



Presence and potential impact of anthropogenic nesting materials on a colonial breeding waterbird

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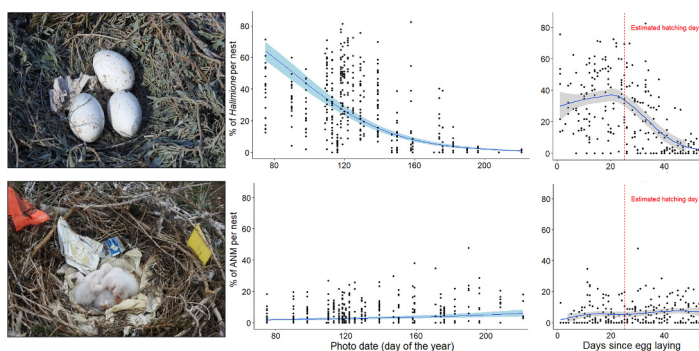
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HIGHLIGHTS

- We quantified anthropogenic nesting materials (ANM) in spoonbill nests.
- Photos balanced precision & disturbance in assessing ANM in the nest lining.
- ANM are part of the spoonbill's nest lining and are mainly plastic sheets.
- The proportion of ANM increased throughout the season.
- No link was found between ANM proportion and breeder age or hatching success.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the vital importance of wetlands globally, these habitats have increasingly received anthropogenic materials, such as plastics, which can impact the wildlife these habitats support. Despite commonly found in the nests of Eurasian spoonbill (*Platalea leucorodia*), the presence of such materials has never been quantified. Here, we monitored the occurrence of anthropogenic nesting materials (ANM) in spoonbill nests in the Camargue wetland in Southern France during two breeding seasons (2021–2022). We also investigated their potential function by associating their presence with breeder experience (based on their age) and hatching success. Out of 439 nests, 39 % contained at least one anthropogenic material item, usually plastic of sheet-like shape and white-transparent colours. Throughout the breeding season, the proportion of ANM in the nest lining increased, coinciding with a decrease in natural materials (i.e. vegetation). A higher proportion of natural green materials was detected during the egg phase compared to other phases of nest development. Our results did not support a correlation between the proportion of ANM and breeder experience, nor with hatching success. Further studies (e.g., physiological through necropsy or fine temperature nest assessment) may disentangle the benefits and adverse effects of such materials on waterbird breeding success.

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1. Introduction

The importance of wetlands for biodiversity and the value of the ecosystem services they provide are recognized worldwide (Millennium Ecosystem Assessment, 2005; Corcoran, 2010; Keddy, 2010). Nonetheless, it is estimated that 87 % of the world's wetlands were lost since 1700, and that many of the remaining wetlands are either degraded or currently under severe anthropogenic pressure (Davidson, 2014; Gardner and Finlayson, 2018). The increasing influx of anthropogenic materials into the general environment, in combination with their slow rate of degradation, is an important threat for natural ecosystems worldwide (Galgani et al., 2013; GESAMP, 2015), including wetlands. Plastic pollution in particular has become such a challenge that the United Nations Environment Programme (UNEP) has classified it as a critical environmental issue (UNEP, 2011, 2014). However, despite global alerts and efforts to reduce societal dependency on (single use) plastics and to remove them from the environment (Azevedo-Santos et al., 2021; Jain, 2017; Kirk and Popattanachai, 2018; UNEP, 2016), their spread and abundance in the natural world continues to increase (Borrelle et al., 2020; Geyer et al., 2017; Plastic, 2020; Wagner and Lambert, 2018).

Anthropogenic materials are solid, human-made objects (Barnes et al., 2009) of different types (e.g., fibre, metal, paper, plastic, rope, and rubber). Many of these materials are often incorrectly disposed and subsequently find their way into the natural environment (Barnes et al., 2009; Eriksen et al., 2014; UNEP, 2016). Plastics are the most common type of anthropogenic debris in the world (Browne et al., 2015), and can be further categorised according to their original purpose into industrial plastics (e.g., nurdles) and user plastics (e.g., sheet-plastics and threadlike plastics) (Franeker and Meijboom, 2007; Provencher et al., 2017). Consequently, interactions between wildlife and anthropogenic materials have been increasingly reported (Azevedo-Santos et al., 2021; English et al., 2015; Hong et al., 2013; Townsend and Barker, 2014). For example, anthropogenic materials have been found in the digestive tract of an increasing number of marine bird species (e.g., Charadriiformes, Pelecaniformes, and Procellariiformes), mammals (e.g., Mysticeti, Otariidae, and Sirenia) and turtles (Kühn and van Franeker, 2020; Laist, 1997; Thiel et al., 2011), and in some cases, they have been shown to influence some species' development and behaviour (Connors and Smith, 1982; Derraik, 2002; Gregory, 2009). Additionally, an increasing number of reports indicate that chemical components (e.g., polychlorinated biphenyls - PCBs) in anthropogenic materials can have long-term effects on animal physiology (Derraik, 2002; Faure et al., 2015; Kühn and van Franeker, 2020; Laist, 1997). For instance, exposure to biphenyls in birds can lead to reproductive disorders, increased risk of disease or mortality, and altered hormone levels (DeLeon et al., 2020; Kuo et al., 2022; Tanaka et al., 2015).

The presence of artificial materials in birds' nests has been increasingly reported across several avian groups (Gil-Delgado et al., 2017; Jagiello et al., 2023; Luna et al., 2022; Townsend and Barker, 2014). Nests are comprised of four main sections: attachment (composed by materials responsible for the attachment of the nest to its substrate), outer layer (decorative), structural layer (support of the structure), and lining (to protect the eggs) (Hansell, 2000). The materials that usually make up the shape of the nest and provide its structural support are considered as structural materials, whereas the lining materials are typically associated with the creation of a suitable microclimate within the nest (Hansell, 2000, 2005). The use of anthropogenic nesting materials (ANM), both for structural and lining purposes, can be associated with the scarcity or absence of natural materials in the vicinity of nesting sites (Antczak et al., 2010; Hiemstra et al., 2021; Reynolds et al., 2019; Witteveen et al., 2017). This suggests a potential trade-off between the time and energy spent searching for natural materials and the collection of more readily available and easily found ANM (Antczak et al., 2010). For example, in South Korea, 71 % of the nests in a colony of black-faced spoonbill (*Platalea minor*) were reported to contain some type of plastic material in 2010 (Lee et al., 2015). However, when natural materials

were provided at nesting sites, the proportion of nests containing plastic materials halved across the subsequent years (2011–37 %; 2012–33 %) (Lee et al., 2015). The presence of specific ANM could also be linked to the similarity with the shape of natural materials used to form and line the nests. The brown noddy (*Anous stolidus*), mostly collects rope and rope-like materials to build its nests, potentially due to the similarity between these materials and elongated fragments of natural materials such as twigs (Ryan, 2020).

Nest characteristics have a key role in determining breeding success (Mainwaring et al., 2014) and the presence of ANM (e.g., fishing nets and gears) is known to increase nestling mortality due to entanglement or ingestion (Lavers et al., 2014; Luna et al., 2018; Ryan, 2018; Seacor et al., 2014; Votier et al., 2011). Furthermore, the proportion and type of ANM could potentially compromise its functional performance (e.g., camouflage, structural integrity, and thermoregulation), which could in turn, impact fitness-related traits (e.g., hatching, or fledging success; Deeming and Reynolds, 2015; Thompson et al., 2020). Nevertheless, some authors have proposed that ANM may be favoured over natural materials in certain contexts (Briggs et al., 2023; Jagiello et al., 2023). As different types of ANM contain very specific properties, these could potentially benefit nests of different species, specifically: i) durability and malleability - for example, the use of artificial plants by the common coot (*Fulica atra*) is thought to strengthen their nests (Hiemstra et al., 2021). While the use of plastic by the great grey shrike (*Lanius excubitor*) was shown to play an important role in strengthening nest structures, particularly during harsh early spring weather, providing additional protection for eggs and nestlings (Antczak et al., 2010); ii) chemical components - discarded cigarette butts retain substantial amounts of nicotine and other compounds, which may act as ectoparasite repellents (Suárez-Rodríguez et al., 2013). Cigarette butts are actively selected by the house finch (*Haemorrhous mexicanus*) (Suárez-Rodríguez and Macías García, 2017) and were seen to improve breeding success, despite the potential for long-term toxic damage (Suárez-Rodríguez and Macías García, 2014); iii) visibility/mate attraction - black kites (*Milvus migrans*) use plastic in their nest structure, probably to signal nest quality (Sergio et al., 2011); iv) availability - the wider availability of ANM in the nesting area might reduce search costs for nest materials and increase the speed of nest building (Antczak et al., 2010). This is a notable example of urban areas, where the availability of anthropogenic materials is increasing while the availability of natural materials is decreasing (Corrales-Moya et al., 2021).

Despite their crucial role as nesting sites for numerous species, wetlands are currently facing significant anthropogenic pressures, including pollution (Barnes et al., 2009; Gardner and Finlayson, 2018). Anthropogenic materials can enter coastal wetlands (such as Camargue, Southern France) through direct input from surrounding urban settlements or via influx from waves, tides and rivers that collect such materials along their course (Geyer et al., 2017; Koehler et al., 2015; Lebreton et al., 2017). The Camargue is an important nesting site for several waterbird species of high conservation value and consists of a mosaic of different natural and anthropogenic habitats (Fraixedas et al., 2019; Galewski and Devictor, 2016). Therefore, the Camargue provides an opportunity to assess the impact of anthropogenic pressures on the presence and type of ANM in waterbird nests. The Eurasian spoonbill (*Platalea leucorodia*, hereafter spoonbill) is a waterbird species that regularly breeds in Camargue, where it forms large colonies (Champagnon and Kralj, 2023). Furthermore, its relatively long breeding season (i.e., from February to early August) and accessible colonies make it an ideal candidate to explore the occurrence and possible function of ANM in waterbird nests. A total of four different islets in Camargue were sampled and monitored to: i) determine the presence and type of ANM in spoonbill nests; ii) explore the use of ANM as a substitute or complement to natural materials and how this varies in relation to season, breeding phase, age of breeders, and varying distance to main human settlements; and iii) assess the relationship between ANM and hatching success. We predict that due to the likely decrease in

the availability and quality of natural nesting materials (e.g., due to the flowering period and vegetation dryness), the number of ANM will increase throughout the season. Furthermore, we predict that nests closer to settlements will have a higher abundance of ANM. During the incubation period, we predict that there will be a higher amount of green lining materials, which are likely to improve nest (and thus egg) temperature regulation (Igc et al., 2009; Wimberger, 1984) and potentially increase the hatching success.

2. Material and methods

2.1. Study population and site

The spoonbill is a colonial waterbird that has recovered from severe population declines during the second half of the 20th century due to intensive recovery programmes, increased or improved nature conservation legislation, bans on the use of pesticides (i.e., organochlorine) and habitat restoration (Champagnon et al., 2019; Overdijk, 2013; Triplet et al., 2008). The species is currently distributed from the East-Atlantic Coast to Southeast Asia (Triplet et al., 2008) and is listed as Least Concern (LC) on the European Red List (BirdLife International, 2019). Yet, it is still threatened by habitat degradation and changes in land use on breeding and non-breeding sites (Jocelyn Champagnon and Kralj, 2023; Mikuska et al., 2023; OTEDD, 2016). Spoonbills breed in wetland areas, either on the ground, on islands or dikes, in reed beds, or in trees, with nearby access to shallow open water or salt ponds (Hancock et al., 2010; Svensson et al., 2023). They construct their nest using branches and stems, lining it with grass and leaves (Isenmann et al., 2010; Matheu et al., 2020), building a new nest each year. In Camargue, they breed on the ground in small islets with abundant shrub and herb vegetation (Kayser et al., 2008). As with other bird species (Peralta-Sanchez et al., 2010; Wimberger, 1984), spoonbills tend to replenish their nests with new material throughout the season, particularly during the incubation period.

During the breeding seasons of 2021 and 2022, we conducted fieldwork at nine different colonies across four different islets of Camargue, with distances ranging from 2 to 29 km between each islet. Seven of the nine colonies were situated on the islets Banaston (41 to

121 nests) and Besson (102 to 236 nests) in the Sensitive Natural Area of the “Etang des Impériaux” (N43°28, E4°28, Fig. 1), close to Saintes-Maries-de-la-Mer (ca. 4–5 km), a small touristic coastal town of >2000 inhabitants, which is highly visited during spring and summer months (Badone, 2008). The two remaining colonies were in Galère (16 nests in 2021) and Vaisseau (14 nests in 2022), which are located within a privately managed area of operational saltworks (N43°22, E4°39, Fig. 1), at a greater distance from Salin-de-Giraud (ca. 7–8 km), another urban settlement of approximately 2000 inhabitants. The colonies on the saltwork islets were considerably smaller (fewer nests) than those in Impériaux (Table A.1). Anthropogenic activities (e.g., agriculture, fishing, hunting, saltworks, and tourism) may result in the introduction of anthropogenic materials into the environment in both areas.

These islets are in close proximity to, or covered by, a vast area of saltmarshes which encompass a total area of 10,000 ha in Camargue (Blondel et al., 2013; Marnotte and Carrara, 2006; Médail, 2018). The main vegetation types identified in the monitored nests were consistent with the dominant perennial plant species present in the area: *Halimione portulacoides* (hereafter *Halimione*); *Sarcocornia fruticosa* (hereafter *Sarcocornia*); and *Suaeda vera* (hereafter *Suaeda*). However, a comprehensive identification of all the vegetal material was not carried out.

Since 2008, the population of spoonbills in Camargue has been subject to a long-term monitoring programme by the Tour du Valat (Blanchon et al., 2010). As part of this monitoring program, ringing of chicks with a white Darvic ring with a unique black alphanumeric identification code was carried out annually allowing for individual identification from a distance. It allowed understanding recruitment process, distribution of individuals during non-breeding period and explore the impacts of non-breeding dispersal on survival (Blanchon et al., 2019; Champagnon et al., 2021; Ferreira et al., 2024b). Individual marking, also enable the determination of individual age (hereafter considered as a proxy for their experience) when a marked individual has been observed breeding.

2.2. Proportion of nests containing at least one anthropogenic item

During early egg incubation (i.e., approximately two weeks after egg laying to limit disturbance and avoid colony abandonment), 439 nests

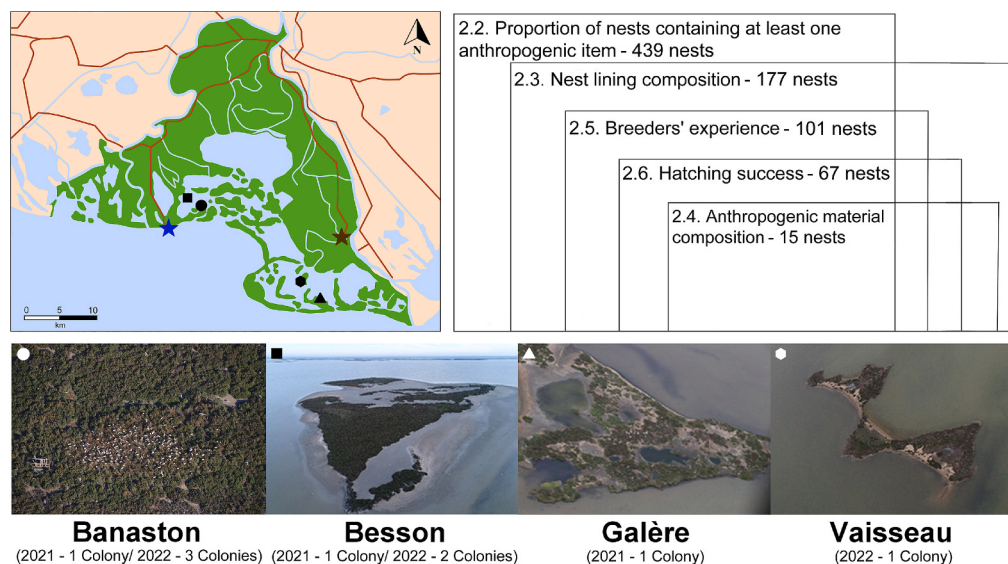


Fig. 1. Map of Camargue area in green and location of the four different islets where spoonbill nests were sampled. At Etang des Impériaux: Islet of Banaston (circular shape; ©Yves Kayser / Tour du Valat) and Islet of Besson (square shape; ©Antoine Arnaud / Tour du Valat). In the saltworks area: Islet of Galère (hexagonal shape; ©Yves Kayser / Tour du Valat) and Islet of Vaisseau (triangle shape; ©Yves Kayser / Tour du Valat). Blue star – Saintes-Maries-de-la-Mer; Black star – Salin-de-Giraud. Rectangular schematic inset represents the different surveys conducted in this study and total number of nests sampled is described per survey. Rectangles that are not contained within the taller ones include nests that were not considered in the other surveys (e.g., all nests in 2.6 were also surveyed for 2.3, but only some nests of 2.6 were also surveyed for 2.5 and 2.4).

distributed across seven out of the nine monitored colonies (Table A.1) were visually examined to determine the presence of at least one anthropogenic item in the nest lining. Spoonbills can display highly asynchronous breeding and there may be months between earliest and latest laying dates in the same breeding site (Aguilera, 1990). In Camargue, the breeding season is quite extended and characterized for having several breeding peaks (within the same islet or in other islets), allowing to separate the different peaks spatially and temporarily into distinctive cohorts (henceforward, colonies). Therefore, the seven colonies monitored for this assessment were surveyed at different times during the season (Table A.1). Only a subset of nests from each colony were considered (for further details regarding colony size, type of survey and survey period, refer to Table A.1). To avoid disturbance at the breeding site, this survey was conducted as swiftly as possible and only once during the initial incubation period of each colony. Due to logistical issues, it was not possible to conduct this survey in two of the nine monitored colonies.

2.3. Nest lining composition

To assess the type of materials (both anthropogenic and natural) used in nest lining in different colonies throughout the season, we randomly selected nests and monitored them until the maximum of 55 days have passed since estimated laying date as it is unlikely chicks are still using the nest. For this aim, we adopted the methodology of Ryan (2020) by photographing the same nests multiple times across the season as described above. Total number of nests monitored by colony was limited by the time available on each colony visit (ca. 40 min) to keep disturbance as low as possible. Furthermore, if fewer than 20 nests were present during the first visit to a colony, when logistically feasible, all available nests were monitored (Table A.1). For colonies with >20 nests at the first visit, 20 nests were randomly selected, ensuring a similar distribution between the nests located in the centre and edges of the colony. Two or three nests adjacent to the initially randomly selected nests were chosen to facilitate the identification of breeders by camera trap monitoring. Total number of photos taken per nest varied according to nest failure (mainly due to predation), nests not having a subsequent visit, limited time at the colony, marking flags being lost, and colony size. Success in following nests since incubation or not, also influenced the number of nests considered in the remaining surveys.

To field test the protocol and due to other surveys being conducted at the same time, the first colony surveyed (Besson 2021) had considerably more nests sampled ($n_{\text{nests}} = 71$) compared to other colonies (Table A.1). On each visit, nests were vertically photographed twice (two replicates) at a height of approximately one metre above the nest, resulting in a total of 870 photos from 177 different nests (Banaston: $n_{\text{nests}} = 54$, $n_{\text{photos}} = 360$; Besson: $n_{\text{nests}} = 99$, $n_{\text{photos}} = 382$; Galère: $n_{\text{nests}} = 14$, $n_{\text{photos}} = 54$; Vaisseau: $n_{\text{nests}} = 10$, $n_{\text{photos}} = 74$), which were individually marked with two small flags. The nests monitored in this survey were not necessarily included in the previous “Proportion of nests containing at least one anthropogenic item” survey. Subsequently, each photo was analysed using the point-count CPc 4.1 software (Coral Point Count with Excel extensions; Kohler and Gill, 2006), which randomly generates sample points within the limitation of the nest lining defined by the user. This sampling method developed by Dumas et al. (2009) and adapted by Thompson et al. (2020), involves the delineation of a square border along the outer edge of the nest photo, targeting the nest lining by incorporating the nest cup and a part of the outer edge. Thus ensuring, an accurate representation of the lining. The space within the created border was converted to a 3×3 squares grid, and six points were generated in each grid square, resulting in 54 random points per photo. We performed an exploratory analysis to determine the appropriate number of random points required to accurately reflect the proportion of anthropogenic and natural materials in the nest lining. In short, 15 photos were randomly selected, and total point sample sizes of 27, 54, and 72, were randomly generated on the selected photos and the

material under these points classified. The classification was based on colour, shape, and consistency, with materials divided into two major categories (Galgani et al., 2013; Provencher et al., 2017): natural materials (feathers, *Sarcocornia*, *Halimione*, *Suaeda*, and other plant material) and ANM (marking flags, papers, user plastics, ropes or strings, and others). Based on this preliminary analysis, 54 points were determined to be a reasonable compromise between processing time and accurate representation of the materials present in the nest lining. When increased to 72 points, the proportion of ANM in the nest lining did not change substantially. Points corresponding to other materials (chicks, eggs, nest identification flags, and unknown elements) were subtracted from the total, as these are not lining materials and could skew the surface density estimate of all other materials of interest. For each photo, the percentage of anthropogenic and natural materials of each type was calculated. The frequency of occurrence (FO) of each material across both nest replicas was then averaged and each unique nest/visit was assigned a single mean value of FO for each type of material. This was done to avoid replication, account for variation across both replicates, and obtain a more reliable representation of the nest lining (Thompson et al., 2020).

Contrary to the *Sarcocornia* and *Suaeda* plant, which possess small leaves and are likely used as structural components of the nest due to their elongated shape, *Halimione* contains lanceolate or linear-lanceolate leaves (Lamare and Pavon, 2021; Marnotte and Carrara, 2006; Thomas et al., 2018) that are likely used as lining material. In fact, *Halimione* fragments appear to be placed at the centre of the nest during the incubation period (Fig. A.1), suggesting a thermoregulation role within the nest. Therefore, and due to scarcity of the remaining vegetal materials in the nest lining, *Halimione* was identified as the main natural lining material.

2.4. Anthropogenic material composition

To assess if photos are a true representation of the use of anthropogenic material in the nest, and to quantify the frequency of occurrence (FO) and the type of ANM composing the nest structure, 15 nests (out of 177) were randomly selected from the islets of *Impériaux* (2021: $n_{\text{Besson}} = 2$; $n_{\text{Banaston}_2} = 7$; 2022: $n_{\text{Besson}_2} = 5$) to be weighed and deconstructed (Table A.2). To avoid any disturbance to the colonies, this survey was carried out once juveniles had left the islets and no active nests were present. The nests were collected, placed in a bag, and weighed at site using a dynamometer (± 25 g). All the ANM were then separated from the natural ones (e.g., branches, leaves, and feathers) in situ and collected for examination at the laboratory. In the laboratory, the total mass of the ANM for each nest was measured (± 1 g) and each material was characterized and classified based on Galgani et al. (2013) and Provencher et al. (2017) classifications, taking into account the colour, type of material, and shape of the material ($n_{\text{materials}} = 423$).

2.5. Breeders' experience

Since 2008, >3000 spoonbill chicks have been ringed with an engraved PVC ring allowing us to individually identify marked birds and to discern their age (ranging from four years old to 14). Camera traps which took photos every ten minutes, were deployed in several nests, and regularly rotated to associate marked breeders with their nests. In total we were able to identify 121 marked breeders associated with 101 (out of 177) photographed nests. Some marked birds were associated with multiple nests ($n_{\text{ind}} = 13$), and in 34 nests both breeders were marked.

2.6. Hatching success

To test whether the presence of ANM was correlated with hatching success, the number of eggs that hatched successfully or not was recorded for each nest. Due to the disappearance of nest identification

flags, predation by Eurasian wild boar (*Sus scrofa*) or too long period between visits (i.e., when the number of eggs and chicks in the nest was less than the number of eggs counted during the previous visit, which prevented us from distinguishing between the disappearance of eggs and the death of successfully hatched chicks), hatching success could not be determined for 114 of the 177 nests photographed. These were therefore discarded, leaving a total of 63 nests for this analysis.

2.7. Statistical analysis

We used generalized linear mixed-effects model (GLMM; package: *lme4*, Bates et al., 2015) with a binomial error structure and a logit link function, in an information-theoretic model selection framework (Burnham and Anderson, 2003) to determine the relationship between nest lining and the variables of interest: spatial (site); temporal (breeder age, nest development phase, season, and year). Two different response variables were considered: i) ANM, how many points in each photo were classified as ANM and how many were not; ii) *Halimione*, how many points in each photo were classified as *Halimione* and how many were not.

To explore the usage of ANM as a substitute to natural materials, seasonality and spatial distribution were considered. Despite the dominant perennial plant species of the area being available all year round (pers. obs.; Aellen, 1938; Marnotte and Carrara, 2006), we considered seasonal variability because of the dryness of the area, which could affect the quality of the vegetation (e.g., thermoregulation potential or colour, spoonbills appear to collect mainly green vegetation). Furthermore, due to the flourishing of *Halimione*, from June to October (Aellen, 1938; Marnotte and Carrara, 2006), the detectability (i.e., during our photo assessment, no *Halimione* flowers were detected) and quality of this plant could also change. Thus, to test for a seasonal effect due to a possible diminishing attractiveness of *Halimione* and an increase availability of anthropogenic debris due to the rise in tourism activity, Julian date was scaled and considered as a continuous fixed effect (*Day* – Julian day). Due to the proximity of some islets to highly touristic areas (Banaston and Besson) and the potential differences in material availability in the immediate surrounding environment, *Site* was considered as a four-level fixed effect (Banaston, Besson, Galère and Vaisseau).

To test whether ANM and *Halimione* could play a role in nest thermoregulation, we considered the development phase of the nest (*Phase*) as a fixed effect of two levels, according to the state of the nest at the time of monitoring (egg – from laying of the first egg until at least one egg hatched vs. chick – at least one chick in the nest until a maximum of 55 days since estimated nest laying date, when its unlikely nests are still being used). Finally, *Year* was considered as a fixed effect of two levels (2021 and 2022), due to possible differences in abiotic conditions and materials availability between seasons. Nest identity (*Nest*) was included as a random term to account for the same nest being sampled at several moments throughout the season. Thus, we obtained the following equations as the initial model:

$$Y_i \sim B(n_i, \pi_i)$$

$$\text{logit}(\pi_i) = \text{Day}_i + \text{Site}_i + \text{Phase}_i + \text{Year}_i + \text{Nest}_i$$

$$\text{Nest}_i \sim N(0, \sigma_{\text{Nest}}^2)$$

(equation 1: anthropogenic material; equation 2: *Halimione*)

Y_i is the number of samples with ANM (eq. 1) in nest i , n_i is the total number of samples in nest i , and π_i is the probability of anthropogenic lining within nest i . While in equation 2 *Halimione* is considered instead of anthropogenic materials as the response.

The different models developed were ranked and selected according to the Akaike's Information Criterion adjusted for small sample size (AICc) (Anderson and Burnham, 2002). When there were multiple models within two AICc points of the top-ranking model, the model with the fewest parameters was selected (i.e., the most parsimonious model,

Anderson and Burnham, 2002). Once the most parsimonious model was selected, we built a forest plot to represent the odds ratio that each point on the photo corresponds to ANM (or *Halimione*) in response to the different variables. Using the *emmeans* package (Lenth et al., 2024), subsequent pairwise Tukey HSD post-hoc test comparisons were conducted to the most parsimonious model to explore differences between the marginal means of each group. A Sidak correction was applied, which adjusts for the family-wise error rate across all tests and provides a more conservative control for Type I errors (Sidak, 1967).

To test if bird experience affects the type of material lining the nest, age (*Age*, numerical variable) was considered as a continuous variable according to the breeder's age to a subset of nests with known breeder. To account for the fact that multiple observations originate from the same nest or individual, we considered including *Nest* and *Breeder* as random intercepts. However, the inclusion of *Breeder* as a random effect led to singular fit, which indicates an overfitted model, likely due to a small number of individuals with multiple nests ($n_{\text{Breeder}} = 13$), and thus *Breeder* was removed. The linear mixed model containing *Age* as a fixed effect was then compared to a null model accounting for ANM (equation 3) or *Halimione* (equation 4) lining, respectively, through an analyse of variance (ANOVA) test.

$$Y_i \sim B(n_i, \pi_i)$$

$$\text{logit}(\pi_i) = \text{Age}_i + \text{Nest}_i$$

$$\text{Nest}_i \sim N(0, \sigma_{\text{Nest}}^2)$$

(equation 3: anthropogenic material; equation 4: *Halimione*)

To test the potential impact of nest ANM and *Halimione* FO on hatching success, further models were tested. In equation 5, a binomial variable of hatching success was considered as the response variable and an interaction of the FO of both ANM and *Halimione* in the nest lining as the fixed effect. The interaction was included to test if a potential relationship between hatching success and nesting materials was different according to type of material. The same process as for the previous equations was carried out.

$$Y_i \sim B(n_i, \pi_i)$$

$$\text{logit}(\pi_i) = \text{Artificial}_i \times \text{Halimione}_i + \text{Nest}_i$$

$$\text{Nest}_i \sim N(0, \sigma_{\text{Nest}}^2)$$

(equation 5: hatching success) Where Y_i is the number of hatched eggs per nest, n_i is the total number of eggs per nest, and π_i is the probability of hatching for nest i . In a preliminary analysis, the two variables showed limited collinearity $r(433) = -0.23, p < 0.001$, which allowed this equation to be tested.

All analyses were carried out in the software R version 4.2.1 (R Core Team, 2022) and plots were generated using the packages *ggplot2* (Wickham, 2016) and *sjPlot* (Lüdtke et al., 2023). Our linear mixed models were estimated using the ML and Nelder-Mead optimisers via the *glmer* function in the "lme4" package (Bates et al., 2015).

3. Results

3.1. Anthropogenic material presence, characterization, and nest composition

Field-based visual inspections of nest structures (including attachment, outer layers, and lining) across the surveyed sites confirmed the presence of ANM in 39 % of nests from all surveyed colonies. On average, 41 % (± 19 % SD, range 17–69 %) of the monitored nests per colony ($n_{\text{nests}} = 439$) contained at least one anthropogenic item (Fig. A.2), while the point-count assessment of the photos ($n_{\text{nests}} = 177$) revealed that 78 % of the nests had some degree of anthropogenic lining at some point during their use.

When considering the 15 deconstructed nests, the total mass proportion of ANM varied between 0.1 % and 2.8 % (Table A.2), representing a minor proportion of the nest total mass ($FO_{mean\ nest\ mass} = 1.4 \pm 0.87\%$ SD; $n_{nests} = 15$). These materials were distributed across all sections of the nest structure but were most commonly found in the centre of the nest (within the nest structure and in the nest lining). The deconstructed nests were mainly composed of dried vegetation. A total of 423 fragments of ANM were found (Fig. A.3 and A.4). Most of these materials were plastics ($FO_{mean\ plastic} = 56 \pm 18\%$ SD, range 20–88 %, $n_{materials} = 259$) and paper ($FO_{mean\ paper} = 35 \pm 19\%$ SD, range 0–67 %, $n_{materials} = 121$). There was a higher occurrence of transparent ANM ($FO_{mean\ transparent} = 22 \pm 18\%$ SD, range 0–50 %, $n_{materials} = 123$) or a light-coloured shade ($FO_{mean\ white} = 33 \pm 28\%$ SD, range 0–100 %, $n_{materials} = 111$; cream: $FO_{mean\ cream} = 17 \pm 26\%$ SD, range 0–75 %, $n_{materials} = 51$).

Based on the photos analysis, the nest lining was found to contain an average of 6 % ($\pm 8\%$ SD, range 0–48 %) of ANM and 26 % ($\pm 21\%$ SD, range 0–82 %) of *Halimione*. Independently of the nest laying dates, the FO of *Halimione* on the nest lining decreased after around 21 days of incubation, while the FO of ANM remained stable (Fig. 2).

The most parsimonious model (Model 2, Table A.3) explaining the presence of ANM (equation 1) only included a seasonal (Day) effect: the odds of encountering ANM lining the nest increased as the season progressed ($\beta = 0.27$, 95 % CI [0.16, 0.37], $p < 0.001$; Fig. 3). There was limited support for differences between years or between the egg and chick phase (models within 2 AIC), and a lack of support for differences between sites (Table A.3).

When considering *Halimione* lining as the response (equation 2), the model containing all variables was the only model supported ($df = 8$, $AIC_c = 5106.7$, $weight = 1$; Model 1, Table A.4). Contrary to the ANM, the odds of having *Halimione* in the nest lining decreased throughout the season (Fig. 4).

Abbreviations: σ^2 – Population variance; τ_{00} – Variance between nests; ICC – Intraclass correlation coefficient; n_{nests} – number of nests; r^2 – r-squared

There is also a spatial difference in the amount of *Halimione* in the nest lining, with nests on Banaston being more likely to contain *Halimione* compared to the other islets (Fig. 5). As for the nest development phase, nests during the egg phase had a higher probability of having *Halimione* lining than during chick phase, independently of nest laying date.

A pairwise comparison between a null model and a model containing Age (equation 3), showed no significant relationship between breeder's age and amount of anthropogenic material lining the nests [$F(1, n_{Breeder*Nest} = 357) = 0.002$, $p = 0.967$]. Likewise, when considering

halimione lining, no significant relation was detected [$F(1, n_{Breeder*Nest} = 357) = 0.076$, $p = 0.078$].

3.2. Impact in survival and hatching success

There were no recorded cases of mortality events of chicks or adult spoonbills caused by entanglement or ingestion of anthropogenic materials in our study. The proportion of ANM or *Halimione* in the nest lining was not correlated with hatching success (equation 5), as the null model was the most parsimonious model when considering the three models within two AICc (Table A.6, Model 2).

In Table 1 a summary of all the significant variables present in the different parsimonious models are highlighted per equation.

4. Discussion

At least one anthropogenic item was found in 39 % of all spoonbill nests monitored in our study, thereby providing the first assessment of ANM presence for the species. This finding supports our initial prediction of a widespread presence of ANM in spoonbill nests. In Camargue, spoonbills appear to use plastic materials with a sheet-like shape (61 %) and white-transparent colours (68 %) as lining materials. Although it is not clear whether they are used to replace or complement natural materials, an increase in the use of ANM throughout the season coincided with a decrease in the proportion of *Halimione* in the nest lining. There was no significant spatial variation in the proportion of ANM in the nest lining between sites. Nevertheless, significant spatial variation was found when considering *Halimione* lining. Similarly, no differences were found in the proportion of ANM in the nest lining between development phases (chick and egg), but a higher amount of *Halimione* was found in the egg phase. Contrary to our predictions and independently of the material considered, breeder experience did not influence nest lining composition. Finally, our findings suggest that nest lining had no significant effect on hatching success, regardless of the material used.

4.1. Presence and type of ANM

On average, 41 % of nests were found to contain at least one anthropogenic item during visual inspection (Fig. A.2). Nevertheless, detailed examination of photos revealed that 78 % of the nests surveyed (139 out of 177) had some degree of anthropogenic material at some point. The frequency of occurrence of ANM in the nests of different waterbird species and colonies has been reported to range from 4 to 80 % (Battisti, 2020; Hartwig et al., 2007; Tavares et al., 2016; Votier et al., 2011; Witteveen et al., 2017), with higher proportions being linked to

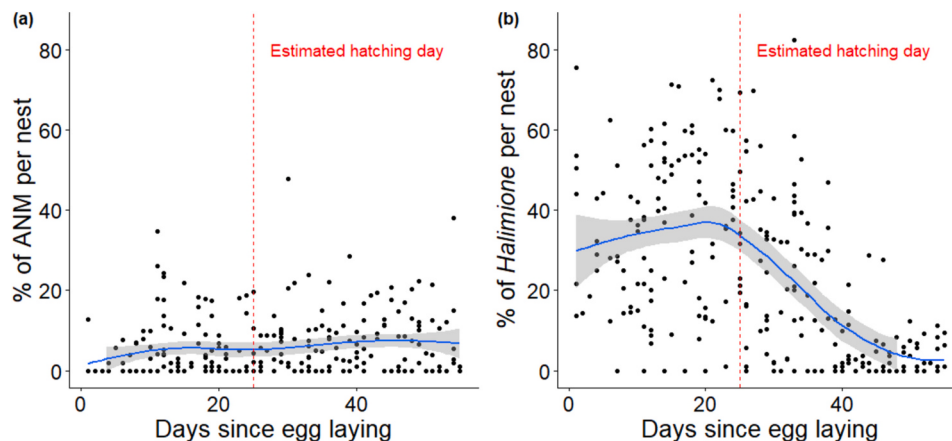


Fig. 2. (a) ANM FO in the nest lining on relation to the nest development phase with 95 % confidence intervals ($n_{photos} = 259$; $n_{nests} = 78$). (b) *Halimione* FO on the nest lining in relation to the Day of the nest with 95 % confidence intervals ($n_{photos} = 259$; $n_{nests} = 78$). The red dotted line indicates 25 days of incubation, the day when usually spoonbills egg hatch. Shaded-grey area represents a confidence interval of 95 %.

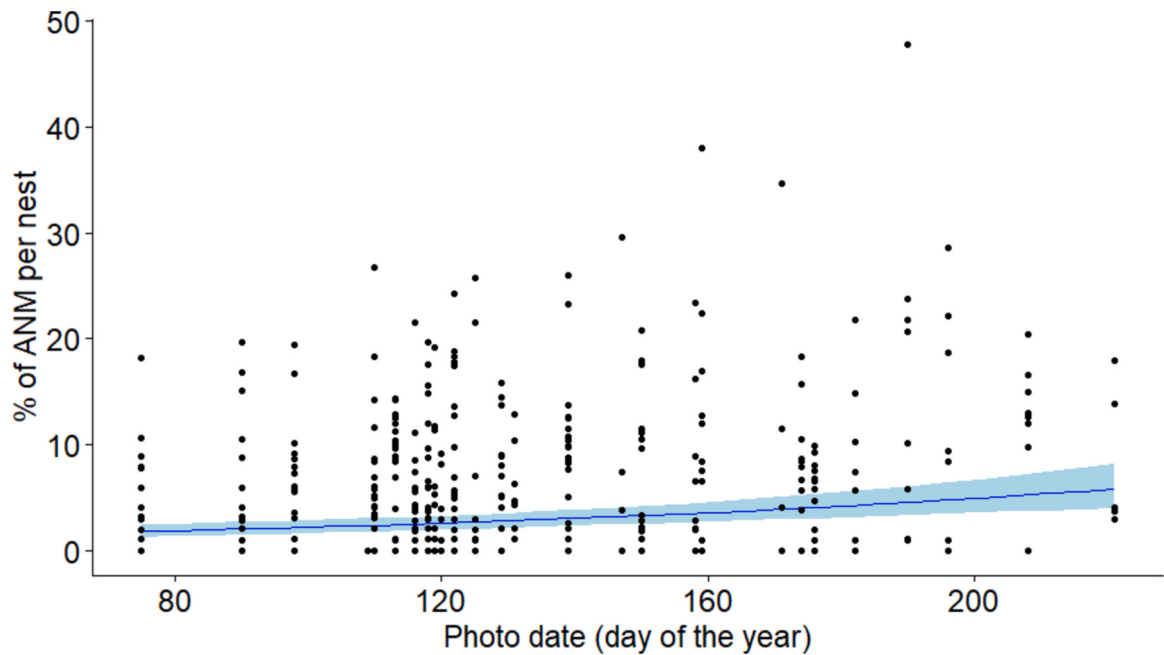


Fig. 3. Predicted variation in anthropogenic material lining over the season according to the most parsimonious model ($n_{photos} = 435$; $n_{nests} = 177$; Table A.3, Model 2). Black dots indicate the actual proportion of anthropogenic material and the shaded-blue area a standard error of ± 1.8 %.

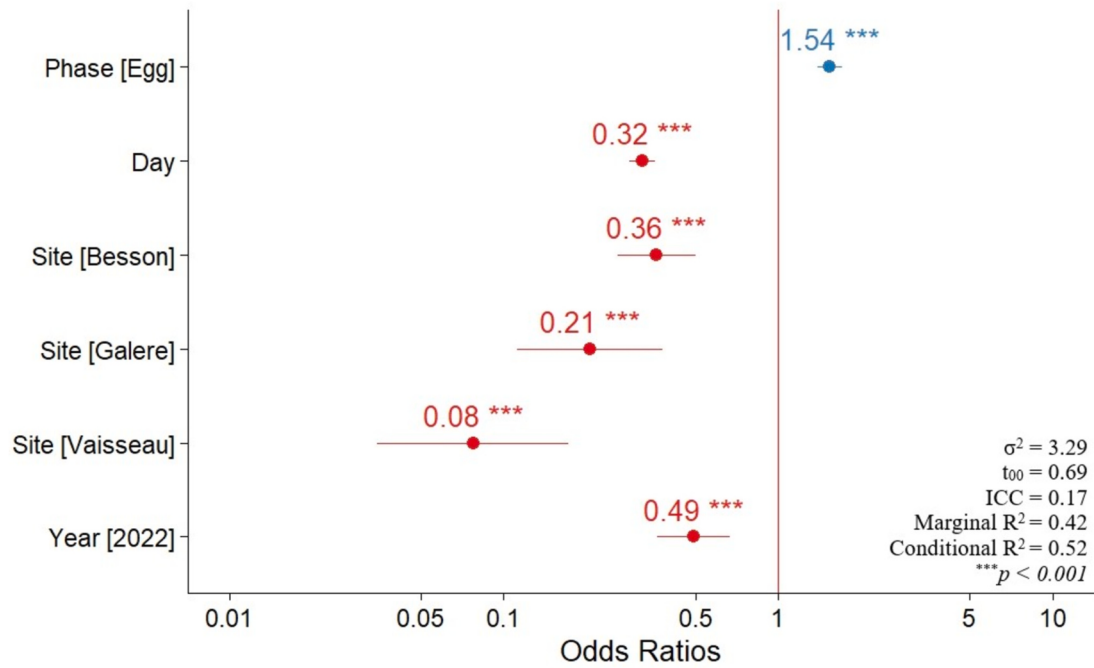


Fig. 4. Odds ratios of a lining material being *Halimione*: as the season progresses (Day); between nest development phase (chick – Reference and egg); site (Banaston – Reference, Besson, Galère and Vaisseau); and year (2021 – Reference and 2022). Red colour represents lower odds of a point being *Halimione* when compared to the reference level, while blue colour represents higher odds. The “neutral” line (red vertical line) indicates no effect when intercepted. Horizontal lines indicate 95 % confidence intervals. For details check Table A.5.

nest reuse over the years and distance from pollution source (Thompson et al., 2020). Contrary to other species such as white stork (*Ciconia ciconia*), spoonbills do not reuse the same nests over the years (Jagiello et al., 2018). However, using materials collected from conspecific nests, including older nests, is possible and a notorious behaviour of Pelecaniformes (Grant et al., 2018; Nelson, 2006). Black-faced spoonbills, which breed on small rocky islands that lack vegetation, were also found to have a high incidence of ANM (71 %), which was halved when natural

materials were provided at nesting sites (Lee et al., 2015). Nevertheless, spoonbills in Camargue are unlikely to experience such a shortage of natural materials. Despite the potential benefits of using photos, the information obtained only reflects the material present at the nest lining, and smaller materials or those present in other nest structures may be missed. Nevertheless, recent studies suggest that the use of photos may be an effective intermediate solution to the precision-disturbance trade-off,

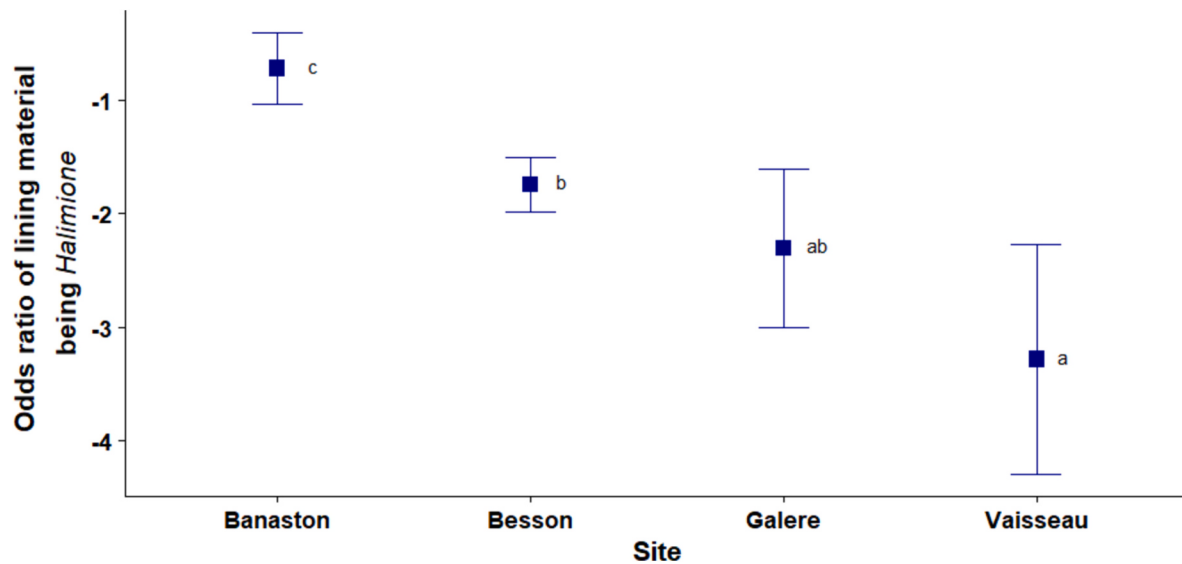


Fig. 5. Post-hoc comparisons of estimated marginal mean of percentage of *Halimione* lining the nests between sites. Vertical lines indicate 95 % confidence, and a Sidak correction was applied. If two or more means share the same grouping symbol, then these are not significantly different ($n_{\text{photos}} = 435$; $n_{\text{nests}} = 177$; Table A.4, Model 1).

Table 1

Summary of supported fixed effects (i.e., present in the most parsimonious model; highlighted in “X” blue bold) for the different fitted GLMMs. If no variable was highlighted, the null model was the most parsimonious model. Variables identified with an “0” were tested but not retained in the final model. Variables identified with an “-” were not included. For details about model selection consult Table A.3, A.4, and A.6.

Equation	Response (probability of)	Age	ANM	Day	<i>Halimione</i>	Phase	Site	Year
1	Anthropogenic lining	-	-	X	-	0	0	0
2	Halimione lining	-	-	X	-	X	X	X
3	Anthropogenic lining	0	-	-	-	-	-	-
4	Halimione lining	0	-	-	-	-	-	-
5	Hatching	-	0	-	0	-	-	-

allowing for a systematic and permanent representation of the nest lining, while considerably reducing disturbance to the colony (Rodríguez et al., 2023; Ryan, 2020). This is supported by our subsample of deconstructed nests, which confirmed that ANM represent only a small proportion of the total nest mass (mean frequency of proportional mass: 1.4 ± 0.2 % SE). Thus, although present in most nests, the mass of anthropogenic material is relatively small. Therefore, it is unlikely that these materials are used as fundamental structural layers of the nest, as they seem to be mainly present in the centre of the nest, incorporated in the nest lining. Additionally, it means that photos of the nest lining are likely to provide a reliable representation of the use of ANM by spoonbills, while avoiding disturbance. Finally, the number of eggs or chicks could obscure some materials in the nest centre and potentially lead to an underestimation of the proportion of ANM present. However, no evidence of such bias was found as there were no clear differences in ANM proportions across nest age, development stage or in relation to hatching success.

Examination of 15 deconstructed nests revealed that most of the anthropogenic material incorporated into the nests corresponded to sheet-like plastic of white-transparent colour (Fig. A.3 and A.4), which are likely to have originated from domestic and agricultural waste. Similar types of material were also found in the nests of other waterbird species, such as white stork (Jagiello et al., 2018), herring gull (*Larus argentatus*, Thompson et al., 2020) and kelp gull (*Larus dominicanus*, Witteveen et al., 2017). Due to their shape and colour, it is unlikely that the plastics used were mistaken for natural materials such as branches and accidentally collected. Furthermore, spoonbills are not known to eat food wrapped in plastic, as some gull species do (Battisti, 2020). In the

Wadden Sea, the loss of a ship container resulted in the contamination of the spoonbill breeding area with small pieces of plastic. However, despite their high availability, no macro plastics (>20–100 mm) were detected in the regurgitates of spoonbills (Foekema et al., 2021). It is therefore unlikely that Camargue’s spoonbills inadvertently collect sheet-like plastics at a higher rate than other ANM. This suggests a potential preference for these materials, but complementary studies focusing on their availability in the area would be needed for testing selection.

4.2. Role of lining materials

Our results indicate that the proportion of ANM in the nest lining increased towards the end of the breeding season (Fig. 3), while the proportion of *Halimione* decreased (Fig. 4). This suggests a replacement of *Halimione* by ANM throughout the season, as was initially hypothesised.

Surprisingly, despite the nests at the *Impériaux* (Besson and Banaston) being closer to a human settlement than the saltwork sites, and presumably having higher availability of ANM, they had the highest proportion of *Halimione* lining (Fig. 5). Furthermore, the significant difference between the islets of Banaston and Besson, which are only two kilometres apart, suggests that spoonbills collect *Halimione* from the immediate vicinity of the colony (i.e. on the islets). In contrast to *Halimione*, the absence of spatial differences in the proportion of ANM in the nest lining, indicates that spoonbills tend to collect anthropogenic materials when building or replenishing their nests, independently of availability in the immediate vicinity of the colony. This behaviour has

previously been documented in great tits (*Parus major*), which appear to actively seek out ANM even when they are uncommon in the area (Hanmer et al., 2017; Surgey et al., 2012). However, the availability of materials in proximity to the breeding sites was not assessed, and thus, an opportunistic source of ANM or an unknown reason for a localised absence of *Halimione* cannot be ruled out. For instance, the progressive increase in anthropogenic lining may be connected to an accumulation of waste in addition to the influx of new materials (i.e., brought to the nest by breeders), rather than a decreasing availability of natural materials. For instance, tomato and melon fields (a growing cultivation practice in Camargue), a likely source of sheet-like plastics, cover 1752 ha of the Camargue land surface area (180,000 ha; Balma, 2023; Galewski and Devictor, 2016). Spoonbills have a wide range of activities (up to 50–60 km from their nesting site Pigniczki et al., 2016; Tenan et al., 2017), including in Camargue (Ferreira et al., 2024a). Thus, it is possible that they collect ANM originating from the many human activities in Camargue (e.g., agriculture, ecotourism, fishing, and settlements) outside of the breeding islets. Similar to spoonbills, a recent study of a gull species (yellow-legged gulls – *Larus michahellis*) with a range similar to that of spoonbills in the Camargue found no significant differences in plastic availability between sites (Almeida et al., 2023), suggesting these materials are equally widespread across the region and readily available anywhere. Finally, despite the uneven sampling across the different islets, the absence of spatial differences in the proportion of ANM lining the nests suggests that this is unlikely to have influenced our results.

Green vegetation materials are included in the nests of several bird species and are considered to play a role in microclimate regulation (Igc et al., 2009; Wimberger, 1984). When plotting the estimated nest development phase (in days since egg laying started), in relation to the proportion of *Halimione* lining, a clear decrease in *Halimione* was observed at around 21 days (Fig. 2). As spoonbills' eggs hatch at around 25–26 days (Lok et al., 2014), this suggests a potential role of *Halimione* leaves in nest thermoregulation during the egg phase (for nests initiated at any time during the breeding season). Indeed, there is higher odds of encountering *Halimione* lining during egg phase than during chick phase (independently of nest laying date). However, this was not the case for sheet-like plastic materials, which have also been suggested, but not proven to have thermoregulatory properties (Thompson et al., 2020). We hypothesise that this could be explained by a fundamental difference between anthropogenic and natural materials. After incubation, it appears that breeders stop replenishing the nest with new material, possibly due to different microclimate requirements (i.e., within the nest) between egg and chick phases, and to reduce their energy costs (Ar and Sidis, 2002; Dawson et al., 2011; Lambrechts et al., 2012; Mainwaring et al., 2014). Consequently, it is possible that vegetation materials present in the nest will rapidly dry out, whereas ANM may remain intact in the nest until removed by wind or other birds (Marchant et al., 1990; Thompson et al., 2020). In addition, unlike other species such as the herring gull, sheet-like plastics were mainly found in the centre of nests, rather than at the periphery, suggesting that this type of material may be more frequently used during the incubation phase. Therefore, it is possible that ANM may have a role in regulating nest temperature, despite the lack of significant differences in anthropogenic lining between nest development phases.

The composition of nest lining does not seem to be influenced by breeder experience (Table 1). However, male spoonbill are thought to be primarily responsible for nest building (Aguilera, 1990). In this study we were not able to sex the breeders. Further data collection, considering the age of male through genetic sampling at the time of ringing may in the future be used to further test this. Alternatively, since no effects of ANM on hatching success were detected, there is no selective advantage for experienced birds to increase their use of ANM as they age. This absence of reinforcement may explain why breeder experience did not influence the proportion of ANM lining the nest.

4.3. Potential impact of lining materials

It is worth noting that in order to test the potential role of lining material in nest thermoregulation, we used seven Onset Computer HOBOWare (Onset Corporation, Borne, MA) thermal loggers to measure nest temperature and humidity. Loggers were placed within the nest and near its surface. However, after the loggers were deployed, spoonbills continued to replenish their nests, sometimes significantly increasing the size of the nest, and moving the eggs away from the loggers. Consequently, temperature recorded by loggers did not represent the temperature experienced by the eggs, and no data analysis was carried out. Therefore, we recommend the use of temperature loggers embedded in fake eggs (e.g., Hope et al., 2018) to further investigate the relationship between lining material and nest temperature.

It is also noteworthy that, despite the high incidence of ANM in spoonbill nests, there are no documented cases of mortality due to entanglement or suffocation of chicks or adults by nest material in Camargue. This might be due to the types of ANM used not being prone to such events. However, to further assess the costs and benefits of the use of ANM, an experimental approach is recommended (e.g., to control the availability of materials, or to assess the impact of different materials in nest temperature). Despite the presence of plastic in nests being potentially a useful indicator of plastic pollution (Bond et al., 2012; Jagiello et al., 2019; Kershaw et al., 2019; O'Hanlon et al., 2019; Tavares et al., 2016), the lack of spatial variation in the amount of ANM used, combined with the fact that spoonbills seem to actively collect certain types of plastic, suggests that other species may be better sentinels of plastic pollution in wetland nesting sites. Nevertheless, spoonbills could act as biovectors (Ballejo et al., 2021; Cano-Povedano et al., 2023), potentially transporting materials such as sheet-like plastics from agricultural fields to natural areas where they nest. While the collected materials may have a limited impact on the breeding success of spoonbills themselves, their presence in these habitats could pose broader environmental risks, affecting other species and ecosystems. Understanding where spoonbills gather these materials and whether they actively seek them is thus a priority for further research. This reinforces the importance of long-term monitoring programmes, which facilitate spatial and temporal comparisons that are essential to understand how animals respond to adverse and increasing anthropogenic influences (e.g., recognition and use of ANM as beneficial nesting material) that show no signs of slowing down. Such insights are fundamental for establishing a baseline to mitigate the negative impacts of such materials in the environment (Avery-Gomm et al., 2018; Jagiello et al., 2019; Sergio et al., 2011).

CRedit authorship contribution statement

Hugo R.S. Ferreira: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana C. Hadden:** Writing – original draft, Visualization, Formal analysis, Data curation. **Jocelyn Champagnon:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Tamar Lok:** Writing – review & editing, Visualization, Validation, Supervision. **Marion Vittecoq:** Writing – review & editing, Visualization, Validation. **José A. Alves:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics approval

All fieldwork procedures were compliant with applicable animal welfare regulations by the Centre de Recherches sur la Biologie des Populations d'Oiseaux (Museum National d'Histoire Naturelle, <https://crbpo.mnhn.fr>), programme personnel 580.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hugo Ferreira reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2025.178588>.

Data availability

Data will be made available on request.

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