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Effects of nest and nest site characteristics on Humboldt penguins' breeding success at Punta San Juan, Peru: Implications for conservation.

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partial fulfillment of the requirements for the degree
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Effects of nest and nest site characteristics on Humboldt penguins' breeding success at Punta San Juan, Peru: Implications for conservation

Abstract

Populations of Humboldt penguins (*Spheniscus humboldti*) in Peru began declining significantly in the 19th century because of industrial guano mining and a later increase in fishing. Guano mining changed the features of the penguins' breeding habitat by eliminating the substrate where this species excavated their burrows. We analyzed the effects of nest and nest site characteristics and monitoring methodologies on Humboldt penguins' breeding performance at Punta San Juan, Peru. We used a binomial generalized linear mixed model with random intercept to measure the probability of success and also analyzed the number of fledglings produced among nests with different characteristics. We identified nests (including artificial nests) and classified them by nest cover (covered or uncovered), nest site habitat (cliff tops and beaches or caves) and nest substrate (guano or other substrate). We also assessed the impact of invasive and noninvasive nest monitoring methodologies on breeding performance. We included year and breeding season as random effects to account for environmental oscillation between and within years. Our results showed that the combination of guano substrate with noninvasive nest monitoring and nest cover were best correlated with penguin breeding success (increased probability of success and number of successful fledglings). The mean number of fledglings produced changed significantly between years and breeding seasons. In a separate analysis, breeding success of artificial nests was similar to natural covered or uncovered nests. Artificial nests in guano substrates were more successful than artificial nests in other substrates. Breeding success between habitats was similar. Our results show that nest and nest site characteristics affect Humboldt penguins' breeding performance. Management decisions regarding penguins breeding habitat can have an effect on

the conservation of Humboldt penguins. A clear experiment is required to disentangle the effects of monitoring methodology and substrate on Humboldt's penguins' breeding performance, but as cautionary measures we recommend careful nest monitoring and avoiding guano extraction from penguins' breeding sites.

Introduction

Habitats differ in environmental conditions and resources through space and time. The interaction between an organism's genotype or phenotype and the environment will lead to differences in the outcomes of reproduction and survival; these differences in individuals' performance (fitness) among environments is the basis upon which natural selection operates (Abrams 2007). Hence, there is strong selective pressure to distinguish good, marginal and poor habitats. For these reasons, knowing the value and relative importance of various habitats for a target species is needed to prioritize conservation efforts and assess management strategies (Johnson 2007).

Environmental conditions can affect organisms in many ways. Environmental factors and organisms' tolerance to freezing and desiccation affect species distribution limits of tide pool and intertidal organisms (Metaxas 1993; Connell 1961). Habitat conditions can also modify community structure and interspecific interactions (Bertrand et al. 2008; Siddon and Witman 2004). In addition, weather patterns such as rainfall can affect population dynamics by increasing reproductive success of Song Sparrows (Chase et al. 2005) and Darwin's finches (Grant et al. 2000). Many environmental conditions present an intrinsic temporal oscillation and/or are subject to stochastic changes. Seasonal, human induced or stochastic changes in habitat quality

can have important effects on entire ecosystems and among various trophic levels, thus affecting species distributions and survival (Bakun and Weeks 2008).

Measuring habitat quality can help us understand and predict how fitness, distributions and population numbers are affected by environmental characteristics (Orians and Wittenberger 1991). There are many ways to assess habitat quality. For birds, Johnson (2007) differentiates two main methods: measuring habitat quality by direct attributes (resources and environmental constraints) or by measuring birds to reveal habitat quality (density, survival, reproduction, distribution and individual condition). Johnson (2007) also points out that many variables should be measured because several aspects of a given habitat can affect life history parameters and performance. When measuring the effects of multiple variables on a target organism, researchers not only need tools for prediction and hypothesis testing, but also tools to assess the relative importance of each of the measured variables. Model selection and parameter estimation allow quantitative predictions of the differential effect of measured factors on an organism's response (Shaffer and Burger 2004). Selection of the environmental variables, biological responses and the scale of the measurements are critical, because the effects of environmental conditions on individual performance are scale-sensitive, thus the scale of the measured conditions can affect the results (Bowyer and Kie 2006).

Measuring the effects of environmental factors on breeding success is a common objective in ecological and conservation studies (Hazler 2004). Breeding success, as an indirect measurement of fitness, can provide information to assess the viability of a given population (Shaffer and Burger 2004; Johnson 1979) and breeding patterns can act as long term indicators of fluctuations of environmental conditions and productivity (Boersma 1978).

For seabirds, breeding success can be highly affected by environmental conditions (Simeone et al. 2002; Crawford et al. 2006; Boersma 1998; Boersma 1978), inter- and intraspecific interactions (Stokes and Boersma 1998; Tenaza 1971) and age and experience (Mougin, Jouanin, and Roux 2002). Seabirds can also change their foraging or reproductive behavior to overcome changes in their environment (Kitaysky et al. 2000). Seabirds live in very diverse environments and experience a very large range of weather patterns (Schreiber and Burger 2002). For these species, breeding site selection is probably based on environmental factors such as degree of shade, wind level or distance to open waters (Schreiber and Burger 2002). Nest site quality has important effects on breeding outcome. The nest, which is a good example of habitat at a small spatial scale, can aid in securing a mate, supporting a stable microenvironment (Paredes and Zavalaga 2001), protecting adults and their brood from adverse environmental conditions such as rainfall (Simeone et al. 2002), and decreasing the risk of predation (Frere, Gandini, and Boersma 1992). Thus, nest selection and nest site availability can have important effects on breeding success.

Nest cover is an important characteristic positively correlated with breeding success (Gandini, Frere, and Boersma 1999; Stokes and Boersma 1998). The nest can have an effect on breeding success by modifying environmental conditions and providing a more or less stable environment (Frere, Gandini, and Boersma 1992). Nesting habitat modification and degradation can affect seabirds breeding performance and is a major threat to their survival (Schreiber and Burger 2002).

Population decline in Humboldt penguins (*Spheniscus humboldti*) is a clear example of how both habitat degradation and efforts to protect their breeding sites can impact population persistence. For this species human alteration of their main breeding habitat contributed to its pronounced

population decline (Boersma and Stokes 1995). Guano extraction during the 19th century practically eliminated the main substrate where this species excavated their nests (Murphy 1936, Cushman 2003, Zavalaga and Paredes 1998). By mining the thick guano deposits from the islands used as breeding colonies of large populations of Guanay cormorant (*Phalacrocorax bougainvillii*), Peruvian booby (*Sula variegata*) and Peruvian pelican (*Pelicanus thagus*), commonly referred as guano birds, the features of these islands changed dramatically, leaving bare rocks and steep slopes where Humboldt penguins used to excavate their burrows (Murphy 1936). In addition to that, guano harvesters also hunted the penguins and collected their eggs, contributing to the population decline (Duffy 1984, Paredes et al. 2003). For these reasons and the later increase in fishing efforts and its adverse interactions with wildlife (Duffy 1983, Duffy 1984, Majluf et al. 2002), urbanization and coastal development of Peru (Duffy 1984) and recurring ENSO events that alter environmental conditions and prey abundance (Bertrand et al. 2008; Culik et al. 2000; Taylor et al. 2002), in less than 200 years Humboldt penguin populations have declined from hundreds of thousands to approximately 30,000 to 40,000 individuals (De La Puente et al. 2013). In more recent years, the government carefully managed guano harvest and paid special attention to guano bird populations, promoting scientific research and providing some of the first examples of large scale sustainable management efforts (Cushman 2003; Cushman 2005).

By protecting guano bird breeding colonies, guano management also protected portions of the breeding habitat of other species (Paredes et al. 2003). Today many penguin, fur seal and sea lion breeding sites are located inside the protected guano islands and headlands, which have been included as part of a natural reserve network (Reserva Nacional Sistema de Islas, Islotes y Puntas

Guaneras (RNSIIPG)) since 2009. Punta San Juan, one of these headlands, is the largest known Humboldt penguin breeding site in Peru (Paredes et al. 2003).

Human presence can also have a detrimental effect on breeding success. For some seabirds, tourism and disturbance associated with research can result in nest desertion, temporary nest abandonment, increased risk of predation and nest destruction (Schreiber and Burger 2002). For Humboldt penguins, the most timid species of the Sphenisciformes (Ellenberg et al. 2006), human disturbance such as direct visual contact in their breeding sites increases their heart rate and can lead to nest abandonment (Taylor et al. 2002, Ellenberg et al. 2006).

Humboldt penguins are listed as vulnerable on the IUCN Red list of Threatened Species and are also protected by the Peruvian law (De La Puente et al. 2013). Current conservation efforts range from protection of their breeding sites and avoiding guano extraction in breeding areas to the use of artificial nests to increase nest availability. Nevertheless, the success of these conservation strategies has yet to be measured. Hence, in order to recommend management decisions regarding priority areas for conservation and the impact of habitat modification on the conservation of this species, we need more detailed information about how environmental characteristics can affect their performance and survival.

Here, the relationship between breeding success environmental conditions and human perturbation are analyzed by measuring the effects of nest and nest site characteristics and monitoring methodology on the breeding success of Humboldt penguins at one of the most important colonies in Peru. The goal of this project is to identify the most important factors that contribute to breeding success of Humboldt penguins at Punta San Juan, Peru.

Objectives

- Measure the effects of nest cover, habitat, substrate and monitoring methodologies on Humboldt Penguin breeding success at Punta San Juan, Peru
- Understand the contribution of each factor to breeding success to assess artificial nests and guano extraction zonation as ways to improve penguin reproductive success

Material and Methods

Study Organism

Humboldt penguins are endemic to the Humboldt Current System (HCS); its distribution ranges from Isla Foca, Peru ($5^{\circ}12'S$) to Metalqui, Chile ($42^{\circ}12'S$) (De La Puente et al. 2013).

Humboldt penguins feed mostly on anchovies, silversides and other pelagic shoal-forming fish, and squids and crustaceans (De La Puente et. al 2013).

Humboldt penguins breed throughout the year in Peru, with two major reproductive peaks in April/May and August/September (Paredes et al. 2002). Females lay two eggs in each nesting attempt, and incubation lasts for approximately 40 days and chick rearing 75 days (Paredes et al. 2002). In Punta San Juan reproduction occurs from March to December; females commonly have two clutches per year (Paredes and Zavalaga 2001). Both parents guard and feed the chicks and take turns foraging at sea (Luna-Jorquera & Culik, 1999; Taylor et al. 2002; Hennicke & Culik, 2005).

Study Site

Punta San Juan (PSJ) (15022´S, 75012´W) is located at the Nazca district, Ica region, Peru. This headland is part of the national reserve network “Reserva Nacional Sistema de Islas Islotes y Puntas Guaneras” (RNSIIPG) since 2009. The 54 ha reserve is protected from terrestrial predators and human perturbation by a 1.2 km long concrete wall 2.5 meters in height. PSJ is an arid zone without vegetation cover where large numbers of guano birds breed between December and March. Humboldt penguin colonies are located throughout the reserve; the colony at S7 and S8 beaches is the largest one (Figure 1).

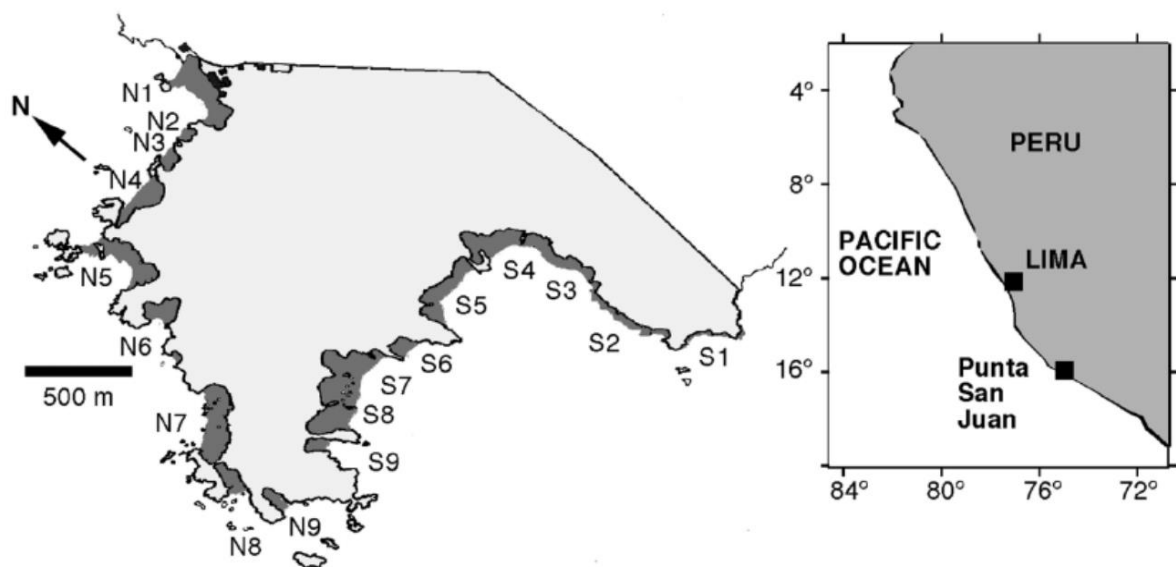


Figure 1. Map of Peru indicating location of the Punta San Juan guano reserve and denoting the location of the 20 beach sites within the reserve. Map from Paredes & Zavalaga (2001).

PSJ is the only guano reserve with scientific personnel continuously monitoring seabird and marine mammal population numbers and protecting the reserve from direct land-based human perturbation since 1983. The Punta San Juan Project (PSJP) and the St. Louis Zoo Wild Care

Institute Center for the Conservation of the Humboldt Penguin in Punta San Juan, with cooperation of other institutions, have been researching and protecting the local fauna present at PSJ, and conducting Humboldt penguin annual censuses along the entire coast of Peru, performing annual health assessments and participating in the past guano extraction campaigns. In addition, artificial nests for Humboldt penguins were built on the cliff tops (S5, S7-S8, N9 and N6. Figure 1.), but the effect of this effort has not been measured. Guano has not been harvested where Humboldt penguins breed at PSJ since 2001, when the first sustainable guano harvest took place.

Monitoring was conducted at PSJ between March and December 2012. During the months of August-October, a guano extracting campaign took place. A database from the Punta San Juan Project of nest content observations and nest characteristics from 2000 to 2011 was also analyzed. The entire dataset followed a very similar protocol and included the same sampling sites.

Study Design

By observation and statistical model building, we assessed the relationship among breeding success and habitat conditions and human perturbation. Immediately prior to the start of the first breeding season, sample sites and nests were identified inside commonly used nesting areas with the help of the reserve personnel to ensure that the monitored areas could be followed. To analyze breeding success, active nests inside monitored areas were checked during the entire breeding period. Intervals between visits and the number of visits varied among years (Appendix 1). High density, superficial nests were checked from 30-100 meters with help of a telescope and binoculars (noninvasive) and low density and more protected nests were checked individually

and directly manipulated with help of a flashlight (invasive). Between 2008 and 2011 only invasively monitored areas were visited. By regular visits the nest phenology was estimated.

Environmental Characteristics

To measure the effects of human perturbation and nest and nest habitat characteristics on breeding success, nests at monitored areas were identified and characterized by nest cover, nest habitat and substrate. The methodology used to check every nest was included as a binary nominal explanatory variable (invasively (I) or noninvasively monitored (II)). Due to the relatively small size of the reserve and the complex characteristics of every sampling site, we assumed that the entire reserve works as a single block where all visited nests are in the same area even though they are physically separate. The sample unit is the breeding attempt. Only one breeding attempt can occur at a nest at any point in time, but the same nest can be reused by the same or another breeding pair for another breeding attempt. Since nest and breeding pair identification was not the same from year to year (nests monitored non-invasively were assigned a separate identification code every year at the beginning of the breeding season and the breeding pair identity could not be recognized) and nest characteristics can change in time, we assumed independence between breeding attempts.

Nest cover was classified into one of three categories depending on the exposure of the nest content where (I) uncovered nests are open scrapes poorly excavated without a roof but sometimes with a back (Battistini and Paredes, 1999), (II) covered nests are protected nests with side and roof cover, and (III) artificial nests are human-made covered nests of concrete. Artificial nests were only monitored during 2012.

Nest site characteristics include nest habitat, substrate and monitoring methodology. Nest habitat was placed into one of two categories: cliff tops (I) or beaches and caves (II). The second category included a wide variety of habitats that are located in a gradient from sea level to cliff tops (landslides, boulders and slopes located in beaches and sea caves), but only a few nests fit this grouping. Substrate was divided into two categories: guano (I) and other substrates (II). The main difference between substrates is that guano substrates are easily excavated while other substrates are much harder to dig.

To account for environmental oscillation between and within years, the year and the breeding season of each breeding attempt were included as explanatory variables. The first breeding season included all nests that started between January and June and the second season included all nests that started between July and December.

Measures of Success

Breeding success was measured in two ways. First, to model the probability of success by a generalized linear mixed model approach, we measured breeding success as a binomial variable; a breeding attempt was considered successful if at least one chick survived for 60 days, otherwise it was considered a failure. Second, we measured the number of fledglings produced by each breeding attempt (fledglings/nest). Each breeding attempt can produce 0, 1 or 2 fledglings. We considered a fledgling to be any chick with an age of 60 days or older.

Database management and age estimation

Data from the 2000-2012 nest content surveys were put into three discrete categories: empty nests, active nests during the incubation period and active nests during the rearing period. When possible, chicks that moved from their nests to other locations were counted as if they remained

in their nests. To determine nest fate, only nests that were found during the incubation period were taken into account. Nests with unknown fate were not considered in the analysis. Because it was more difficult to correctly determine nest content and age using the noninvasive approach (especially during the incubation period, early rearing or when nests had cover), adult behavior and chick molting stages were taken into account as cues to assess whether the chick was alive or dead; nevertheless, in most cases the length of the survival period of the breeding attempt was also compared with the observed content to assess if age was consistent with the content (incubation takes approximately 40 days and rearing 70 to 90). If the nest content did not match the stage (incubation or rearing) for its time of survival, that particular breeding attempt was removed from the analysis.

The non-invasive methodology increased error in correctly assigning early rearing stages to the rearing period because of the distance from which nests were monitored and the size of newly hatched chicks, resulted in a mismatch in the duration of the incubation and rearing periods between monitoring methodologies. Because of our monitoring methodology, incubation time of nests where chicks were observed was significantly longer ($t=-38.39$ $p<0.01$) for breeding attempts monitored noninvasively than for the breeding attempts monitored invasively (group mean: 39.6 days for invasively monitored breeding attempts, 62.2 days for noninvasively monitored breeding attempts). Hence, the age of actual fledglings from successful noninvasively monitored nests would be underestimated, thus decreasing the number of chicks considered as successful fledglings for this category. This problem was corrected using the reference incubation time of 42 days for the incubation period in all nests where chicks were seen. The incubation period was assumed to be 42 days and additional days (previously accounted as incubation) were assigned to the rearing period. In this way underestimation of success was

avoided (appendix 2). We also used this reference period of incubation for invasively monitored nests; these nests were visited less frequently so that days of rearing could also have been erroneously considered inside the incubation period depending on the interval between the last visit when eggs were seen and the first visit when chicks were seen.

Analysis

The effects of nest and nest site characteristics, human perturbation, year and breeding season on the probability of breeding success were analyzed using binomial generalized linear mixed models with random intercept. These models estimate parameters for each explanatory variable using restricted maximum likelihood estimation solved by iteratively weighted least squares. The differential effects of each category of the explanatory variables on the probability of success will be represented as a parameter, but one of the categories of each explanatory variable will be used as the baseline and included in the intercept. The main assumption of this model is that all breeding attempts survive or fail independently of one another.

To avoid unbalanced sampling sizes (avoid lack of observations for certain combination of categories of explanatory variables), the smallest sample size considered for each fixed effect was 10 breeding attempts by breeding season, otherwise the breeding attempts from the particular breeding season were not taken into account (Appendix 3: sample size).

Our preliminary analysis showed that monitoring methodology and substrate were highly correlated and their effects could not be disentangled. For this reason the following analysis represents both variables as one categorical variable with 4 levels (methods-substrate).

Nest cover, habitat and the combination of monitoring methodology and substrate were included in the model as fixed factors and year and breeding season as partially crossed random factors.

Breeding attempts from natural nests through all the monitoring period and natural and artificial nests during 2012 were analyzed as two separate data subsets using different models. The main subset which includes most of the gathered data measures the effects of nest cover, habitat and the combination of monitoring methodology and substrate on the probability of success and the mean number of fledgling from natural nests between 2000 and 2012. The second subset assesses the differences in breeding success of natural and artificial nests, and substrates of noninvasively monitored nests on cliff tops during 2012 (Appendix 3).

Model selection was performed using likelihood ratio tests and AIC values from the models that included all the explanatory variables and their simplified version with only significant factors (Appendix 4: model selection).

We test between groups of nests for differences in the number of successful fledglings produced and the probability of success (fitted model values) using the Mann-Whitney U test (for comparing two groups) and the Kruskal-Wallis rank sum test (for comparing more than two groups).

All the analysis was performed using R (R Development Core Team 2008). Model fitting and graphs were performed using lme4 (Bates et al 2009) and ggplot2 (Wickham 2009) packages respectively.

Results

From a total of 4248 monitored breeding attempts found during incubation, 3806 were analyzed (3665 breeding attempts analyzed in the main model, 563 breeding attempts in the model including artificial nests); the rest were discarded because of unknown fate or because the small sample size of the categories they represented. Due to its small sample size (47 breeding attempts

and less than 10 for each breeding season) invasively monitored nests in guano substrate were not included in the analysis. The 2011 second breeding season of noninvasively monitored nests in guano substrate was also discarded due to its small sample size ($n < 10$). Only artificial nests were noninvasively monitored in other substrates ($n = 70$) and were not considered in the main model. For this reason, the variable representing the combination of monitoring methodology and substrate in the main data subset contains 2 categories: invasive monitoring methodology/other substrate and noninvasive monitoring methodology/guano substrate (figure 2).



Figure 2. Nest distribution at PSJ. Upper panel: distribution of covered and uncovered nests within monitoring methodology/substrate categories. Lower panel: distribution of nests in different habitats within monitoring methodology/substrate categories.

Natural Nests 2000-2012

Nest cover and the combination of monitoring methodology and substrate had a significant effect on the probability of success and were selected in the most supported model (table 1.). The variable representing the combination of nest monitoring methodology and substrate had the highest and most significant contribution to the probability of success (monitoring methodology/substrate: $F=134.06$, $p\text{-value}<2e-16$; nest cover: $F=11.91$, $p\text{-value}=0.0005$).

Main Model				
random effects: year+ breeding season				
fixed effects: monitoring methodology/substrate+nest cover				
baseline: Invasive/other + covered				
parameters	Estimate	Std. Error	z value	Pr(> z)
baseline	-1.7313	0.49742	-3.481	0.0005
non invasive/guano	0.74404	0.10565	7.042	1.89E-12
uncovered	-0.26785	0.09801	-2.733	0.00628

Table 1. Most supported main model. Data includes 3665 breeding attempts monitored between 2000 and 2012

Random Effects: Year and Breeding Season

The mean number of fledglings produced varied significantly by year ($K= 227.1619$, $df = 12$, $p\text{-value} < 2.2e-16$). Breeding attempts during the first breeding season had a significantly higher number of fledglings than breeding attempts during the second breeding season (first season= 0.62 ± 0.82 . second season= 0.24 ± 0.57 ; $U= 2125025$, $p\text{-value}<2.2e-16$). During the second breeding period of 2001 and 2004 no noninvasive monitored nest in guano substrate was successful (sample size: 2001=70, 2004=45) (Fig. 3).



Figure 3. Top panel: Number of fledglings from non-invasively monitored nests in guano substrate by year and breeding season. Middle panel: Number of fledglings from invasively monitored nests in other substrate by year and breeding season. Bottom panel Number of fledglings by year and method-substrate category including artificial nests noninvasively monitored in other substrate. Whiskers represent confidence intervals.

Fixed Effects: Monitoring Methodology/Substrate, Nest Cover & Habitat

Nests in guano substrate and monitored noninvasively had higher fitted values of the probability of success and mean number of chicks produced than those in other substrates and monitored invasively (figure 4) (Probability of success: $U = 664972$, $p\text{-value} < 2.2e-16$; mean number of fledglings: $U = 1401424$, $p\text{-value} < 2.2e-16$). The parameter representing the contribution of noninvasively monitoring methodology/guano substrate was positive and significantly different from 0 (noninvasive/guano = 0.744, $p = 1.89e-12$). Noninvasively monitored nests in guano

substrate during 2000-2007 and 2012 produced on average 0.57 ± 0.78 fledglings and invasively monitored nests in other substrate only 0.35 ± 0.72 (Figure 4).

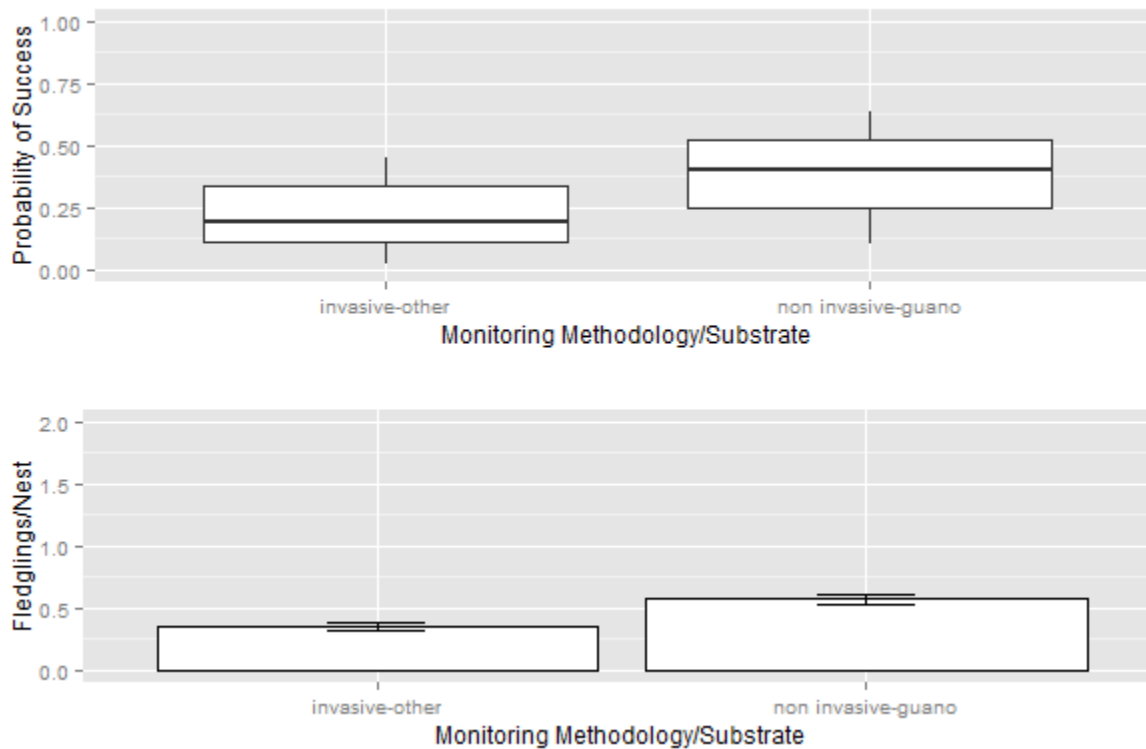


Figure 4. Upper panel: Fitted values of the probability of success from breeding attempts noninvasively monitored in guano substrate and invasively monitored in other substrate. Fitted values from most supported model. The horizontal line in each box represents the median, the box defines the hinge and the whiskers are 1.5 times the hinge. Bottom panel: mean number of fledglings produced from breeding attempts noninvasively monitored in guano substrate and invasively monitored in other substrate. The whiskers represent the confidence interval.

The parameter representing the contribution of uncovered nests to the probability of success was negative and significantly different from 0 (uncovered nests: -0.26 p -value = 0.006). Covered nests in noninvasively monitored areas with guano substrate had higher fitted values of the probability of success and produced a higher mean number of fledglings than uncovered nests in the same areas (probability of success: $U=577186$ p -value $< 2.2e-16$; mean number of fledglings:

covered=0.66±0.81, uncovered=0.53±0.76; $U=477392.5$, $p\text{-value}=0.0002837$), but covered and uncovered nests invasively monitored in other substrate had similar fitted values of the probability of success and mean number of fledglings produced (probability of success; $U=64362$, $p\text{-value}=0.279$; mean number of fledglings: covered=0.35±0.72, uncovered=0.36±0.72; $U=59458.5$, $p\text{-value}=0.8621$) (Figure 5). Covered and uncovered nests monitored noninvasively in guano substrates produced significantly more fledglings than similar nests invasively monitored in other substrates (covered nests: $U=397909$, $p\text{-value}<2.2e-16$; uncovered nests: $U=45621.5$, $p\text{-value}=0.03$) (Figure 5).

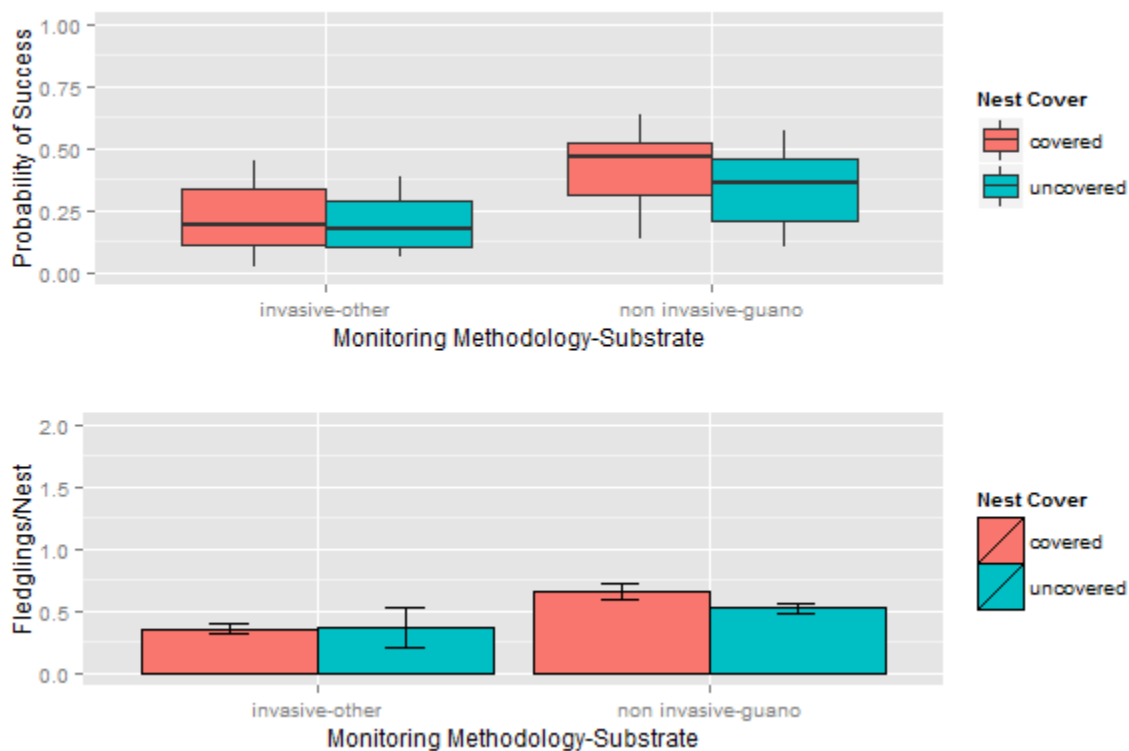


Figure 5. Probability of success and mean number of fledglings between superficial and covered nests invasively and noninvasively monitored in guano and other substrates.

Habitat categories had a similar contribution to the probability of success and this variable was dropped during model selection. For these reasons, this variable was not included in the most supported model. In addition, beach/cave noninvasively monitored nests in guano areas were only monitored during 2012, while cliff tops in the same monitoring methodology/substrate category were monitored between 2000 and 2007 and in 2012. Invasively monitored nests in other substrates were monitored in both habitats during the entire research period, but we only found covered nests in cliff top habitats in this monitoring methods/substrate category.

Our results show that there was a significant difference in the mean number of fledglings produced between cliff top and beach/cave habitats between 2000 and 2012 (cliff top= 0.52+/- 0.77, beach/cave=0.4+/-0.73; U=1447460, p-value=1.309e-08), but this difference was not significant for breeding attempts monitored during 2012 only (U=63564.5 p-value=0.06). When analyzed separately between monitoring methodology/substrate categories, we found no significant differences in the mean number of chicks produced between neither habitats, but noninvasively monitored covered nests in guano substrate were the most successful nest type and produced significantly more chicks than uncovered nests in the same habitat and monitoring methodology/substrate categories (Table 2).

Monitoring Methodology/Substrate	Habitat	Nest Cover	n	Fledglings	sd	p-value	
Non Invasive/Guano	Cliff Top	covered	559/114	0.65/0.79	0.81/0.82	<0.001/0.59	*/0.88
		uncovered	1246/308	0.5/0.74	0.75/0.79		
	Beach/Cave	covered	*/82	*/0.7	*/0.8	*/0.5	
		uncovered	*/124	*/0.79	*/0.83		
Invasive/Other	Cliff Top	covered	445/52	0.41/0.46	0.77/0.8	0.67/0.59	0.08/0.27
	Beach/Cave	covered	1133/70	0.33/0.28	0.7/0.64		
		uncovered	76/13	0.36/0.46	0.72/0.87		

Table 2. Mean number of fledglings produced. Values on the left side of the slashed line indicate results from the entire 2000-2012 period, while values on the right side of the slashed line indicate results from 2012 only. P-values

from Mann-Whitney U test

Artificial Nests

Artificial nests did not have a significantly different contribution to the probability of success than natural covered or uncovered nests. The parameter representing the effect of other substrates to the probability of success was negative and significantly different from zero (Table 3).

Artificial, covered and uncovered nests in guano substrate produced a similar number of fledglings, but artificial nests in a guano substrate produced significantly more fledglings than artificial nests in other substrates (Table 4).

Artificial Nest Model				
random effects: breeding season				
Fixed effects: nest type+substrate				
baseline: guano substrate				
parameters	Std.	Error	z	value
artificial nests	0.10918	0.33666	0.324	0.7457
natural covered nests	-0.02839	0.29809	-0.095	0.92413
natural uncovered nests	-0.08285	0.25656	-0.323	0.74674
other substrates	-1.00273	0.36085	-2.779	0.00546

Table 3. Artificial nest model. Data includes 563 breeding attempts from noninvasively monitored nests in cliff tops during 2012.

Substrate	Nest type	n	Fledglings	sd	p-value
guano	natural covered	114	0.79	0.82	0.79
	natural uncovered	308	0.74	0.79	
	artificial	79	0.7	0.75	
other	artificial	62	0.4	0.66	0.009

Table 4. Mean number of fledglings produced. P-values from Mann-Whitney U test for artificial nests between substrates and Kruskal-Wallis Chisq between nest types in guano substrates

Discussion

Our findings suggest that the combination of monitoring methodologies and substrate have the strongest effect on the breeding performance of Humboldt penguins (table 1, figure 4); however, we were not able to disentangle their effects. Breeding attempts noninvasively monitored in guano substrate were significantly more successful than breeding attempts invasively monitored in other substrates (figure 4). In addition, artificial nests in guano substrates were more successful than artificial nests in other substrates. Together, these results and the fact that most nests at PSJ are located on guano, show that substrate might have an effect on breeding performance, but these results must be supported by a clear experiment with balanced sample sizes for all the present categories. Although guano is considered as Humboldt penguins' historical breeding substrate, nowadays many of the Humboldt penguin colonies in Peru lack big guano deposits. An ongoing management strategy where guano extraction cannot mine where penguins reproduce was first tested at PSJ during 2001 and represents the commitment of the guano management to perform sustainable extraction activities. In addition, guano depth is positively correlated with the number of covered nests (Paredes and Zavalaga 2001) and previous research (Paredes and Zavalaga 2001) and our results show that the most successful breeding attempts were located on cliff tops and used noninvasively monitored covered nests with guano as substrate (Table 2). Human perturbation has a detrimental effect in other seabird species (Schreiber and Burger 2002) including Magellanic penguins (Schreiber and Burger 2002) and it could have an important effect for Humboldt penguins as well, especially because of their known lower tolerance to human presence (Ellenberg et al. 2006). Besides, research methodologies should be carefully implemented to avoid harming the target species and interaction with other factors which could affect the research goals. The nest distribution at PSJ

is skewed towards noninvasively monitored guano areas (PSJP unpublished data) and the frequency of covered nests is much higher in invasively monitored/other substrate areas than in noninvasively monitored guano ones (table 2, figure 2, upper panel).

The combination of monitoring methodology and substrate represents ongoing management decisions that can be easily modified in order to protect Humboldt penguins. Nevertheless, more information is required to measure the effect of substrate on Humboldt penguins breeding performance and correctly assess the conservation significance of guano zonation management strategies. Future research should also seek to identify the stages when most breeding attempts fail or lose nest content and relate it to differences in habitat categories and monitoring methodologies. Invasive monitoring is important to assess a number of habitats where nests could not be assessed otherwise; maintaining this methodology for these situations while avoiding its detrimental effects would be important for Humboldt penguins' research. This will require a clear understanding of the temporal or spatial conditions under which invasive monitoring is harmful, and gaining this understanding will require carefully designed experimental monitoring.

In addition, our results suggest that nest cover affects Humboldt penguins' breeding performance (table 1). Covered nests noninvasively monitored were more successful than uncovered nests in the same guano areas (Figure 3), especially for breeding attempts in cliff top habitats, where the largest breeding colonies at PSJ are located (table 2). For invasively monitored nests in other substrates there was no clear difference. A possible explanation for this could be that the combination of invasive monitoring methodology and other substrates have such a detrimental effect on penguins' breeding performance at PSJ, that the effects of nest cover and habitat are overwhelmed. The frequency of breeding attempts using uncovered nests in the invasive

monitoring methodology/other substrate category is relatively small and dispersed through time which can also affect the significance of the p-values (table 2, Figure 2).

Previous research highlighted the importance of burrow nests and guano deposits for the conservation of this species. Penguins using burrow nests have significantly higher reproductive success than those using other nest types (Paredes and Zavalaga 2001). Our findings are consistent with and support these results. For Magellanic penguins (*Spheniscus magellanicus*), a closely related penguin species that shares part of their range with Humboldt penguins, nest type, location, substrate, density and rain are factors that affect this species reproductive output (Frere, Gandini, and Boersma 1992; Stokes and Boersma 2000; Stokes and Boersma 1991; Boersma 2008). For Magellanic penguins nest cover positively affected fledging success by diminishing predation risk and exposure to extreme temperatures (Stokes and Boersma 1998; Stokes and Boersma 2000; Gandini, Frere, and Boersma 1999; Frere, Gandini, and Boersma 1992). During the study I observed kelp gulls (*Larus dominicanus*) and turkey vultures (*Cathartes aura*) predate superficial nests, thus supporting these findings.

The number and location of covered nests may depend on the site characteristics (Stokes and Boersma 1991) and there is a positive relationship between the thickness of the guano layer and the numbers of nests in burrows at PSJ (Paredes and Zavalaga 2001). Hence guano can increase site quality by allowing the excavation of burrow nests. Without the right soil or without guano, burrows cannot be dug (Stokes and Boersma 1998). Besides, at PSJ nest preferences might be driven by the strong effects of the combination of monitoring methodology and substrate and penguins nesting in invasively monitored areas with other substrates might be actively choosing covered nests. Future research should measure the effects of monitoring methodology, habitat and substrate on the distribution of Humboldt penguins' nests at PSJ.

We did not find any difference in habitat breeding performance within monitoring methodology/substrate, but cliff top habitats were more successful than beach/cave ones when all data was pulled together. Habitat was highly unbalanced between years and methodologies; beach/cave habitats were noninvasively monitored only during 2012 and have a relative small sample size (figure 2). To better measure the role of habitat, noninvasively monitored beach/cave habitats should be included as sampling areas in long term projects at PSJ. In Peru, Humboldt penguins nest on islands and rocky coasts, using natural crevices and sea caves, burrowing holes in guano or constructing open nests on the ground's surface. In Chile, Humboldt penguins' natural breeding habitat is in rock crevices under cliffs or rocks on the shore, but they also use human modified habitats like breakwaters, where they nest under the rocks (Simeone and Bernal 2000). On Chanaral Island, the major colony throughout their breeding range, Humboldt penguins nest in large numbers under dense shrubs and cacti (Mattern et al. 2004).

Sea caves are an available habitat for Humboldt penguins inside and outside protected areas, but there is little information about penguins' breeding success inside these places (Birdlife 2008). Although sea cave nests might have always been a natural breeding environment, only a small proportion of the large historical population used these nests (Murphy 1936). Besides, previous studies in Peru show that low altitude nests are prone to flooding and therefore have lower reproductive success (Paredes and Zavalaga 2001). In addition, guano is mostly available on cliff tops since guano birds nest there. Guano slopes are the result of the accumulation of byproducts (feathers and poor quality guano) from previous guano extraction campaigns and suggest that the correct disposal of these byproducts might increase high quality habitat, nevertheless more information and research on this topic is also required.

Breeding performance in artificial nests did not differ from that of natural nests (table 3 and 4). Artificial burrows in Namibia increased high quality nests for the African penguins (*Spheniscus demersus*) (Kemper, Underhill, and Roux 2007). Since artificial nests do not have a significant negative contribution to the probability of success and there is no difference in the mean number of fledglings between noninvasively monitored superficial, covered or artificial nests in guano substrates during 2012, our results suggest that artificial nests do not have any visible detrimental effect on breeding performance of Humboldt penguins' at PSJ and that they might increase the number of available nests.

The mean number of fledglings varied significantly between years and the first breeding period was the most successful one. One of the major environmental fluctuations that take place in the waters off the Peruvian coast is the "El Niño Southern Oscillation" (ENSO), which affects the entire ecosystem and the related human activities (McPhaden, Zebiak, and Glantz 2006). These fluctuations between strong upwelling, cold nutrient-rich and weak upwelling, warm nutrient-poor conditions and the consequent changes in productivity are an extremely strong climatic signal that can be felt worldwide (Bertrand et al. 2008; McPhaden, Zebiak, and Glantz 2006). The last ENSO events occurred during 2002-2003 and 2006-2007.

Humboldt and Galapagos penguins are adapted to ENSO related environmental variability. Galapagos penguins breed as many times as they can when the conditions are optimal and Humboldt penguins do not breed in harsh environmental conditions (Boersma 1978). Although these organisms are well adapted to deal with environmental uncertainty (Boersma 1978), current fishing pressures that decrease food supply can compromise the organisms' ability to recover from these events (Myers et al. 1995; Boersma 1998)

As top predators, Humboldt penguins' breeding success, chick development and population trends, can be helpful to assess changes in their environment (including management and conservation strategies), thus acting as marine sentinels (Boersma 1978; Furness and Camphuysen 1997; Boersma 2008). Because they are also a charismatic species, they can raise awareness of environmental problems and its conservation could benefit other species that share their habitat.

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