTRIBUTIONS

L.C.M.F.; E.B.: Supervision; Resources; Project Administration; Funding Acquisition Conceptualization; Investigation; Methodology; Software; Formal Analysis; Writing – original draft; Writing – review & editing.

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