Universidade de Aveiro
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Sucesso reprodutivo de limícolas em salinas: impactes da perturbação antropogénica

Breeding success of waders in salt-pans: impacts of anthropogenic disturbance

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor José Augusto Alves, Investigador Auxiliar do Departamento de Biologia e CESAM da Universidade de Aveiro

Dedico este trabalho aos meus pais, que sempre me apoiaram.

o júri

presidente	Doutor Eduardo Manuel Silva Loureiro Alves Ferreira Investigador Doutorado (nível 1) Universidade de Aveiro
arguente principal	Doutor Pedro Correia Rodrigues Professor Adjunto Convidado, Instituto Politécnico do Porto
orientador	Doutor José Augusto Belchior Alves Investigador Doutorado (nível 1), Universidade de Aveiro

agradecimentos

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palavras-chave

Pernilongo, borrelho-de-coleira-interrompida, sucesso de incubação,comportamento parental, perturbação antrpogénica.

resumo

As salinas são frequentemente consideradas bons locais de reprodução para algumas espécies de limícolas. No entanto, o aumento da perturbação humana nestas áreas pode ter um efeito negativo no sucesso reprodutivo das espécies destas. Este estudo tem então como objetivo estabelecer os níveis de sucesso de eclosão de pernilongos (*Himantopus himantopus*) e borrelhos-de-coleira-interrompida (*Charadrius alexandrinus*), assim como o investimento comportamental associado no cuidado das ninhadas de pernilongos em salinas com variados níveis de perturbação na Ria de Aveiro. Para isso, monitorizamos os ninhos de ambas espécies e realizamos observações de cuidados parentais em seis salinas, três com baixa presença humana e três com maior perturbação humana causada por visitantes. Não conseguimos encontrar diferenças significativas entre o sucesso de eclosão em ambas as espécies entre salinas com diferentes níveis de perturbação, embora um grande número de desfechos de ninhos desconhecidos possa ter mascarado os resultados. Durante o cuidado parental, os pernilongos nas salinas com maiores níveis de presença humana mostraram mais comportamentos defensivos relacionados com perturbação humana, e estiveram geralmente mais distantes das suas crias do que nas salinas com níveis mais baixos de presença humana. O aumento da presença humana poderá ter efeitos negativos nas taxas de sobrevivência das crias em salinas perturbadas, no entanto, mais estudos são necessários para avaliar de forma adequada o efeito da perturbação antropogénica, particularmente focando no sucesso das crias (produtividade).

keywords

Black-winged stilt, kentish plover, hatching success, parental behaviour, anthropogenic disturbance.

abstract

Salt-pans are often considered good breeding sites for some wader species, despite being an artificial habitat. However, increasing human disturbance on these areas could have a negative effect on breeding success. This study aims to establish the levels of hatching success of black-winged stilts (*Himantopus himantopus*) and Kentish plovers (*Charadrius alexandrinus*), and associated behavioural investment on brood care of black-winged stilts in salt-pans with varying levels of disturbance in Ria de Aveiro. We monitored nests of both species and conducted parental care observations in six salt-pans, three with low human presence and three with higher human disturbance due to visitors. We did not find differences between hatching success on both species across salt-pan varying in disturbance level, although a high number of unknown nests fates may have masked the results. During brood care, black-winged stilts on salt-pans with higher levels of human presence showed more human related disturbance behaviours and were overall more distant from their chicks than in salt-pans with lower levels of human presence. Increased human presence could have negative effects on chick survival rates on disturbed salt-pans, and more studies are necessary to properly assess anthropogenic disturbance effects, particularly focusing on fledging success (productivity).

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Introduction

Breeding success is a crucial demographic parameter influencing species abundance. During the breeding season, low productivity can lead to population declines, and at the individual level, high breeding success indicates high fitness.

Migratory waders, like other migratory birds, show variation on breeding success which can be attributed to several factors. Predation, for example, can depress wader productivity severely, due to the removal of eggs or chicks from the clutch or broods, respectively. And, high rates of nest predation seem to be associated with wader's low productivity values (Galbraith, 1988; Green, 1988; Baines, 1990; Grant *et al.*, 1999; Malpas *et al.*, 2013). In fact, most studies of wader nest survival reviewed in (Macdonald and Bolton, 2008) identified predation as the major cause of nest failure in Europe. As waders are ground nesting species, both nest concealment and crypsis are two strategies employed to avoid predation (Macdonald and Bolton, 2008). Despite concealing their nest to avoid detection, incubating adults must also be able to see their surroundings in order to not be caught off guard (Giitmark *et al.*, 1995). Hence, the balance between different strategies to decrease nest detection is complex and some species simply do not attempt to conceal their nest, relying entirely on egg pattern camouflage which blends with the nest material (Troscianko *et al.*, 2016). Such "opens nesters" also depend on early predator detection and therefore breed in sites with a good field of view (Amat and Masero, 2004) which allows leaving the nest well in advance, when the potential predator is still at a considerable distance, thus decreasing the probability of detection (Burrell and Colwell, 2012).

Breeding success of waders is also affected by the timing of laying clutches, which is determinant to their productivity. Early breeders tend to have higher breeding success as is the case of common redshank (*Tringa totanus*), that after 25 days from the start of the season incurred higher nest predation probability (Thyen and Exo, 2005). Similarly, Icelandic black-tailed godwits (*Limosa limosa islandica*) showed higher chick recruitment for chicks hatched early, rather than later in the season (Alves *et al.*, 2019). Although factors influencing wader breeding success are multiple, seasonality plays an important and underlying role on patterns of nest survival (Morrison *et al.*, 2019). Egg size is also relevant for productivity, as larger eggs grant more nutrients for chick development and hence, produce larger chicks, with higher survival rates (Grant, 1991).

Despite breeding mostly in natural habitats, many wader species also use artificial habitats to lay their clutches with varying degrees of success. At northern temperate latitudes, several species breed in agricultural landscapes. In Northern Ireland, Eurasian curlew (*Numenius arquata*) breeding success was estimated to be 0.14-0.26 fledglings per pair in marginal farmland, interspersed among improved agricultural grasslands habitat (Grant *et al.*, 1999). And in Canada, northern lapwing (*Vanellus vanellus*) used both improved (intensive production regime) and unimproved (extensive production regime) agricultural habitats to breed, but, productivity was 63% lower in improved sites in relation to unimproved (Baines, 1990). At more southerly latitudes, man-made habitats as salt-pans, artificial lagoons, or agricultural land can also be used as nesting sites for waders. In fact, salt-pans were considered among the most important breeding sites in the Canary island for Kentish plover (*Charadrius* alexandrinus) (Antonio and Emmerson, 1995). In Portugal, little ringed plover (*Charadrius dubius*) used agricultural ponds to breed, with 57% of nest success (Guilherme *et al.*, 2019). In Spanish rice-fields, the nest success of Kentish plover and black-winged stilt (*Himantopus himantopus*) was estimated to be 45% and 50%, respectively (Toral and Figuerola, 2012). Other waterbirds, also breed in artificial wetlands as is the case of the greater flamingo (*Phoenicopterus roseus*) which breeds in commercial salt-pans in the south of France, with an estimated productivity of 0.46 ±0.41 fledglings per pair (Bechet and Johnson, 2008). As natural wetlands are converted, salt-pans become particularly important breeding habitat for waders. Such sites can act as an alternative to natural wetland habitat for nesting, while serving as complementary foraging sites (Aymerich, 1997), as these are rich in invertebrate fauna (Britton and Johnson, 1987).

In Portugal, a total of six species of waterbirds were recorded breeding in Ria Formosa's salt-pans (Fonseca, Grade and da Fonseca, 2005). Furthermore, it is estimated that about 70% of Portugal's black-winged stilts population breed in salt-pans (Rufino *et al*. 1984 cited in Fonseca, Grade and da Fonseca, 2005). These man-made sites are also important for Kentish plovers, which have been recorded to achieve high levels of nesting success in this habitat: 75.7% in Ria de Aveiro's, 36.9% in Mondego's salt-pans (Neves, 1997 and Ribeiro, 2001 cited in Norte and Ramos, 2004); and 25% in Tagus estuary salt-pans (Rocha *et al.*, 2016). Which is considerably higher than in natural breeding sites as sandy beaches, with 12% of breeding success (Norte and Ramos, 2004).

One of the disadvantages of breeding in artificial habitats is the potentially higher levels of anthropogenic disturbance. Although, natural breeding habitats, such as beaches, are also being increasingly disturbed by humans (M. Pienkowski, 1992). Anthropogenic activity can affect breeding waders in various ways: egg destruction and chick mortality due to trampling (by humans, cattle or machinery), intensification of erosion processes of breeding sites by off-road walking or driving, or by altering parental care regimen due to frequent disturbance, resulting in a potential decrease in overall productivity (M. W. Pienkowski, 1992; Verhulst, Oosterbeek and Ens, 2001). Concerning trampling, the overall proportion of northern lapwing clutches lost due to machinery on improved agricultural sites was higher when compared with unimproved ones (Baines, 1990). Regarding erosion, African oystercatcher (*Haemontopus moquini*) and white-fronted plover (*Charadrius marginatus*) increased the number of breeding pairs, and Damara tern (*Sterna balaenarum*) advanced its breeding phenology, following the ban off-road driving in South-Africa beaches, showing the detrimental effect of such activity on ground nesting waders and terns (Williams, Ward and Underhill, 2004). Finally, human presence not only decreased the time spent by Eurasian oystercatcher (*Haematopus ostralegus*) incubating their clutch but also reduced the amount of food allocated to chicks (Verhulst, Oosterbeek and Ens, 2001), indicating the potentially negative effects of human disturbance on productivity.

Despite the apparently higher breeding success in salt-pans when compared to natural habitats (e.g. beaches), these have been increasingly converted for recreational activities, such as eco-museums, bathing facilities, or gastronomic experiences. However, the potential impacts of such activities in the breeding season of waders, remains unexplored.

This study aims to establish the levels of hatching success of black-winged stilts and Kentish plover, and associated behavioural investment on brood care of black-winged stilts in salt-pans with varying levels of disturbance. Comparing nesting success and brood investment across two levels of human disturbance, we aim to quantify the effects of this factor. We expect a higher nesting success in undisturbed salt-pans, due to the higher levels of visitors in disturbed salt-pans, which may interfere with incubation patterns more often (likely leaving the nest unattended for longer) than in undisturbed salt-pans. Moreover, we expect that adults in disturbed salt-pans spend more time in brood defence behaviours, such as being vigilant or producing alarm calls, than in undisturbed salt-pans. These behavioural differences could potentially influence brood care regimen, possibly leading to greater distances between adults and chicks in disturbed salt-pans.

Methods

Study area

Fieldwork was undertaken between early May and late June of 2020. Data was collected in six different salt-pans located in Aveiro, Portugal. These were Ecomuseu Marinha da Troncalhada (40º38’42’’N, 8º39’44’’W), Marinha da Noerinha (40º38’45’’N, 8º39’34’’W), Marinha da Pasã (40º38’53’’N, 8º39’38’’W), Marinha da Grãn-Caravela (40º38’36’’N, 8º39’50’’W), Marinha da Senitra (40º38’35’’N, 8º39’56’W’) and Marinha da Peijota (40º38’23’’N, 8º39’56’’W).

Anthropogenic disturbance data

Two types of salt-pans were established based on the presence or absence of recreational activities: disturbed (Ecomuseu Marinha da Troncalhada, Marinha da Noerinha and Marinha da Grãn-Caravela) and undisturbed (Marinha da Pasã, Marinha da Senitra and Marinha da Peijota) (Fig. 1). Disturbed salt-pans were also characterized by the presence of structures that facilitated human presence, such as improved accesses, large and comfortable walking paths, resting areas with shading and information signs. Furthermore, these salt-pans offered services such as tours, bathing pools (within salt-pan area) and shops. Conversely, undisturbed salt-pans had none of the previously mentioned characteristics, but did have salt-extraction workers, similar to the disturbed ones. Nonetheless, both salt-pan types had similar natural resources available.

Figure 1. Map of study area: Disturbed salt-pans (red): A-Marinha da Grã-Caravela; B-Ecomuseu Marinha da Troncalhada; C-Marinha da Noerinha. Undisturbed salt-pans (blue): D- Marinha da Pasã; E- Marinha da Senitra; F-Marinha da Peijota.

Nests data

Black-winged stilt and Kentish plover nest's possible locations were assessed by observation of bird behaviour from the salt-pans perimeter, with the assistance of a telescope and binoculars (Omegon 20x60 and Olympus 10x50 DPS I, respectively). After locating a nest, it was approached and marked with a skewer stick within a meter radius. Each nest was given a code and its GPS location was registered. Following nest finding, nest success was monitored twice a week, first by checking from a safe distance if an adult was incubating (i.e. distant enough so that the bird would not abandon the nest), in which case the nest would not be approached to avoid disturbance. If the adult was absent (often indicating hatching or nest loss), the nest was checked and the following information was registered: if the eggs were being incubated (warm, cold, cracked, holed, or hatched) or if the nest was flooded, predated, deserted, trampled, or with unknown fate. Nest visits on the same salt-pan were spaced with by at least one full day, unless it was necessary to confirm nest fate (i.e. hatching was eminent – see below).

At first visit (i.e. nest finding) each egg was marked with a number and had its length and breadth measured with a calliper to the nearest 0.05 mm. The enumeration of eggs allowed determining if an egg was laid before the next visit to the nest. If not cracked or holed, egg development was quantified using the flotation method (Liebezeit *et al.*, 2007), which allows determine laying date, and thus the predicted hatching date given an incubation period of 23 days for black-winged stilt and 25 for Kentish plover (Cramp, S. & Simmons, 1983), this process do not negatively affect the egg hatchability (Hansen, Schmidt and Reneerkens, 2011). A nest was considered successful if at least one egg hatched, which was confirmed visually (i.e. by recording at least one hatchling inside or in the vicinity of the nest cup). If at the predicted hatch date (+ 2 days) the nest cup was empty, evidence of potential outcome such as egg shells, hatchlings, or predation signs were searched on and near the nest, which was deemed of unknown fate when outcome was impossible to determine.

Parental behaviour during brood care

After chicks hatched, black-winged stilt parental behaviour was assessed through focal observations of families. These were made with the telescope, far enough from the family's location to ensure that the observer was not a cause of disturbance. In each observation, a random pair of adults with chicks was chosen and the parental behaviour recorded every 30 seconds during 1 hour (totalling 120 instants). The distance between the furthest adult and the chicks, and the number of visitors in the salt-pan were recorded every 5 minutes. Furthermore, the number and development stage of chicks in the focal family were recorded as well. Chick development was divided into three stages according to its height: 1- chick does not reach the adult's ankle; 2- the chick's height is between the adult's ankle and its ventral area; 3- chick is taller than the adult's ventral area. The various behaviours of adult females and males were organized with different codes (Table 1), modified from (Kubelka, 2019). A total of 45 observation bouts were made, 22 in disturbed and 23 on undisturbed salt-pans.

Behaviour	Code	Description
MAINTENANCE AND FORAGING		
Walking	W	Walking individual, obviously slower movement than running.
Running	RU	Running individual, obviously faster than walking.
Pecking/drinking	PE	Pecking, typically, at a food item or probing (foraging technique typical for many sandpipers and allies), or dipping the bill to the water, drinking.
Flying	FL	Bird is flying without any display.
Standing	STAND	Standing individual in relaxed position, often on one leg or with hidden head.
Sit	SIT	Sitting individual (sometimes with hidden head) on the ground, not on nest.
Preening	PR	Maintenance of feathers (often ruffled) by bill.
Head scratching	HS	Head scratching by leg as a part of maintenance behaviour.
Wing stretching	WS	Wing stretching as a part of maintenance behaviour.
Leg stretching	LS	Leg stretching as a part of maintenance behaviour.
Head flicking	HF	Head flicking to the side (over the shoulder).
Bathing	BATH	Bathing in the shallow water with ruffled feathers, feathers soaking and often wings flapping.

Body shacking	BS	A movement lasting a few seconds of body shaking with ruffled feathers, often after bathing of stretching.
BROOD ATTENDANCE		
Brooding the chicks	B	Chick(s) is/are ate least partially hidden in adult's breast feathers and under wings. Adult can stand or sit on the ground.
Chick calling	CC	At least one adult calls for the chick(s) (different from alarm calls), and usually chicks tend to approach the adult.
BROOD DEFENCE- against natural predators or humans, but not against observing researchers		
Chasing conspecific (unknown sex) Chasing conspecific male Chasing conspecific female Chasing heterospecific individual Chasing multiple conspecific individuals (note species and the sex if possible)	CHC CHCM CHCF CHCH CMC	A quick aggressive behaviour against another animal, typically a waterbird to chase it away from the feeding ground.
Standing head-up	HU	Alerted individual, often scanning the surrounding for potential danger.

Standing extreme head-up	EHU	Alerted individual with stretched neck (vertically elongated), often scanning the surrounding for danger.
Alarm calling	DC	Calling/warning from distance, typically from the ground, giving potential predator away, but without closer approach to the predator.
Injury feigning	IF	Adult is pretending to be injured, e.g. “broken wing display” “wing flapping”, resembling easy prey for potential predator, to allure potential predator away brood location.
Other displacement activities	DIAC	False feeding, false swimming, pseudo-sleeping, or false maintenance behaviour as a part of the distraction display to allure potential predator away from real brood location.
Ungulate display	UNG	To deter a big animal (often ungulate) from vicinity of nest or brood, adult shorebird faces the animal with fully raised wing(s) and loud calling.
Aggressive circling, scolding, or attack	AC	Intense flight above or in close vicinity of approaching predator whilst emitting alarm calls (mobbing), without physical contact. Or intense mobbing connected with physical contact with intruder/predator, often repeated and predominantly air strikes complemented with loud vocalization.
OTHER		

Unseen	UNS	Focal bird is covered from view, e.g. behind vegetation.

Table 1. Codes used to organize different parental behaviours during observations.

For the purpose of analysis related behaviours were pooled: vigilant “VIG”, includes “HU” and “EHU”; chasing “CH”, combines “CHC”, “CHCM”, “CHCF”, “CHCH”, “CMC” and “UNG”; distress “D”, incorporates “DC”, “DIAC” and “IF”; maintenance “MAI”, includes “W”, “RU”, “PE”, “FL”, “STAND”, “SIT”, “PR”, “HS”, “WS”, “LS”, “HF”, “BATH”, and “BS”.

Causes of aggressive circling, scolding or attack (AC) and other active defensive brood behaviours (DC; DIAC; IF) were divided into three categories: natural, if the behaviour was caused by a natural predator; worker, if a salt-pan worker was the cause of the behavioural response; visitor, if the behaviour was caused by a visitor.

Statistical analysis

All data was tested for normality and for homogeneity of variance with Shapiro-Wilk and Levene’s tests, respectively. Significant differences between groups were therefore tested with parametric and non-parametrics test, according to data characteristics resulting from the tests above.

In order to test if hatching success and predation differed between disturbed and undisturbed salt-pans we used a logistic regression for both species. Clutch investment, quantified as egg volume (cm^3) per egg, between disturbed and undisturbed salt-pans was tested with Student’s t-test (black-winged stilt) and Mann-Whitney-Wilcoxon test (Kentish plover. To assess weather percentage of time spent on brood defence behaviours (VIG; CH; B; D; AC; CC) differed between disturbed and undisturbed salt-pans Kruskal-Wallis test was used, followed by Mann-Whitney-Wilcoxon as post-hoc for pairwise comparisons. The same procedure was applied regarding causes of active defensive behaviour (D; AC) between both salt-pans types. Maintenance behaviours (MAI; UNS) were singularly tested between disturbed and undisturbed salt-pans with Student’s t-test. In order to test if the cause of disturbance varied between salt-pan types Chi-Square test was used, and resulting Pearson residuals were plotted for visual inspection of the relations between disturbance causes and salt-pan type. Furthermore, the frequency of occurrence of each cause was calculated, while accounting for the total number of observations, as these were different between salt-pan types. Finally, potential differences in the distances between parents and chicks were explored in a Generalized Linear Model with Poisson distribution with two factors, salt-pan type, and chick development stage. All mentioned statistical procedures were performed in R (version 1.2.5033) software.

Data review

Between November 2019 and January 2020 SCOPUS and Web of Science databases were searched using English terms to find relevant studies on wader breeding success. The terms “waders AND productivity” where first searched in SCOPUS resulting in 185 hits, and then in Web of Science returning 162 hits. Finally, “shorebird AND productivity” were searched in SCOPUS resulting in an additional 92 hits. All titles and abstracts that contained information on wader/shorebird productivity were selected for data extraction resulting in 106 papers (appendix 1). The following parameters were extracted whenever present: (1) species nest success; (2) species fledgling success; (3) method used for estimations; (4) survey’s year; (5) study location.

We filtered the results of the data search, removing non wader species and hatching success values obtained with different methods than the one used in this study, direct observation. Then, each species (subspecies were not discriminated) was converted into their average body mass (of females when available), which values (in g) extracted from BTO Bird Facts (Robinson, 2005), or from Encyclopedia of Life website (EOL; http://eol.org/) for missing species, except for snowy plover (*Charadrius nivosus*), which was absent from both websites and its data was retrieved from (Gómez, Palacios and Eduardo, 2015) and for St. Helena plover (*Charadrius sanctaehelenae*) which body mass we could not find. Finally, we combined this data with the obtained from our study in a single graphic allowing to visually compare our results and place those in the wider context of wader breeding success. For studies spanning multiple years we averaged hatching success across years.

Results

Nest investment and success

A total of 50 Kentish plover and 42 black-winged stilt nests were found and surveyed.

For Kentish plover, 24 nests were found in disturbed salt-pans and 26 in undisturbed. In disturbed salt-pans, 21% of nests were successful, 46% predated, 8% trampled, and 25% had unknown fate (Fig. 2A). In undisturbed salt-pans 27% of nest were successful, 19% predated, 4% flooded, and 50% had unknown fate. Hatching success was not significantly different between salt-pan types (z = 0.503, P = 0.615, n = 50), but predation was significantly higher in disturbed salt-pans (z = -1.967, P = 0.049, n= 50). Average egg volume (± SE) in undisturbed salt-pans was 8.9 ± 0.1 cm^3 and 8.8 ± 0.1 in disturbed, and this was not significantly different between salt-pans types (W = 1731, P = 0.968, n = 118).

For black-winged stilt, 18 nests were found in disturbed salt-pans and 24 in undisturbed ones. In disturbed salt-pans 61% of nests were successful, 11% predated, 6% flooded, 6% deserted and 17% had unknown fate (Fig. 2B). In undisturbed salt-pans 50% of nests were successful, 4% predated, 4% flooded and 42% had unknown fate. Hatching success also did not vary significantly between salt-pan types (z = -0.714, P = 0.475, n = 42), nor did predation (z = -0.833, P = 0.405, n = 42). Average egg volume (± SE) in undisturbed salt-pans was 22.0 ± 0.3 cm^3 and 22.2 ± 0.4 cm^3 in disturbed, and again these differences were not significant ($t_{(94.083)}$ = 0.4007, P = 0.69, n = 134).

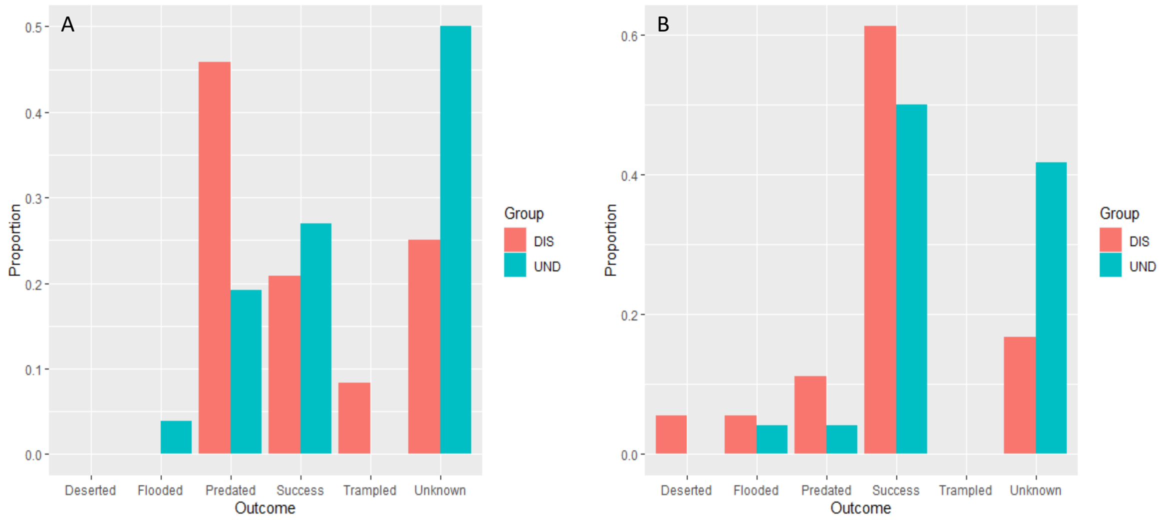

Figure 2. Nest outcomes of (A) Kentish plover (n = 24 for disturbed, red; n = 26 for undisturbed, blue) and (B) black-winged stilt (n = 18 for disturbed, red; n = 24 for undisturbed, blue) in Ria de Aveiro salt-pans.

Black-winged stilt parental behaviour

Brood defence behaviour was overall different on disturbed and undisturbed salt-pans (Kruskal-Wallis, $x^2_{(11)}$ = 113.48, p < 0.0001, n = 270). Time spent on "chick calling" (CC) was significantly higher in undisturbed salt-pans, but for all other behaviours this was the opposite except "brooding" (B) and "chasing" (CH) which did not differ significantly (Table 2A, Fig. 3A).

	Disturbed mean (SE)	**Undisturbed** mean (SE)	**Statistic**	**Df**	**P value**
A					
VIG	15.38 (2.29)	6.27 (1.15)	398.5		**0.0009**
AC	4.83 (1.33)	0.85 (0.27)	404		**0.0004**
D	6.69 (1.49)	0.85 (0.73)	402.5		**< 0.0001**
CC	0.49 (0.23)	1.32 (0.27)	124.5		**0.003**

B	1.99 (1.03)	0.18 (0.13)	281		0.282
CH	0.47 (0.17)	4.44 (2.9)	188.5		0.111
B					
MAI	39.13 (4.04)	65.42 (4.36)	-4.4207	42.874	**< 0.0001**
UNS	31.02 (3.45)	20.67 (3.24)	2.1849	42.687	**0.034**

Table 2. Comparison of percentage time spent on each behaviour by breeding black-winged stilts between salt-pans types. Significant differences of Wilcoxon (A) and Student's (B) tests are indicated with p-value in bold.

Black-winged stilt adults of undisturbed salt-pans spent significantly more time in "maintenance" (MAI) behaviour than those in disturbed salt-pans (Table 2B). Overall, adults spent significantly less time unseen (UNS) in disturbed salt-pans than in undisturbed ones (Table 2B, Fig. 3B).

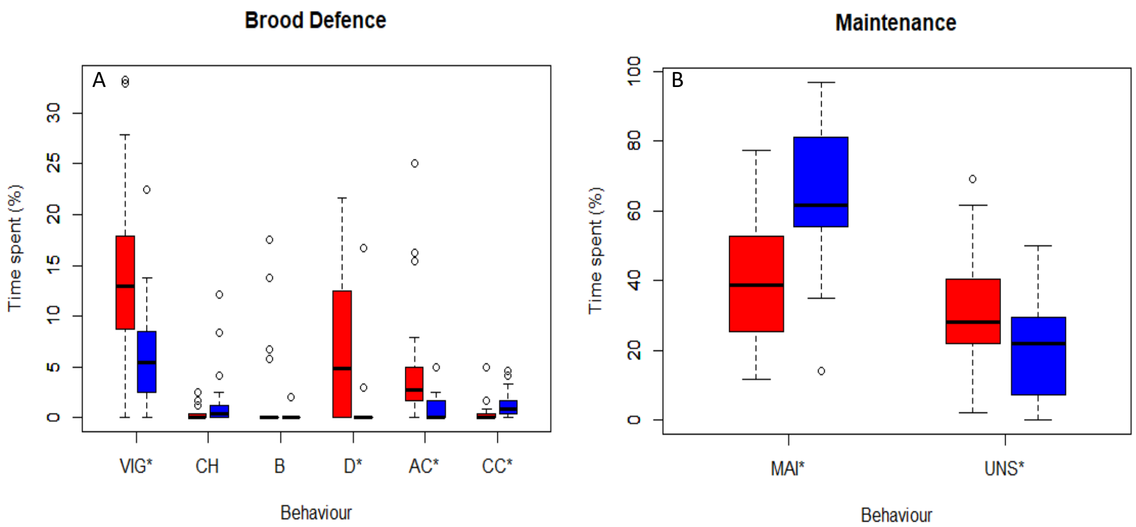

*Figure 3. Variation on percentage of time spent by black-winged stilts adults in: (A) brood defence and (B) maintenance on disturbed (red) and undisturbed (blue) salt-pans. Behaviour codes marked with * indicate significant differences between salt-pan type (Table 2).*

The causes of disturbance related behaviours varied significantly between disturbed and undisturbed salt-pans (Kruskal-Wallis, $x^2_{(5)} = 29.574$, $p < 0.0001$, n = 135; Fig. 3). But significant differences were only detected for percentage time spent dealing with disturbance caused by visitors, being higher on disturbed salt-pans (W = 414, P < 0.0001, n = 45), while no differences were found for natural (W = 298, P = 0.24, n = 45) and worker (W = 309.5, P = 0.066, n = 45) related causes of disturbance (Fig. 4).

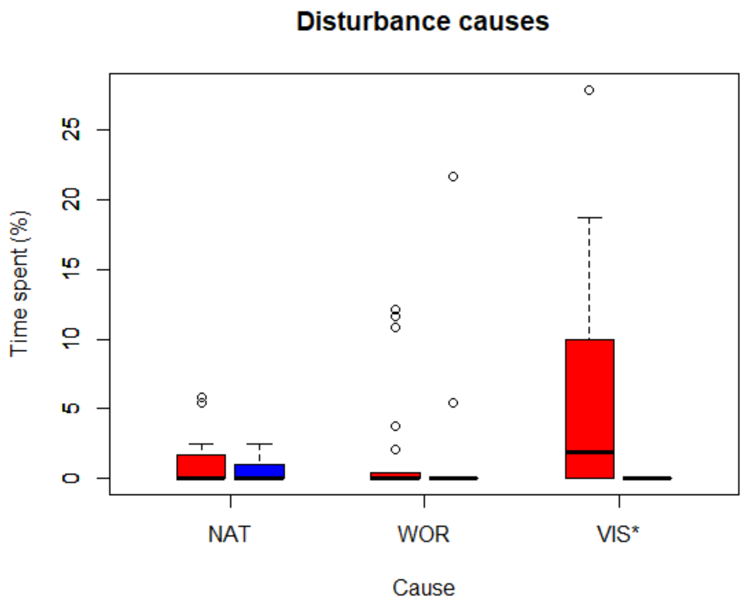

*Figure 4. Variation on percentage of time spent by black-winged stilts adults in disturbance associated behaviours ("AC" and "D") caused by natural predators, workers, or visitors in disturbed (red) and undisturbed (blue). Causes of disturbance associated behaviour marked with * are significantly different between salt-pan type.*

Moreover, there were significant differences in total occurrences of each disturbance cause in disturbed and undisturbed salt-pans (Chi-square, $x^2_{(2)} = 104$, P< 0.0001, N = 687; Fig. 5A). Pearson residuals suggests positive association between disturbed salt-pans and visitors disturbance, and negative association with natural and worker disturbance, while the reverse applies to undisturbed salt-pans (Fig. 5B).

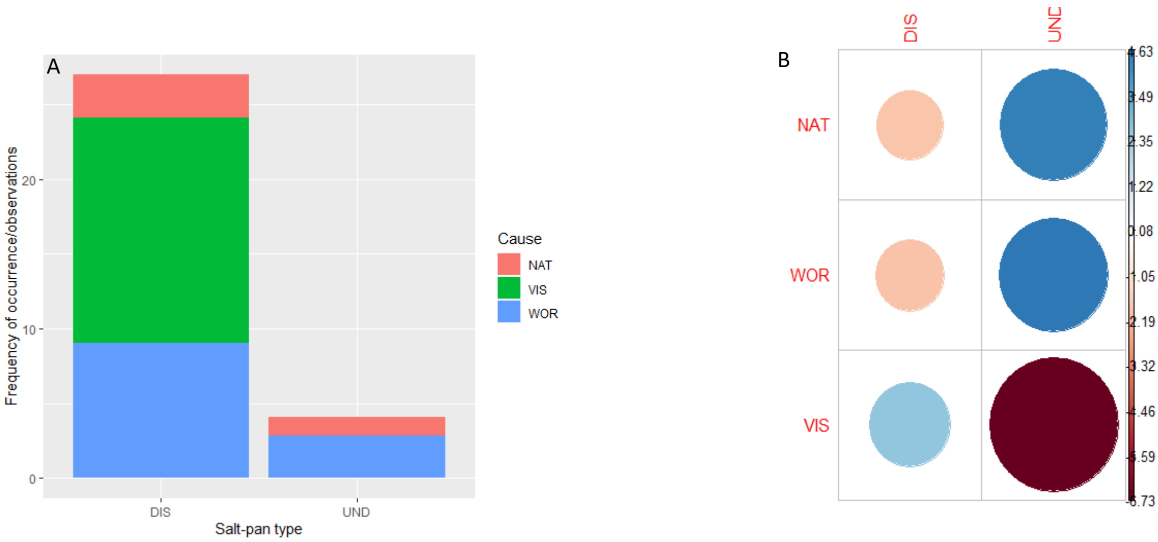

Figure 5. (A) Frequency of occurrence of different causes of disturbance divided by the total number of observations of each salt-pan type. (B) Representation of pearson residuals from Chi-square test: positive residuals (blue) indicate positive associations, and negative residuals (red) indicate negative associations between disturbance causes and salt-pan type. The size of the circle is proportional to the amount of the cell's contribution.

Distances between adults and chicks

The maximum distances between parent and chicks were significantly larger overall in disturbed salt-pans ($P < 0.0001$), being consistent throughout all chick development stages: stage one ($P = 0.0059$); stage two ($P < 0.0001$); stage three ($P < 0.0001$) (Fig.6). Distances were significantly smaller in stage one than in stage two ($P < 0.0001$) and stage three ($P < 0.0001$), but no significant differences were found between stages two and three ($P = 0.758$) (Fig.6).

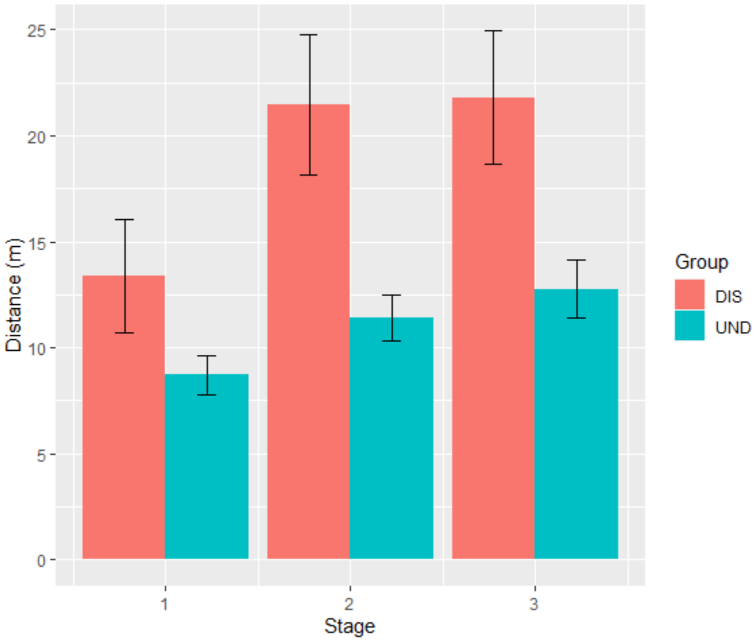

Figure 6. Variation on the average (±SE) maximum distance between adults and chicks across different development stage in disturbed (red) and undisturbed (blue) salt-pans.

Discussion

Nests outcomes

Kentish plover hatching success did not differ significantly between disturbed (21%) and undisturbed (27%) salt-pans in this study. However, the percentage of nests with unknown fate was substantially high, especially in the undisturbed salt-pans (50%), which may have masked differences. This high percentage of unknown fate may have been caused by several factors. Kentish plover adults were observed removing eggshells from the nests shortly after hatching, a common practise in many bird species (Nethersole-Thompson, D., & Nethersole-Thompson, 1986), which seems to be associated with nest concealment, as the interior of eggshells are mainly white, it is easily seen by potential predators (Tinbergen *et al.*, 1962) . Moreover, chicks of most wader species are precocial (Walters, 1984; Schekkerman, Teunissen and Oosterveld, 2009), hence they tend to move away from their nests one to three days after hatch (Wilson and Colwell, 2010). As mentioned in the methods, it was impossible to check each nest at the predicted hatch date, so in many cases, there was not enough evidence to confirm the nest's fate. In future studies, visits on or closer to predicted hatching must be conducted to improve the determination of nest outcome. Predation, on the other hand, was significantly higher in disturbed salt-pans, being the most common nest fate in this salt-pan type. This is usually the major cause of nest failures in waders (Baines, 1990; Valkama, Currie and Korpimäki, 1999; Rönkä *et al.*, 2006; Macdonald and Bolton, 2008; Burrell and Colwell, 2012). In fact, evidence of small mammal presence was found in the most predated salt-pan, which was probably the main predator in that site. Kentish plover hatching success in Ria de Aveiro was (24%) overall lower than in other regions of the world: 47% and 33% in USA (Conway, Smith and Ray, 2005); 58.4% in North Africa (Hanane, 2011); 45% in Spain (Toral and Figuerola, 2012) and 45.3% in Hungary (Székely, 1990). It also was one of the lowest of its body mass category regarding other species (Fig 7). As for Portugal, it was higher than in natural sandy beaches (12%) (Norte and Ramos, 2004), lower than Mondego's salt-pans (36.9%) (Neves, 1997 and Ribeiro, 2001 cited in Norte and Ramos, 2004) and did not differ from Tagus's salt-pans (25%) (Rocha *et al.*, 2016). Despite of this, it was substantially lower than in previous studies in Ria de Aveiro (75.7%) (Neves, 1997 and Ribeiro, 2001 cited in Norte and Ramos, 2004). Despite our results, Ria de Aveiro, including our study area, has been increasingly requalified, offering improved touristic conditions, which may have had a negative effect on hatching success over the years, perhaps not directly, as shown in this study, but instead indirectly. As mentioned, predation is usually the most common cause of nest failure, and man-made structures seem to benefit certain predators, specifically avian, for example by providing perching opportunities (Wallander, Isaksson and Lenberg, 2006; Kristan and Boarman, 2007). Indeed, the predator identified more frequently by visual observations was of this group, the black kite (*Milvus migrans*). Thus, the increasing touristic interest in Ria de Aveiro may benefit predation.

Black-winged stilt hatching success also did not differ significantly between disturbed (61%) and undisturbed (50%) salt-pans. Nevertheless, as in Kentish plover, the percentage of unknown's fates was substantially high, particularly, again, on undisturbed salt-pans (42%). As Kentish plovers, black-winged stilt adults were also seen removing egg shells hatching episodes, as this is a common behaviour in similar species like black-necked stilts (Sordahl, 1994). However, the factors mentioned above could also occur on disturbed salt-pans, but for both species, nests with unknown fates were more frequent on undisturbed salt-pans. This may be associated with the salt-pans characteristics, as undisturbed salt-pans lacked proper paths, so in order to determine nests fate, observers had to walk over man-made mud divisions inside the site. This became an obstacle at some hatching dates, as it coincided with the salt-pan cleaning and, some areas were filled with water, hence, workers did not allow observers to walk over the mud divisions, resulting in a one to two-day nest check delay. Predation was not significantly different between salt-pan types, and it was substantially lower than in Kentish plover nests, this may be explained by the differences in body mass and hence, defensive responses against predators between species, as waders with higher body weight are more likely to attack predators than smaller species (Larsen, 1991). Therefore, as black-winged stilts are considerably heavier and larger than Kentish plover (180 g and 48 g, respectively), they may experience lower vulnerability to nest predation. Hatching success in Ria de Aveiro was overall lower than in other world regions: 70% in Iran (Barati, Etezadifar and Sharikabad, 2012); 89.6% in Senegal (Diallo, Ndiaye and Ndiaye, 2019) and 89.7% in China (Yanwei *et al.*, 2003). However, it was similar to results from Spain, 50% (Toral and Figuerola, 2012) and 59% (Cuervo, 2005). Contrary to Kentish plover, black-winged stilts in Ria de Aveiro had a hatching success in the upper end of those recorded for waders of similar body mass (Fig 7).

Overall, hatching success did not differ between disturbed and undisturbed salt-pans on both species. This goes against our initial prediction and also results from other waterbird in breeding colonies disturbed by humans, as western gulls (*Larus occidentalis*) (Robert and Ralph, 1975), black-crowned night-herons (*Nycticorax nycticorax*) (Tremblay, J., & Ellison, L, 1979), black skimmers (*Rynchops niger*) (Safina and Burger, 1983) and northern lapwings (Baines, 1990), all of which had lower hatching success values associated with an increase of human disturbance.

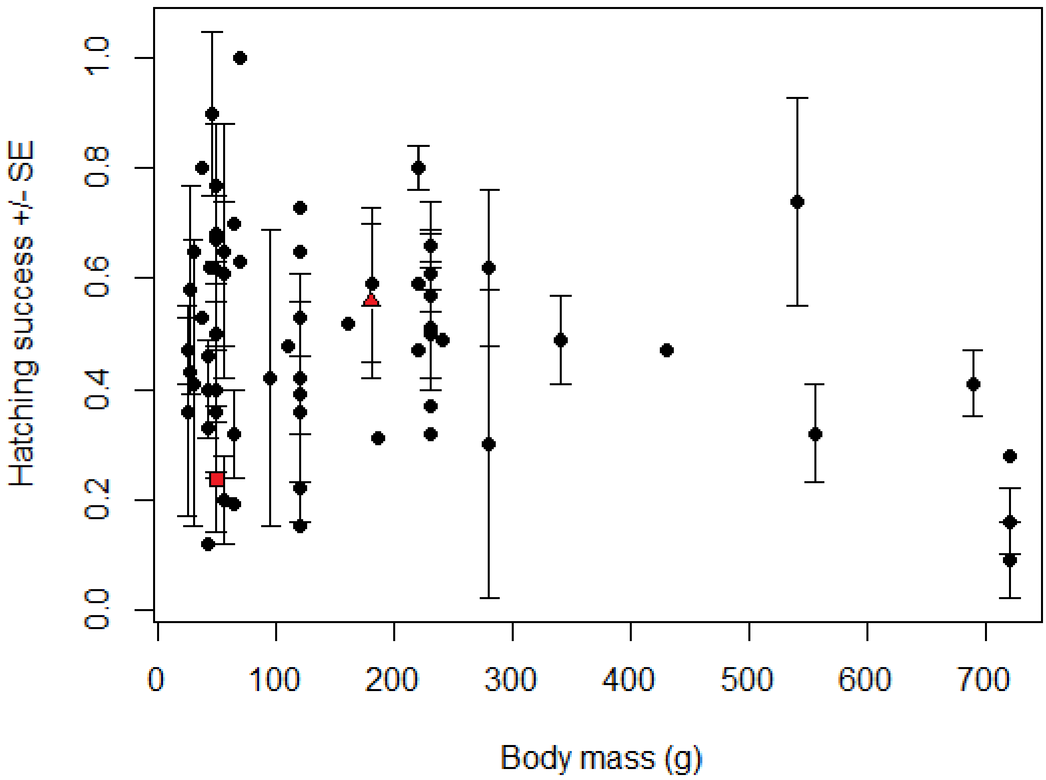

Figure 7. Variation on Hatching success relatively to body mass across waders compiled form a data review. Data from this study is marked with a red square (kentish plover) and triangle (black-winged stilt). References are found in Appendix 1.

Egg volume

Regarding egg volume, neither of the species in our study varied significantly between salt-pan types, suggesting equal investment on the eggs laid. Kentish plover average egg volume (8.9 ± 0.1 and 8.8 ± 0.1 cm^3) in Ria de Aveiro was similar to other studies in Portugal (8.96 ± 0.64, Norte and Ramos, 2004) and in Spain: 9.1±0.5 and 9.0 ± 0.7 cm^3 (Amat, Fraga and Arroyo, 1999); 8.95 ± 0.64 cm^3 (Amat, Fraga and Arroyo, 2001a). As for black-winged stilts, average egg volume in our study (22.0 ± 0.3 and 22.2 ± 0.4 cm^3) was slightly higher than in Iran (18.3 cm^3, Gholami *et al.*, 2017), North Africa (21.61 ± 0.3 cm^3, Adamou *et al.*, 2009), but within the average for the species (22 cm^3, Cramp, 1983; Pierce, 1996 cited in Rodrigues and Tavares, 2014). Egg dimension seems to be relevant for chick survival, as larger eggs produce larger and healthier hatchlings (Grant, 1991; Amat, Fraga and Arroyo, 2001b) but there is no information about its role on hatching success on waders. However, (Krist, 2011) meta-analysis found egg size positively related with hatching success, chick survival and growth rate of birds in general. This author also highlighted the lack of studies regarding egg size and its relationship with hatching success. Egg dimension appears to vary depending on the breeding site quality, as a declining tendency on egg size was associated with a progressive drying of the study site in black-winged stilts (Adamou *et al.*, 2009). Thus, our results overall suggest that Ria de Aveiro's salt-pans are a good breeding habitat for these two species as adults produce standard sized eggs and that this characteristic seem to be unaffected by human disturbance.

Black winged stilt parental behaviour

Although the similar results between salt-pan types in hatching success and egg volume, black-winged stilt adult´s behaviour during brood caring showed several differences between disturbed and undisturbed salt-pans. Our results were similar to our initial prediction, indeed, adults in disturbed salt-pans spent significantly more time on brood defence behaviours, as was the case of "vigilant" (VIG), "aggressive circling, scolding, or attack" (AC) and "distress" (D). "Chick calling" however, was more frequent on undisturbed salt-pans, which may be linked with our results on maximum distances between parents and chicks (see below). "Brooding" besides not being different between types, it was substantially low on both site types, probably because this behaviour is usually associated with a harsh cold or extreme heat conditions (Kubelka, 2019) which do not occurred during the observations. Regarding "chasing", which also did not differ between salt-pans, it seemed to be associated with territory defence regarding other black-winged stilts and other species, including Kentish plover, occurring when the observed family was on a relatively densely populated area, which was uncommon on both disturbed and undisturbed salt-pans. All behaviours related to an active response to the disturbance, as "aggressive circling, scolding or attack" (AC) and "distress" (D), were more common on disturbed salt-pans. These results seem to support our hypothesis of disturbed salt-pans being associated with more disturbance related behaviours, but it is also important to assess the causes of disturbances on both salt-pan types. On undisturbed salt-pans, the disturbance originated only from natural causes and workers. During observations, undisturbed salt-pans had no visitors, conversely, only one observation bout on disturbed salt-pan had no visitors, having an average of 14 (± 0.17 SE) visitors per observation bout. This factor could explain the differences between salt-pan types regarding the percentage of disturbance caused by visitors, as undisturbed had no visitors at all. Nonetheless, visitor's disturbance was seemingly the most relevant cause in disturbed salt-pans, being overall the most frequent (15.05 occurrences per observation), and this value was greater than the frequency of natural and workers disturbance combined (11.91). These differences between salt-pans types could be relevant regarding chick survival rate. On oystercatchers, anthropogenic disturbance during brood care proved to cause negative alterations on the quantity of food allocated to chicks (Verhulst, Oosterbeek and Ens, 2001). But, this would not affect black-winged stilts, as chicks of this species feed on their own after leaving the nest cup, shortly after hatching (Cuervo, 2003). However, parents play an important role in brood defence, as they do not only protect the eggs during incubation but also guard the chicks until fledging (Cuervo, 2003; Ashoori, 2011). So, more frequent disturbance due to visitors could lead to parents spending less time protecting their brood against predators. It could be that the same occurs with workers, after all, besides their disturbance times not being different between salt-pans types, they do cause a non-natural disturbance. However, during observations, black-winged stilts adults seemed to be used to them, apparently reacting less and later to workers when compared to visitors. This seems plausible with our results, as worker disturbance was less common than visitors, but unfortunately, this study did not quantify fledgling success or assessed responses characteristics for different disturbance causes (as the minimum distance between adult and worker/visitor before reaction, for example), so we these hypotheses cannot be confirmed.

Regarding maintenance behaviours and time spent unseen, these were also significantly different between salt-pans, this last being more common on disturbed salt-pans, whilst the contrary is true for maintenance Waders, including black-winged stilts, spend most of their time foraging (Nol *et al.*, 2014), which was included in "maintenance" (MAI) code, where most behaviours were pooled. Hence it could be expected that most time was spent in this overall behaviour. As for the differences between salt-pan types, adults from undisturbed sites were overall less disturbed, so, they could spend more time on non-stress related behaviours, such as foraging or preening. On the contrary, adults on disturbed salt-pans had less time to invest in those activities as they had to invest more on brood defensive behaviours. For this reason, it is likely that adults in undisturbed salt-pans are probably in better condition than those on disturbed one, as they have more time for foraging and preening, while spending less energy in defensive behaviours. It is therefore likely that they may also be more efficient in defending the brood from a given attack by predators. When an adult was impossible to see it could be to different reasons, specifically to purposefully remaining hidden, or due to salt-pan characteristics and observer's viewpoint. During observations, a few families of disturbed salt-pans showed a peculiar behaviour: adults were seen hiding among tall vegetation, calling the chicks until all became hidden, and this would occur during disturbance episodes, including with avian predator's presence. Stilts usually tend to be aggressive towards predators, especially on the breeding season (Pierce and Lake, 1986; Ashoori, 2011), and this hiding strategy was observed only a few times as it was not the common response to the disturbance for the majority of the studied families. Most of the times that an adult was unseen it was due to visual barriers, as tall vegetation and tall divisions inside the salt-pan. Nonetheless, it seemed that this scenario was more frequent after flights, and as disturbed salt-pans had more "aggressive circling, scolding or attack" (AC) behaviours associated, this could explain the differences between the time spent unseen between salt-pans types. However, this cannot be confirmed and probably these differences could be explained by the combination of those various factors.

Distances between adults and chicks

The maximum distance between black-winged stilts parents and chicks were affected significantly by two factors: salt-pan type and chick development stage. Regarding the last, stage one had significantly smaller distances than stages two and three, this being consistent on both disturbed and undisturbed salt-pans. Waders chick survival rate increases rapidly with age (Loegering and Fraser, 1995; Colwell *et al.*, 2007; Ackerman *et al.*, 2014), with predation occurring usually on the first week after hatching (Colwell *et al.*, 2007). Thus, it makes sense that adults were closer to chicks during stage one, which is the more dangerous for the chicks. Colwell *et al.* (2007) reported a relation between improved survival of older chicks of snowy plovers and an increased distance between them and their parents as they grown , which seemed to also be the case of our study. Distances between chick development stage two and three did not differ on either salt-pan type, probably because in these stages chicks are more than a week old. Although the distances differences between stages were consistent on both salt-pan types, these were significantly larger, on every stage, in disturbed salt-pans. Black-winged stilts in undisturbed salt-pans spent more time performing "chick calling" (CC), which may have influenced these differences. However, time spent on that behaviour was overall substantially low, so the most likely reason for the disparities on distances between parents and chicks between salt-pan types may be the increased disturbance, mainly by visitors on disturbed salt-pans. Anthropogenic disturbance effect on wader productivity seems to depend on disturbance intensity. Pearce-Higgins *et al.* (2007) reported that golden plover (*Pluvialis apricaria*) nests location, clutch survival rates and chick growth were not related with the proximity to a footpath, and likewise Morse, Powell and Tetreau, (2006), found no relation between human recreational disturbance and black oystercatcher (*Haematopus bachmani*) productivity, but in both cases, authors pointed that they considered the studied disturbance as slight, and expected worse results with higher levels. On the other hand, Yasué and Dearden (2006) concluded that the tourism development on Thai beaches negatively affects habitat availability and productivity of Malaysian plovers (*Charadrius peronii*). Likewise, great blue heron (*Ardea herodias*) productivity also decreased due to human disturbance (Vennesland and Butler, 2004). Visitors could have a peculiar effect on breeding black-winged stilts, as they tend to have an aggressive posture towards the disturbance origin, or try to divert the attention to themselves, by acting injured or by emitting loud calls, while they get further away from nest or chicks (Goriup, 1982; Pierce and Lake, 1986; Ashoori, 2011; Kubelka, 2019). Hence, in a frequently disturbed site, parents would be more time defending their brood, and whichever technique they use it would lead to getting further from the chicks. This could potentially affect the survival rate of chicks, particularly in their first week of age. As mentioned above, most of the chick predation occurs during that period, and if adults are more distant from the brood due to visitors, predation chances may be higher.

In sum, there is no evidence indicating that human disturbance decreases the hatching success on neither studied species. However, there are large differences in breeding adult behaviour of black-winged stilt during brood care, between undisturbed and disturbed salt-pans. Although we did not have fledgling success values to indicate fitness consequences of such behavioural differences, these differences could lead to higher chick predation rates and lower productivity. Moreover, our data was collected during a pandemic, which, according to salt-pan's workers, it decreased the number of visitors, especially on disturbed salt-pans where it was common to have school's trips, so we expect that our results would differ from those on a regular year. However, we noticed that human disturbance is difficult to assess, so in order to have a better understanding of its effect on Ria de Aveiro's salt-pans more studies, with more precise nest data and including more parameters, specifically fledgling success, are crucial. Nonetheless, we suggest a more controlled visit program on disturbed salt-pans during the breeding season.

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Appendix 1

Species	Body mass (g)	Location	Year	Nest success	SE	Source
Actitis macularia	45	Canada	2002	0,9	0,15	67
Arenaria interpres	120	Canada, USA	2008-2014	0,53		71
Calidirs alpina	48	Scotland	1996-1998	0,36	0,11	98
		Sweden	2008-2013	0,77		68
		USA	2008-2014	0,67	0,08	71
		Scotland	1985-1997	0,5	0,13	99
		Sweden	1993-2004	0,68	0,2	86
Calidris bairdii	43	Canada, USA	2008-2014	0,62		71
Calidris ferruginea	69	Russia	1991	1		55
Calidris fuscicollis	42	Canada	2005-2009	0,12		70
		Canada, USA	2008-2014	0,46		71
Calidris mauri	27	USA	1993-1995	0,58	0,19	103
		USA	2008-2014	0,43		71
Calidris melanotos	68	Canada, Russia, USA	2008-2014	0,63		71
Calidris pusilla	29	USA	1993-1995	0,41	0,26	103
		Canada, USA	2008-2014	0,65		71
Calidris temminckii	24	Finland	1983-2001	0,36	0,19	92
		Finland	1993-1997	0,47	0,06	102
Charadrius alexandrinus	48	USA	1998-1999	0,4	0,16	44
		Portugal	2020	0,24	0,1	This study
Charadrius hiaticula	64	Scotland	1985-1997	0,32	0,08	99
Charadrius melodus	55,6	USA	2006-2007	0,61	0,13	15
		USA	2006-2009	0,2	0,08	64
		USA	2008-2015	0,65	0,23	3

Charadrius nivosus	41,1	USA	2007-2009	0,4	0,09	82
		USA	2001-2016	0,33		6
Charadrius semipalmatus	47	Canada, USA	2010-2014	0,62		71
Charadrius vociferous	95	USA	1998-1999	0,42	0,27	44
Haematopus bachmani	555	USA	2001-2004	0,32	0,09	66
Haematopus ostralegus	540	Scotland	1996-1997	0,74	0,19	99
Haematopus palliatus	720	USA	2006-2010	0,16	0,06	60
		USA	1998-2008	0,28		12
		USA	2010-2011	0,09	0,07	9
Himantopus himantopus	180	Spain	1990-1991	0,59	0,14	2
		Portugal	2020	0,56	0,14	This study
Himantopus mexicanus	186	USA	1998-1999	0,31		44
Limnodromus scolopaceus	110	Canada, Russia, USA	2008-2014	0,48		71
Limosa limosa	340	Poland	2009-2015	0,49	0,08	74
Numenius Americanus	689	USA	2015-2016	0,41	0,06	69
Numenius phaeopus	430	Canada	2010	0,47		71
Phalaropus fulicarius	63	Canada	2005-2006;2008-2009	0,19		70
		Canada, Russia, USA	2008-2014	0,7		71
Phalaropus lobatus	36	Sweden	2008-2013	0,8		68
		Canada, Russia, USA	2008-2014	0,53		71
Pluvialis apricaria	220	England	1986-1988	0,59		90
		Sweden	2008-2013	0,47		68
		Scotland	2009-2013	0,8	0,04	78
Pluvialis dominica	160	Canada, USA	2008-2014	0,52		71
Pluvialis squatarola	240	USA, Canada, Russia	2008-2014	0,49		71
Recurvirostra avosetta	280	Spain	1990-1991	0,62	0,14	2
		USA	1998-1999	0,3	0,28	44

Tringa totanus	120	Scotland	1985-1997	0,42	0,19	99
		Sweden	1998	0,73		97
		Germany	2000-2001	0,15		94
		Germany	2006-2007	0,36	0,2	83
		Poland	2009-2015	0,39	0,07	74
		Sweden	2008-2013	0,65		68
		England	2013	0,22		7
Vanellus vanellus	230	England	1996-1998	0,66	0,03	96
		Scotland	1996-1997	0,57	0,17	99
		Scotland	1996-1998	0,61	0,07	98
		England, Wales	1996-2005	0,37		65
		Germany	2003-2009	0,51	0,11	72
		Poland	2009-2015	0,5	0,08	74
		England	2013	0,57		7
		England	2014	0,32		8

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