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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

Nest-type associated microclimatic conditions as potential drivers of ectoparasite infestations in African penguin nests

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Abstract

Nest design and characteristics can influence the microclimatic conditions in the nest. Nest-dwelling ectoparasites are sensitive to temperature and moisture and as such the conditions in the nest can influence parasite infestations. The endangered African penguin (*Spheniscus demersus*) breeds in different nest types and as yet little is known with regard to the microclimate and parasite infestation within these nests. This study characterized the microclimatic conditions in natural open, natural covered (with vegetation) and artificial nests, and assessed the relationship between nest characteristics (type, age, distance from the coast, orientation and entrance opening) and in-nest ectoparasite infestations and the health of African penguins in Stony Point, South Africa. Penguins (50 adults and 192 chicks) and their nests ($n = 308$) were sampled in 2016 and 2017. Soil temperature was higher in artificial than in natural nests, and soil and nest material moisture was lower in artificial and natural covered nests than natural open. Ectoparasite infestations were higher under warmer and drier conditions, in artificial nests and nests near the coastline. Penguin (adult and chick) body mass and chick body condition were lower in warmer nests and total plasma protein (in adults and checks) was lower in drier nests. Given the potential adverse effects of ectoparasites on host species, it is recommended that conservation agencies implement a monitoring programme to assess the ectoparasite infestation in artificial nests across multiple colonies. This information will facilitate a more holistic penguin conservation management plan that may prevent further detrimental effects on this endangered penguin species.

Keywords Nest characteristics · Soil temperature · Soil moisture · Ticks · Fleas · African penguin

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Introduction

Nests are used by multitudes of species that belong to taxa such as reptiles, birds, mammals and even fish. Nests form important components of animal ecology as they are the physical structure where animals complete reproductive tasks, rest and find protection from climatic conditions and predators (Hansell 2005; Mainwaring et al. 2014). For example, the eastern fence lizard (*Sceloporus undulatus*) oviposits in open nests (Angilletta Jr et al. 2009), while the eastern grey squirrel (*Sciurus carolinensis*) use tree cavities to bear and care for their young and rest at night (Broughton 2020). In the case of birds, nests are equally important and the nest type (i.e. design), building material (e.g. soil or feathers) and location (e.g. on the ground or in tree cavities) vary depending on the bird species (Hansell 2000).

The level of protection that a nest provides against predators and external climatic conditions varies with nest type and location (Hansell 2005). For example, open surface nests (i.e. on the ground) located on flat areas with no vegetation are more exposed to predators and climatic conditions (wind, solar radiation and rain) than open nests that are covered with vegetation or natural burrows (Hansell 2000; Mainwaring et al. 2014). The internal nest microclimate also varies depending on nest location, physical structure (Deeming and Mainwaring 2015) and orientation of the nest opening (Mainwaring et al. 2014; Michielsen et al. 2019). For example, Vleck et al. (1983) found that ground nests of terns and gulls were more humid than more ventilated tree nests of herons, egrets and ibis. Furthermore, enclosed nests (nests with walls and a roof) restrict ventilation and provide more protection against heat loss compared with open nests (Martin et al. 2017). Likewise, the thermal conductivity of building materials and the thickness of the nest walls confer different degrees of nest insularity (rate of heat moving across the nest material) and water penetration (Heenan 2013).

Human population increases have resulted in loss of natural habitat and resources, and a change in climate to the extent that breeding population sizes of wildlife are significantly affected (Barnosky et al. 2011). This is particularly relevant to seabirds of which several species are threatened (Paleczny et al. 2015). In an attempt to mitigate the threat of poor breeding habitat, artificial nests (i.e. man-made nest that mimic natural nests) are used as a conservation management tool to create suitable nest sites and ultimately improve the breeding success of threatened species (Bolton et al. 2004; Kemper et al. 2007). For example, the improved breeding success recorded for yelkouan (*Puffinus yelkouan*) and Scopoli's (*Calonectris diomedea*) shearwaters has been facilitated by the use of artificial burrows made from cement, plastic and other materials (Bourgeois et al. 2015). Artificial nest boxes and burrows have been constructed from plastic, wood, cement and fibreglass and also have several designs (e.g. square boxes, A-frame and dome-shape) (Du Feu 2005). Although the general aim is to provide suitable nesting and roosting habitat, the design of these nests also increases protection against predators and climatic conditions (Lambrechts et al. 2010; Sumasgutner et al. 2020). In particular, the physical characteristics of artificial nests (e.g. materials, wall thickness and entrance size) can influence the nest microclimate (Lambrechts et al. 2010), to the extent that artificial nests are warmer, drier and have a reduced air flow than natural nests (Lei et al. 2014). A recent study on marsh tits (*Poecile palustris*) found that wooden nest boxes were warmer and drier than nests in tree cavities. However, the study also found that nest boxes were poorly insulated against the ambient temperatures and therefore cannot replicate the thermal buffering effect of natural tree cavities (Maziarz et al. 2017). The high temperatures reached in nest boxes have been attributed to poor ventilation and therefore reduced heat loss by convection (Ropert-Coudert et al. 2004). In addition, the use of artificial nests over consecutive years, in addition to limited air flow in the nest, can facilitate the accumulation of nest material, which can create microhabitats for ectoparasitic fauna (Tomás et al. 2007).

Birds are parasitized by a diverse array of ectoparasite species that include fleas, soft and hard ticks (Argasidae and Ixodidae, respectively), mites and lice (Lehmann 1993). The ectoparasites differ in host association that ranges from permanent (lice) to temporary parasites (fleas, ticks and mites) (Marshall 1981; Lehmann 1993). The latter taxa are nidicolous, i.e. ectoparasites that live in or near to the host dwelling in for example, cracks, under stones and among the nest material (Sonenshine 1993; López-Rull and Macías Garcia 2015). Although all the life stages of fleas (egg, larva, pupa and adult) occur in the nest, only the adults consume blood, while most or all of the tick life stage (egg, larval, nymph and adult), depending on the species, require a blood meal (Sonenshine 1993; Bitam et al. 2010). Fleas and ticks, being arthropods, are ectothermic and thus sensitive to the microclimatic conditions in the nest (López-Rull and Macías Garcia 2015). For example, the abundance of the soft tick *Ornithodoros capensis* decreased with an increase in nest moisture in African penguins (*Spheniscus demersus*) (Daturi 1986). Furthermore, the abundance of hen flea (*Ceratophyllus gallinae*) larvae was positively correlated with the presence of the host in the nest and the duration of the warm period, which in turn influenced within-nest temperatures in nests of blue tits (*Cyanistes caeruleus*) (Tripet and Richner 1999). As mentioned above, artificial nests are generally drier and warmer compared with natural nests and it is possible that the microclimatic conditions associated with these nests can facilitate parasite infestations (Wesołowski and Stańska 2001; Hebda and Wesołowski 2012). For example, hen fleas were more prevalent in drier conditions associated with artificial nest boxes of great tits (*Parus major*) (Heeb et al. 2000). Other nest characteristics that can also influence microclimatic conditions and thus parasite infestations include the nest age (in relation to the accumulation of nest material), spatial location, opening size and orientation (George 1959; Mazgajski 2007; Moon et al. 2018).

Ectoparasites can directly affect their bird hosts through their physical presence or feeding behaviour, causing damage to the skin and feathers, stress, weakness and irritation (Lehmann 1993). Blood-sucking arthropods can cause anaemia (i.e. reduce the number or proportion of red blood cells) (Campbell and Ellis 2007), and the multiple injuries caused by ectoparasites can increase metabolic expenditure to compensate for the damage, thus altering host fitness which is reflected in the loss of host body condition and body mass. For example, Cassin's auklet (*Ptychoramphus aleuticus*) chicks with severe infestation of ticks (*lxodes uriae*) recorded lower wing growth and reached peak body mass later than chicks with fewer ticks (Morbey 1996). High ectoparasite infestations can induce adult birds to abandon eggs and chicks, such as seen in Peruvian seabirds deserting their nests in colonies highly infested with ticks (*Ornithodoros amblus*) (Duffy 1983). Ectoparasites, and especially ticks, and fleas also act as vectors for pathogens such as rickettsia, protozoa and viruses (Sonenshine 1993; López-Rull and Macías Garcia 2015) that can affect the health of the bird (Heylen et al. 2015). High ectoparasite infestations in the nests can therefore affect the population size and survival of endangered bird species (Williams et al. 2013).

African penguins, one of the most threatened seabird species in South Africa (BirdLife International 2018), generally return to the same colony and nest site to breed (Randall et al. 1987; Whittington et al. 2005). Adult penguins regularly lay two eggs that are incubated in the nest by both parents, and chicks remain in the nests for ca. 80 days after hatching while the parents rotate to feed at sea during the day and return to the nest in the evening (Cooper 1980). The deliberate removal of guano, the principal substrate used to excavate burrows in their natural habitats, brought about a change in the species' nesting behaviour to the extent that exposed open nests (on the surface) are now commonly used (Frost et al. 1976). In addition, penguins actively use artificial nests (made from cement, wood or fibreglass) that are introduced by colony managers in an attempt to improve breeding success (Sherley et al. 2012). However, in recent years, the conservation authority at Stony Point, one of the largest colonies along the south-western coast of South Africa, raised concerns about the incidence of soft ticks and a suspected concomitant decrease in the condition of penguin chicks at this colony. At Stony Point, African penguins actively use three nest types: natural open, natural covered and artificial nests. The artificial nests (comprising of fibreglass or cement) have been in use, in this colony, for more than 3 years. Given the diverse nest types and the uncertainly of which nests harboured higher ectoparasites infestations, it was important to establish if ectoparasites were randomly distributed among the different nests in the colony and if not random, to establish the nest-associated characteristics (type, age, distance from the coast, orientation and entrance opening) that influence ectoparasite infestations. The aims of the study were therefore to (1) record the microclimatic conditions associated with different nest types (natural open, natural covered and artificial nests) in the Stony Point colony; (2) determine the relationship between various nest characteristics (type, age, distance from the coast, orientation and entrance opening) and the in-nest tick and flea abundance and prevalence; and (3) establish the relationship between nest characteristics and incidence of tick-transmitted pathogens and the general health of African penguins. It was predicted that the microclimatic conditions will differ between the three nest types, given that they differ in design and the level of protection against weather conditions. In addition, it was predicted that warmer and drier nests will facilitate higher flea and tick infestations.

Materials and methods

Study site and sample size

This study was carried out in the Stony Point African penguin colony (34.3741° S, 18.8917° E), Betty's Bay, South Africa (Fig. 1). The colony lies along the south-west coast and receives winter rainfall with cold and wet conditions common from May to September. Since its establishment in 1982 (Whittington et al. 1996), the colony progressively grew in numbers to reach 2533 breeding pairs by 2015 (CapeNature unpubl. data). Management interventions have included the use of artificial nests and the addition of local vegetation to natural nests in an attempt to improve breeding sites by mimicking natural burrow nests.

Since the presence of a bird in the nest (nest occupancy) can positively affect ectoparasite demography by providing food in addition to heat and moisture to the nest microenvironment (Marshall 1981), nests occupied by a visually healthy chick and/or adult penguin (active nests) were included in the study. The nests and the penguins inside the nests were randomly selected and sampled during three sampling seasons: June/July (autumn/winter) 2016 (109 nests, 22 adults and 83 chicks), October/November (spring) 2016 (81 nests, 8 adults and 24 chicks), and June/July (autumn/winter) 2017 (118 nests, 20 adults and 85 chicks). During each sampling period, the nests comprised three different nest types: artificial (fibreglass or cement-fibre nests (ca. 10% cement nests)) (Fig. 2a), natural covered (nests sheltered by vegetation, i.e. *Tetragonia fruticosa*) (Fig. 2b) and natural open (open nests with or without a few dry branches covering on top) (Fig. 2c). A total of 84 penguins (adults and chicks) from artificial, 87

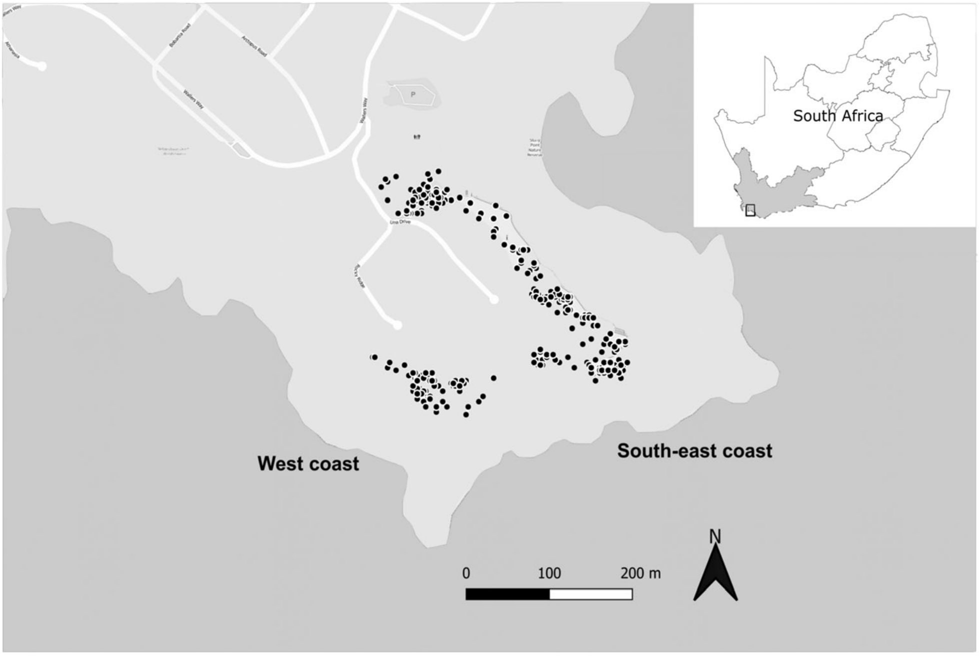

Fig. 1 The Stony Point penguin colony in Betty's Bay, South Africa. Black dots are African penguin nests sampled during the three sample periods (i.e. autumn/winter 2016, spring 2016 and autumn/winter 2017)

penguins from natural covered and 71 penguins from natural open nests were sampled across the seasons (Table 1). Sampling took place from 9:00 to 16:00 h.

Assessment of nest microclimatic conditions

iButton data loggers (Thermochron®; temperature range − 40 + 85 °C and resolution 0.5 °C) were inserted in the soil of the sampled nests at 5-cm depth. This was done to measure the temperature of the environment where nest ectoparasite live (i.e. among the nest material and ground surface; López-Rull and Macías Garcia 2015). Each iButton was set to record temperature at 60-min intervals and placed in the nests for a 30-day period. iButtons were dipped in a rubber coating (PLASTI DIP®) (three coats per device) for waterproofing prior to deployment. iButtons were tested before and after the coating to ensure that the temperature reading was not affected by this procedure. This step was included in the second (October/November 2016) and third field season (June/July 2017) due to the failure (as a result of moisture) of 16 iButtons during the first field season (June/July 2016). Each iButton was subsequently placed in a small plastic pipe (opened and with holes on one side for contact with the soil), adhered to the tip of a wooden stick to facilitate recovery after 30 days (Fig. 2d, e). It is worth noting that at the time of sample collection, some of the nests that were active when iButtons were deployed 30 days before the sampling were found deserted. The data from the iButtons were used to calculate the mean and standard deviation (SD) of the soil temperature. After the 30-day period of logging soil temperature, soil samples were collected to calculate the moisture content in the respective nests. At the same time, nests and penguins were sampled for parasites and the health condition of penguins recorded (methods in sections below). The soil moisture content was calculated by obtaining a 100-ml nest material sample (from the top layer) and 50-ml soil sample (beneath

Table 1 Sample size per bird age (adult penguins and chicks) in each of the nest types across seasons in the Stony Point penguin colony, South Africa

Nest type	Autumn/winter 2016	Spring 2016	Autumn/winter 2017	Total
Artificial nests				
Adults	10	1	9	20
Chicks	28	8	28	64
Natural covered				
Adults	5	4	9	18
Chicks	29	12	28	69
Natural open				
Adults	7	3	2	12
Chicks	26	4	29	59

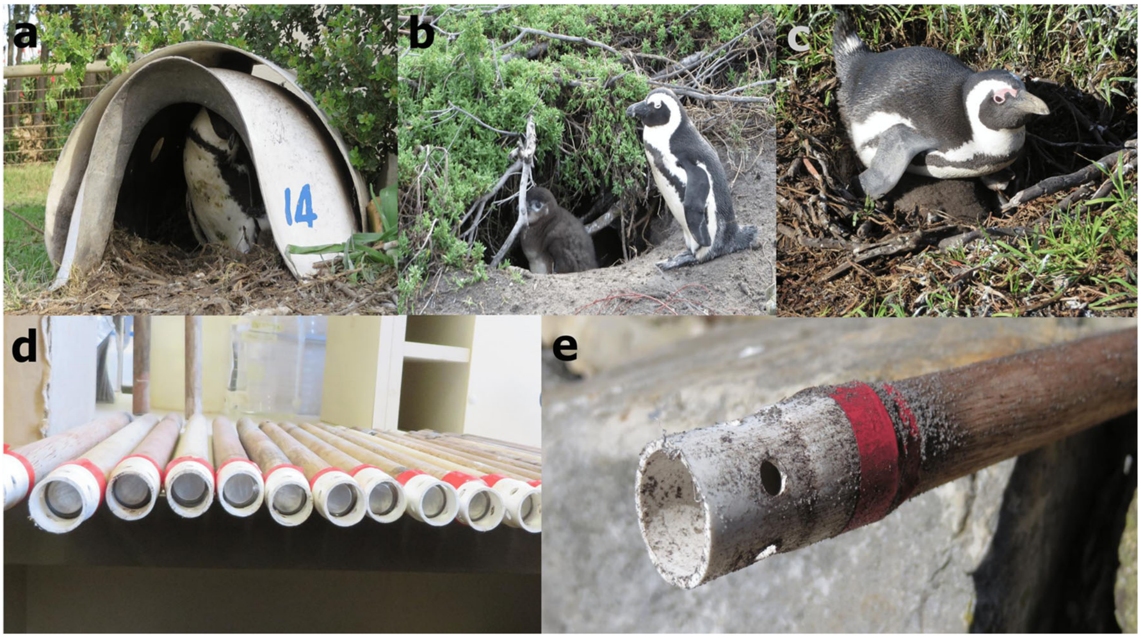

Fig. 2 Nest types that were included in the study: **a** artificial, **b** natural covered and **c** natural open nests. **d** Front and **e** side view of plastic pipe used to insert the iButtons in the nest soil. The colour version of the figure is only available online

the nest material, 2-cm depth) from each nest. Both samples were individually placed in pre-weighed glass bottles and sealed with a lid. The glass containers with fresh soil and nest material were weighed (combined wet and jar weight) using an electronic scale (PS 4500/C/2, Radwag Wagi Elektroniczne, Radom, Poland). The weight of the empty jar was subtracted from the combined weight to obtain the wet weight of each sample. Thereafter, the samples were dried in an oven for 24 h. Soil samples were dried at 105 °C, while nest material was dried at 60 °C in order to avoid burning the nest material content (e.g. leaves, feathers, seaweed and stones) (Gardner 1965). The sample bottles were reweighed and the empty jar was subtracted to obtain the dry weight of the soil and nest material. The gravimetric (dry) nest material and soil water concentration were then calculated for each nest material and soil sample (International Standards Organization 1993):

$$W_{\mathrm{d}} = (\text{weight of moist soil (g)} - \text{weight of dry soil (g)})/\text{weight of dry soil (g)}$$

where W_{d} is the gravimetric (dry) soil water concentration.

Assessment of nest characteristics

Apart from the nest type data (artificial, natural covered and natural open nests), the age of each nest was characterized (based on colony records) as follows: “1”—new nests or established within a year, “2”—nests established more than one but less than 3 years, and “3”—nests established more than 3 years. We recorded the distance of each nest to the south-east coast and to the west coast of the colony due to the fact that Stony Point predominantly receives wind from two different directions during the year (north-west winds in winter and south-east winds in spring). The geographic location of each nest was referenced using a GPS and the coordinates were used to calculate the distance (meters) by drawing a straight line from each nest to the south-east and west coast using the measure tool of Google Earth Pro (image date 30/12/2017 ©2018 DigitalGlobe). In each case, the directions of the lines were in the same direction and parallel to each other. This gave an indication of the exposure to wind and moisture from sea sprays for each nest (Monahan 1968). The orientation of the nest entrance was recorded from artificial and natural covered nests. Nests entrances were denoted as “windward” or “leeward” pending the predominant wind direction (“windward” in winter north-east and spring south-east and “leeward” the nests facing the remaining directions). The size of the nest entrance was recorded horizontally from side-to-side at the widest point of the entrance opening using a measuring tape for artificial and natural covered nests.

Collection and processing of ectoparasites from nests and penguins

For the extraction of ticks and fleas from the nests, a standardized 200-ml sample was collected from the centre of the nest using a spade. The sample, which consisted of nest material (e.g. sticks, seaweed and stones) and soil, was immediately transferred into a plastic jar and sealed with a lid. In the laboratory, nest samples were placed in a modified Berlese funnel for 24 h using naphthalene mothballs as repellent and subsequently inspected using a stereomicroscope to ensure the complete removal of ectoparasites from nest samples (details of the ectoparasite extraction from penguin nests can be found in Espinaze et al. 2019a).

A standardized method for parasite collection was used for adult penguins and chicks. During an 8-min period, ectoparasites were collected from the abdominal area by brushing the plumage for 1 min with a soft brush and from around the eyes using forceps, and stored in 70% ethanol until examination in the laboratory within 1 week from sample collection. The body regions were selected based on their association with ectoparasites from previous studies (Dr Nola Parsons pers. comm. 2016). Ectoparasites collected from nests and penguins were counted and identified morphologically to species level using taxonomic reference keys (Jordan 1942; Kohls et al. 1965).

Penguin health parameters and haemoparasite presence

Body mass, chick body condition, haematocrit and total plasma protein were recorded from African penguins. Penguin (adults and chicks) body mass (kg) was recorded using a handheld electronic scale (25 kg/50 lb Sensation). The head length (mm) of chicks was measured from the back of the head to the tip of the beak using an electronic calliper (Grip 150 mm Digital Vernier). The body mass and head length were used to calculate the body condition of chicks (Lubbe et al. 2014). The method by Lubbe et al. (2014) uses quantile regression equations of minimal and maximal normal growth for successful fledglings to calculate the body condition of a chick with an expected body mass for a given head length. To obtain haematocrit and total plasma protein values, blood was collected from the dorsal aspect of the foot of all penguins using a 23-gauge needle, and placed in 80-ul heparinized microhematocrit capillary tubes. Capillary tubes were centrifuged (on site and on the same day) using a portable centrifuge (Hawksley & sons Ltd.) at 14000 rpm for 5 min (Travis et al. 2006). Haematocrit (packed cell volume) was measured using a microhaematocrit reader (Hawksley & sons Ltd.) and total plasma protein (TPP) was determined using a handheld refractometer calibrated before use (Bellingham and Stanley Ltd. and Schmidt + Haensch). To record the presence of haemoparasites, a drop of blood was collected to make thin blood smears which were fixed with methanol on site and later stained with eosin-methylene blue stain (RapiDiff kit). Presence of tick-transmitted haemoparasites was recorded in 150 fields per slide under a light microscope (Leica Microsystems, Wetzlar, Germany) at × 100 magnification (Palinauskas et al. 2008). Haemoparasites were identified to order level based on morphological characters (Campbell and Ellis 2007).

Statistical analysis

We ran three sets of analysis. Firstly, we fitted a generalized linear model (GLM) with a Gaussian error distribution using the glm() function in R to each nest microclimatic variable as the response variable (including, respectively, mean soil temperature (°C) over a 30-day period from each nest; SD of the recorded soil temperature, soil moisture (W_d) and nest material moisture (W_d)). For explanatory variables, we included nest characteristics (nest type, age, distance from the coast, orientation and opening), together with nest occupancy (active and non-active) and sampling period (SP1: autumn/winter 2016; SP2: spring 2016; and SP3: autumn/winter 2017).

Secondly, we fitted a zero-inflated GLM using the zeroinfl() in "pscl" R package (Jackman 2017) for transformed abundance data ($\log(N + 1)$) to reduce skewness. The response variable, parasite abundances, is the numbers of individual parasites in nests regardless of whether the nest is infested (Bush et al. 1997). Nest characteristics, nest microclimatic conditions, nest occupancy and sampling period were used as explanatory variables. Moreover, we fitted a binomial GLM for the presence and absence of ectoparasites using the glm() function in R. Parasite prevalence (i.e. presence) was considered for nests infested by one or more individual parasites (Bush et al. 1997). Again, nest characteristics, nest microclimatic conditions, nest occupancy and sampling period were used as explanatory variables. For the categorical explanatory variable nest type in these two sets of regression models, we took each nest type as the intercept, although we only presented tables with artificial nests as the intercept for simplicity.

Lastly, we assessed the correlation between in-nest ectoparasites (fleas and ticks combined) and on-host ectoparasites using a Spearman correlation test, after testing for data normality with a Shapiro test. Subsequently, we fitted a GLM with a specific error distribution to each penguin health indicator as the response variable (including, respectively, penguin body mass with a negative binomial distribution, chick body condition with a Gaussian error distribution, haematocrit plasma protein with a Poisson distribution, and total plasma protein with a Gaussian error distribution). The GLM included nest characteristics and microclimatic conditions that were significant in the previous analysis (i.e. nest type, distance from the coast, mean soil temperature, soil moisture and nest material moisture), penguin age (except for chick body condition), on-host ectoparasites and sampling period as explanatory variables. We used the glm() function in R, while we ran the GLM with a negative binomial distribution using the function glm.nb() in the "MASS" R package (Venables and Ripley 2002). Although full models (all explanatory variables included) were used for hypothesis testing, a backward model selection test was performed using the Akaike information criterion (AIC) and a chi-squared test to compare the full models with the best models from the AIC. Haemoparasite prevalence in adult penguins and chicks between the different nest types were compared using proportion tests. All statistical tests and plot design were conducted in R 3·4·3 (R Core Team 2017).

Results

Nest characteristics and microclimate

Mean values and proportions obtained from nest microclimatic conditions and characteristics recorded per nest type and sampling season are presented in Table 2. Nest microclimatic conditions explained by nest characteristics are presented in Supplementary Table S1. None of the full models used in the analysis differed significantly from the best models estimated with the AIC (Supplementary Table S2). Mean soil temperature was significantly higher in artificial nests (17.78 ± 0.24 °C) than in natural covered (16.81 ± 0.22 °C, t-statistic $p < 0.001$) and natural open (16.96 ± 0.27 °C, t-statistic $p = 0.003$) nests. The same pattern was recorded for soil temperature SD (t-statistic $p = 0.003$ and t-statistic $p = 0.047$, respectively). Soil moisture and nest material moisture in artificial nests were not significantly different from natural covered nests (t-statistic $p = 0.135$ and t-statistic $p = 0.217$). However, artificial and natural covered nests were significantly drier than natural open nests (soil moisture t-statistic $p < 0.001$ and t-statistic $p < 0.001$, respectively, and nest material moisture t-statistic $p < 0.001$ and t-statistic $p = 0.009$, respectively). Mean soil temperature (t-statistic $p = 0.002$) and nest material moisture (t-statistic $p = 0.010$) were higher in active (17.12 ± 0.18 °C and 0.42 ± 0.02 W_d) than in non-active nests (17.33 ± 0.23 °C and 0.29 ± 0.02 W_d). Soil moisture was higher in older nests (t-statistic $p = 0.026$) and in nests further away from the south-east coast line (t-statistic $p = 0.003$). Mean soil temperature (t-statistic $p = 0.031$) and temperature SD (t-statistic $p = 0.014$) were significantly lower in nests with wider openings. When comparing between seasons and years, soil temperature was lower (t-statistic $p < 0.001$) and nest material wetter (t-statistic $p < 0.001$) in autumn/winter 2016 (15.51 ± 0.19 °C and 0.41 ± 0.02 W_d) than in spring in 2016 (19.13 ± 0.16 °C and 0.23 ± 0.02 W_d), while soil

Table 2 Nest characteristics assessed for African penguins in the Stony Point colony, South Africa. The mean value (± SE) of and proportion (%) per nest type and sampling season are presented. Sampling seasons: autumn/winter 2016 (SP1), spring 2016 (SP2), and autumn/winter 2017 (SP3)

Nest characteristics	Nest type			Sampling season		
	Artificial	Natural covered	Natural open	SP1	SP2	SP3
Microclimate (mean ± SE)						
Soil mean temperature (°C)	17.78 (± 0.24)	16.81 (± 0.22)	16.96 (± 0.27)	15.51 (± 0.19)	19.13 (± 0.16)	16.65 (± 0.18)
Soil temperature SD	1.91 (± 0.11)	1.47 (± 0.09)	1.62 (± 0.08)	1.76 (± 0.11)	1.62 (± 0.09)	1.65 (± 0.09)
Soil moisture (Wd)[a]	0.31 (± 0.02)	0.35 (± 0.03)	0.56 (± 0.04)	0.41 (± 0.03)	0.42 (± 0.04)	0.39 (± 0.03)
Nest material moisture (Wd)[a]	0.32 (± 0.02)	0.36 (± 0.02)	0.44 (± 0.02)	0.41 (± 0.02)	0.23 (± 0.02)	0.49 (± 0.03)
Occupancy (%)						
Active	74.51	74.76	70.87	82.57	46.91	83.05
Non-active	25.49	25.24	29.13	17.43	53.09	16.95
Age (%)						
1 (< 1 year)	7.84	16.50	16.50	17.43	9.88	12.71
2 (1–3 years)	12.75	43.69	37.86	36.70	30.86	27.12
3 (> 3 years)	79.41	39.81	45.63	45.87	59.26	60.17
Nest opening orientation (%)						
Windward	31.37	43.69	NA[b]	25	59.26	34.67
Leeward	68.63	56.31	NA[b]	75	40.74	65.33
Opening diameter (cm) (mean ± SE)	29.88 (± 0.37)	48.75 (± 1.43)	NA[b]	41.27 (± 1.58)	38.41 (± 1.99)	38.03 (± 1.65)
Distance to the south-east coast (m) (mean ± SE)	169.93 (± 10.64)	169.08 (± 10.06)	165.87 (± 10.19)	157.17 (± 10.09)	169.05 (± 11.27)	178.03 (± 9.61)
Distance to the west coast (m) (mean ± SE)	344.41 (± 11)	353.54 (± 10.21)	332.18 (± 10.97)	346.57 (± 9.76)	341.75 (± 11.86)	341.54 (± 10.74)

[a] W_d gravimetric dry soil

[b] *NA* not applicable

temperature (t-statistic $p < 0.001$) was lower and nest material was drier (t-statistic $p = 0.020$) in autumn/winter 2016 than in autumn/winter 2017 (16.65 ± 0.18 °C and 0.49 ± 0.03 W_d).

Ectoparasite abundance and prevalence

The flea *Parapsyllus humboldti* and soft tick *O. capensis* sensu stricto were recorded in nests and on penguins. Parasite infestations (mean abundance and prevalence) in nests were 13.36 ± 1.68 and 64% for fleas and 11.38 ± 3.84 and 74% for ticks. Parasite infestations (mean abundance and prevalence) on penguins (adults and chicks) were 5.20 ± 0.34 and 81% for fleas and 0.57 ± 0.11 and 21% for ticks. Haemoparasites were recorded in 56% of the penguins (adults and chicks). There was a significant positive correlation between ectoparasite infestation in nests and on penguins ($r_\mathrm{Spearman} = 0.31$, $p < 0.001$).

Nest characteristics and ectoparasite abundance and prevalence in nests

Mean abundance and prevalence of ectoparasites per nest type are presented in Table 3. In-nest ectoparasite (combined flea and tick) and flea and tick abundance and prevalence explained by nest characteristics are presented in Supplementary Table S3. The majority of the full models used in the analysis did not differ significantly from the best models estimated with the AIC (Supplementary Table S4). Artificial nests harboured a significantly higher abundance and prevalence of ectoparasites (z-statistic $p < 0.001$ and z-statistic $p = 0.003$, respectively) (Fig. 3a), fleas (z-statistic $p < 0.001$ and z-statistic $p < 0.001$, respectively) and ticks (z-statistic $p < 0.001$ and z-statistic $p = 0.009$, respectively) than natural open nests. Artificial nests also harboured a higher abundance of ticks (z-statistic $p = 0.017$) than natural covered nests, while natural covered nests harboured a higher abundance of ectoparasites (z-statistic $p < 0.001$) and a higher abundance and prevalence of fleas (z-statistic $p = 0.007$ and z-statistic $p < 0.001$, respectively) than natural open nests. Furthermore, active nests harboured a higher abundance of fleas (z-statistic $p < 0.001$) than non-active nests (Fig. 3b). Ectoparasite abundance was higher in nests closer to the south-east coast (z-statistic $p = 0.048$) (Fig. 3c) and flea prevalence was higher in nests closer to the west coast (z-statistic $p = 0.021$). Mean soil temperature was positively correlated with nest ectoparasite abundance (z-statistic $p = 0.014$) (Fig. 3d) and flea prevalence (z-statistic $p = 0.016$). In addition, nest soil moisture was negatively correlated with total ectoparasite abundance (z-statistic $p < 0.001$) (Fig. 3e) and flea abundance and prevalence (z-statistic $p < 0.001$ and z-statistic $p < 0.001$, respectively). A similar pattern was recorded for nest material moisture, which was negatively correlated with ectoparasite abundance (z-statistic $p = 0.007$) (Fig. 3f) and flea prevalence (z-statistic $p = 0.011$). Nest microclimatic conditions were not significantly correlated with tick abundance and prevalence.

Table 3 Mean abundance (± SE) and prevalence (%) of all in-nest ectoparasites, fleas and ticks per nest type in the Stony Point penguin colony during autumn/winter and spring 2016 and autumn/winter 2017

Parasites in nests	Nest type		
	Artificial	Natural covered	Natural open
Abundance (mean ± SE)			
All ectoparasites	45.19 ± 11.97	18.71 ± 3.32	10.51 ± 2.38
Fleas	18.03 ± 2.98	14.78 ± 3.27	7.31 ± 2.35
Ticks	27.16 ± 11.45	3.93 ± 0.58	3.2 ± 0.57
Prevalence (%)			
Total ectoparasites	93.14	86.41	81.55
Fleas	78.43	66.99	45.63
Ticks	81.37	71.84	68.93

Nest type, health parameters and haemoparasite infestations

Clinical parameters of penguins explained by nest characteristics are presented in Supplementary Table S5. All the full models used in the analysis did not differ significantly from the best models estimated with the AIC (Supplementary Table S6). Nest type was significantly correlated with body mass of adults and chicks, where all penguins in natural open nests recorded a lower body mass than penguins in artificial (z-statistic $p = 0.012$) and natural covered nests (z-statistic $p = 0.015$). Nest soil temperature was negatively correlated with adult and chick body mass and chick body condition (z-statistic $p < 0.001$ and t-statistic $p = 0.009$, respectively). Nest material moisture was positively correlated with body mass (z-statistic $p = 0.009$) and total plasma protein (t-statistic $p = 0.030$) of adults and chicks. In addition, body mass and total plasma protein of adults and chicks were lower in spring 2016 than in autumn/winter 2016 (z-statistic $p = 0.015$ and t-statistic $p < 0.001$, respectively), and lower in autumn/winter 2017 than in autumn/winter 2016 (z-statistic $p < 0.001$ and t-statistic $p < 0.001$, respectively).

There was no significant difference in the prevalence of haemoparasites (Piroplasmorida/Haemospororida and Spirochaetales combined) in adults and chicks between the nest types (Fig. 4).

Discussion

Artificial nests were significantly warmer than natural nests (covered and open) in the present study. The artificial nests

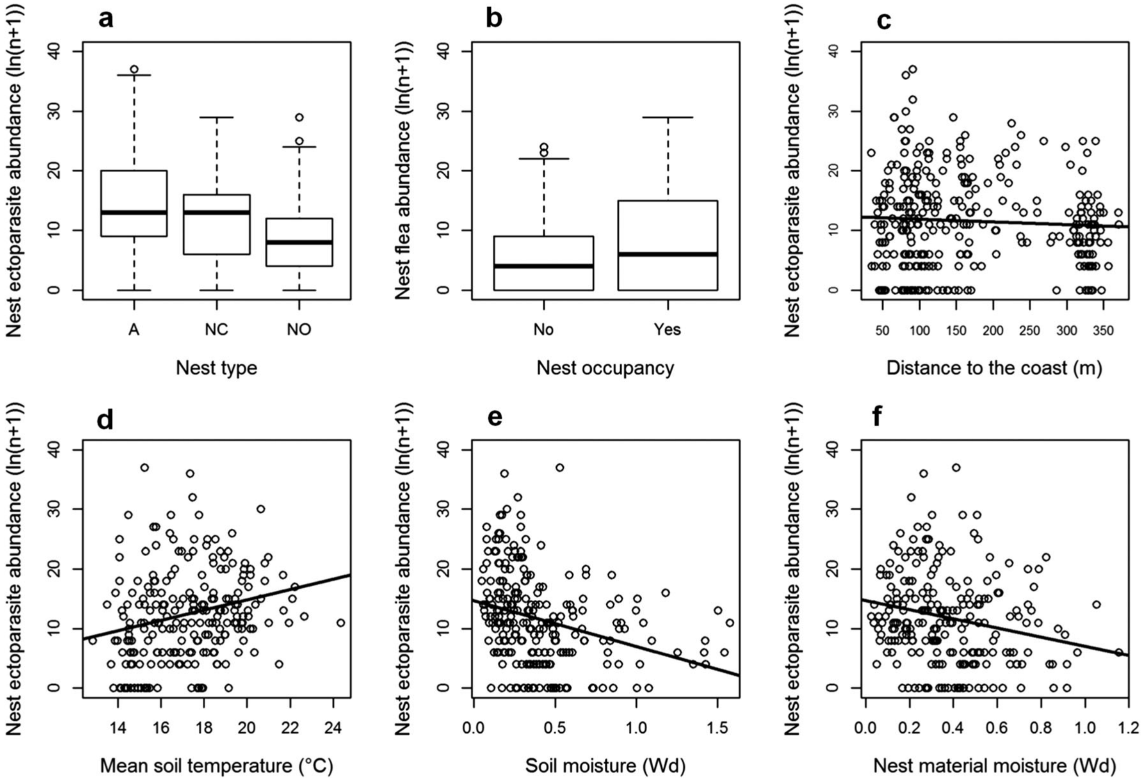

Fig. 3 Relationship between nest characteristics and ectoparasite abundance in African penguin nests. **a** Nest type: A, artificial; NC, natural covered; NO, natural open. **b** Nest occupancy: "no" non-active nests and "yes" active nests. **c** Distance to the south-east coast. **d** Mean soil temperature in nest. **e** Moisture of soil in nest. **f** Moisture of nest material

were made of fibreglass or cement-fibre that is less porous than soil and vegetation used to cover natural nests. This increases the insularity by preventing heat loss in the artificial nests (Deeming and Mainwaring 2015). The higher nest soil temperature is further enhanced by the narrow entrance opening of artificial nests whereas natural surface nests are completely open. The size of the opening can influence airflow in the nest and nests with narrow opening will have poorer ventilation (Martin et al. 2017). A study that recorded the ambient temperature in different nest types of African penguins also recorded consistently higher temperatures (> 30 °C, when *Spheniscus* penguins start being heat-stressed) in fibreglass nests than in natural burrows and natural open nests (Lei et al. 2014). This pattern is not unique to the artificial nests used in African penguin colonies. Ambient temperature in artificial nest boxes (made of plywood), and especially in unshaded artificial nest boxes (which reached > 35 °C), was also found to be higher than natural burrows of Cassin's auklets (Kelsey et al. 2016). Furthermore, a study on long-tailed skinks (*Eutropis longicaudata*) found that the ambient temperature in nests made in artificial habitats (e.g. inside concrete walls) was three times higher than in natural habitats (1.5 and 0.5 °C, respectively) (Huang and Pike 2011). In the present study, artificial nests also experienced higher soil temperature ranges (SD) than natural nests (covered and open). This is in agreement with Kelsey et al. (2016) who found a

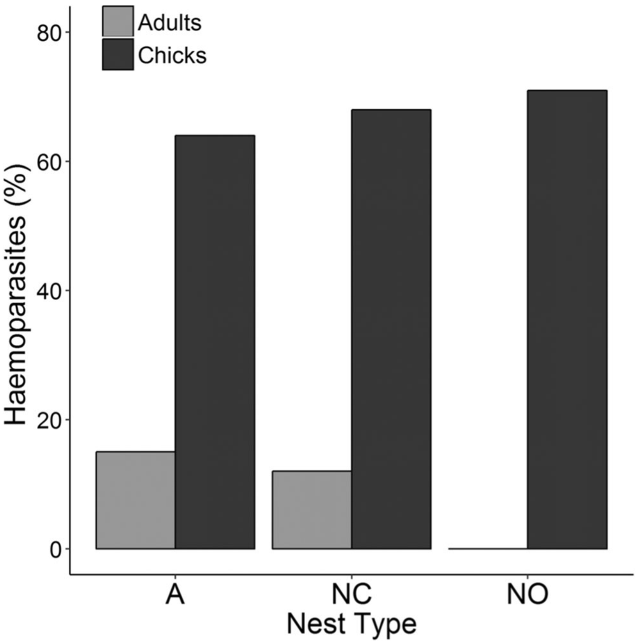

Fig. 4 Prevalence (%) of haemoparasites (order Piroplasmids/Haemospororida and Spirochaetales) in adult African penguins and chicks per nest type (A, artificial; NC, natural covered; NO, natural open) at the Stony Point colony in 2016 and 2017. Sample sizes: A = 20 adults and 63 chicks, NC = 17 adults and 66 chicks and NO = 11 adults and 58 chicks

higher variation of ambient temperature in unshaded artificial nests compared with natural burrows of Cassin's auklets.

In addition to being warmer, the present study also recorded drier conditions (soil and nest material) in artificial nests and natural covered nests than in natural open nests. Both the former artificial material and natural vegetation and soil provided protection against rain, while natural open nests are prone to being flooded during the rainfall season (Seddon and van Heezik 1991; Kemper et al. 2007). The drier conditions associated with artificial nests is confirmed in a recent study where nest boxes of peregrine falcons (*Falco peregrinus*) provide greater protection against wet conditions experienced during rainy seasons compared with open nests on buildings, natural cliffs and quarries (Sumasgutner et al. 2020). It is clear that the microclimatic conditions vary between different nest types and it is possible that these differences can affect nest-dwelling ectoparasites.

In the present study, flea and tick infestations inside the nests correlated significantly with nest type. In particular, artificial nests harboured more fleas and ticks than natural open nests and more ticks than natural covered nests. Furthermore, natural covered nests harboured more ectoparasites (ticks and fleas combined) and more fleas than natural open nests. A similar pattern was recorded in passerine birds, with a higher proportion of artificial nest boxes infested with fleas (*Ceratophyllus* spp. and C*tenophthalmus agyrtes*) than natural nests made in tree cavities (Hebda and Wesołowski 2012). In addition, a similar response was recorded for blowflies (*Apaulina* sp.) in artificial nest boxes occupied by eastern bluebirds (*Sialia sialis*) (Pinkowski 1977). The cover provided by natural material also seems to benefit opportunistic parasites as is seen in the present study and a study on royal penguins (*Eudyptes schlegeli*) (Murray and Vestjens 1967). In the latter, tick (*I. uriae*) infestations were higher on rock stacks covered with vegetation (tussocks) and dry mud within the colony, and in vegetation around the colony, than in the open floor of the colony (Murray and Vestjens 1967). The higher parasite infestations in artificial nests are most probably the result of more favourable microclimatic conditions. Fleas and soft ticks are nidicolous ectoparasites that spend most of their life cycle in the nest of their host (Jordan 1942; Sonenshine 1993). As mentioned before, fleas and ticks are ectothermic and as a result their development and survival is influenced by temperature and humidity in the nest. The development rate of several invertebrate taxa is positively correlated with temperature (López-Rull and Macías Garcia 2015), which results in a shorter generation time (Marshall 1981; Vial 2009), thereby increasing the abundance of fleas in the nest. In particular, fleas can increase their frequency of blood intake and digestion of blood meals when ambient temperature increases (Krasnov 2008), while a rise in temperature (> 24 °C) can trigger the emergence of imagos from cocoons (Humphries 1968). Furthermore, higher temperatures can facilitate the survival of flea larvae such as the larvae of the stick tight flea (*Echidnophaga gallinacea*) which dies when the temperature is below 10 °C (Marshall 1981), while an increase in temperature stimulates mating of some flea species (e.g. northern rat flea *Nosopsyllus fasciatus*) (Iqbal and Humphries 1970). Soft ticks have a similar response to higher nest temperatures, and in particular pre-oviposition and oviposition periods shorten with an increase in temperature (Diehl et al. 1982), which will result in higher tick abundance in these nests. Soft ticks are also morphologically adapted to resist higher temperatures since components of their epicuticle (cuticulin, polyphenol, wax and cement layers) provide an effective barrier against water loss (Lees 1947).

Drier conditions in the nest can also facilitate ectoparasite infestations. Studies on rockhopper (*E. chrysocome*) and royal penguins indicated that fleas (*P. maguellanicus heardi*) and the nidicolous tick (*I. uriae*) require a relatively dry habitat to breed and often avoid wet or flooded places (Murray and Vestjens 1967). Although the environmental water content is a crucial factor for flea development (Rothschild and Clay 1952; Marshall 1981), not all flea species benefit from high humidity. For example, the abundance of adult and larval stages of hen fleas infesting great tits was negatively affected by nest moisture (Eeva et al. 1994) and they often colonized nests with lower humidity (Heeb et al. 2000). These findings, which are consistent with the negative correlation between nest moisture and flea infestations in the present study, might indicate the existence of a moisture threshold above which flea populations start to decrease. Soft ticks, included in the total ectoparasite abundance, also recorded a negative correlation with nest moisture in the present study. In general, nidicolous ticks prefer xeric habitats and/or dry microhabitats (Gray et al. 2013). More specifically, the larvae of the soft tick *Ornithodoros turicata*, which inhabits nests of rodents, birds, reptiles and domestic livestock, are more effective at absorbing water from air when the relative humidity is lower (75%) than when it is higher (93%). Therefore, it is able to better resist desiccation in dry environmental conditions (Yoder and Dutton 1998). Based on these studies and the present, it is clear that the microclimatic conditions associated with artificial nests, and natural covered nests to some extent, facilitates flea and tick infestations in the nest. This can have direct consequences for the penguins as higher parasite infestations in the nest can relate to higher parasite infestations on penguins. In fact, this relationship was recorded in the present study. Fleas and ticks are obligate haematophagous and high host infestations can affect the condition and health of the host (Lehmann 1993). Although ticks and fleas are known vectors of some haemoparasites (Sonenshine 1993; Bitam et al. 2010), we did not observe a higher incidence of haemoparasites in the penguins that occupied artificial nests. However, it is recommended that this result be confirmed using molecular techniques as it is possible that the microscopic method that we used is not sensitive enough (Campbell and Ellis 2007).

Nest age and nest occupancy also correlated with microclimatic conditions and/or ectoparasite infestations. The higher moisture content in older than newer nests is possibly due to the accumulation of substrate (e.g. guano and nesting material) from previous breeding events that might prevent dissipation of water vapour (Rahn et al. 1983; Podofillini et al. 2018). However, there was no significant relationship between nest age and ectoparasite infestation. The higher soil temperature and moisture in active nests than non-active nests may be attributed to the heat and moisture radiation from the host's body and faeces (Rothschild and Clay 1952; Marshall 1981). Active nests also harboured higher flea infestations, which is consistent with the intimate relationship between fleas and hosts (Bitam et al. 2010; Espinaze et al. 2019b). Flea larvae indirectly benefit from nest occupancy as their diet includes host organic refuse (Rothschild and Clay 1952). Nest occupancy was not significantly correlated with tick infestations, which agrees with Daturi (1986) who also found no relationship between soft tick (*O. capensis*) abundance and occupancy in African penguin nests. Soft ticks can survive without a blood meal for extended periods (months to years) and is thus able to remain in the nest without the presence of a host (Sonenshine 1993; Espinaze et al. 2019b).

Another, potentially colony-specific nest characteristic was also observed. In particular, microclimatic conditions and ectoparasite infestations in the nests also varied spatially. Although the surface of the colony mainly comprise of bare rock and soil, natural vegetation (predominantly shrubs and trees) is found more inland and further away from the southeast shore. Nests that occurred under the vegetation canopy were wetter than the nests closer to the shore. It is possible that the vegetation prevents water vapour convection, caused by coastal wind, in these nests. A similar relationship was recorded by Abdallah and Chaieb (2012) that found higher soil moisture levels under Acacia tree canopy than in open exposed areas. The drier conditions recorded in shore nests (regardless of the nest type) is a possible explanation for the higher ectoparasite infestations in these nests than nests further inland. Another potential factor may be the seasonal presence of a large population of Cape cormorant (*Phalacrocorax capensis*) nesting on rocky areas in close proximity (< 15 m) to the shore nests. This can increase the seabird host density in the area and facilitate interspecies transmission of both the tick and flea (Duffy 1983; Daturi 1986).

Contrary to our expectation, there was no direct relationship between artificial nests and any of the health parameters that we recorded for penguins in the present study. However, we did record an indirect relationship. In particular, penguin (adult and chick) body mass was lower in warmer and drier nests, and total plasma protein of penguins (adults and chicks) was lower in drier nests. In addition, chick body condition was poorer in warmer nests. It is possible that these patterns are related to the high ectoparasite (and specifically flea) abundance and prevalence in warmer and drier nests. High ectoparasite infestations develop stress and irritation in their bird hosts, which reduces food consumption and may lead to a decrease in body mass, body condition and total plasma protein (Ots et al. 1998; Norte et al. 2013). In addition, ectoparasite infestations induce blood loss and tissue damage, which increases resource consumption in metabolic processes and results in reduced body mass and condition (Norte et al. 2013). Fitness costs in birds associated with ectoparasite infestations have also been found in great tit nestlings, which showed lower body mass and condition in nests with high abundance of hen fleas (Dufva and Allander 1996). In turn, the body mass of adults and chicks was lower in natural open nests than natural covered and artificial nests. It is likely that this pattern is the result of physiological responses to direct exposure to the prevailing climatic conditions (variation in temperature, solar radiation, rain and wind), which can lead to heat stress or heat loss (Frost et al. 1976) and loss in body mass (Catry et al. 2011). Additionally, natural open nests are more exposed to predators (e.g. small grey mongoose *Galerella pulverulenta* and water mongoose *Atilax paludinosus*; Whittington et al. 1996). This particular stress factor can result in the mobilization of energy reserves and negatively affect body mass (Schoech et al. 1997). An alternative explanation may be that open nests are suboptimal and that lighter and/or younger birds breed in open nests. African penguins exhibit high nest and mate fidelity (Crawford et al. 1995; Ancel et al. 2013) and it is possible that young animals that return to the colony for their first breeding attempt end up using open nests.

Artificial nests are widely used in bird conservation initiatives to counter the current reduction of natural breeding sites and to facilitate improved breeding success (Newton 1994; Sherley et al. 2012). For example, the introduction of artificial nests reduced intra- and inter-specific competition for natural breeding sites (burrows and crevices) in Madeiran storm petrels (*Oceanodroma castro*), which resulted in increased breeding success over three successive seasons (Bolton et al. 2004). In addition, improved breeding success was reported for little penguins (*Eudyptula minor*) that used artificial nests in Australia and New Zealand (Perriman and Steen 2000; Sutherland et al. 2014). In general, artificial nests have also facilitated improved breeding success in African penguin colonies in Namibia (Kemper et al. 2007) and in South Africa (Sherley et al. 2012). However, there have also been reports that the frequency of reuse (after deployment) by penguins decreased over time (Kemper et al. 2007). It is possible that this may be attributable to their colonization by nest parasites over time (Loye and Carroll 1998). In Stony Point, most of the sampled nests have been in the colony for many years and cement nests in particular have been used for more than 3 years. This is supported by studies on passerine birds, where the cost of reusing nest boxes from previous seasons in cavity-nesting birds has resulted in the increase of flea (*C. gallinae*), blow fly (*Protocalliphora azurea*) and mite (*Dermanyssus gallinoides*) populations with a negative impact on bird's health and breeding success (Tomás et al. 2007). This disadvantage needs to be weighed against the benefits reported for artificial nests in different bird species.

This study highlights the importance of nest characteristics in shaping the microclimatic conditions within penguin nests. In particular, the microclimatic conditions associated with artificial nests made from fibreglass and cement-fibre promote higher infestations of nest parasites that can affect the overall health and survival of the endangered African penguin. In light of our findings, it is recommended that a monitoring programme should be introduced in all colonies to record the ectoparasite populations in artificial and natural nests. The value of fibreglass and cement-fibre nests should also be re-evaluated by comparing the breeding success data per nest type over consecutive years. Finally, the design of new artificial nests must consider the use of more porous material and/or better ventilation, which may assist creating less ideal microclimatic conditions for ectoparasites.

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Authors' contributions All authors conceived the study idea and developed the study design. MPAE conducted field and laboratory work. SM, CH and LW supervised the study. MPAE and CH performed the statistical analyses. MPAE wrote the draft versions of the manuscript. SM, CH and LW contributed critically to all draft versions and the final version of the manuscript.

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Data availability The data will be made available through an online repository or as supplementary material upon acceptance of the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate This study followed the ethical guidelines for the use of animals and permits were obtained from the Animal Ethics Committee of University of Stellenbosch (SU-ACUD15-00114), the Division of Environmental Affairs (RES2016/95 and RES2017/02), the Threatened or Protected Species of the Biodiversity Act (07962) and CapeNature (AAA007-00191-0056).

Consent for publication No humans were used in the study.

Code availability The relevant code is presented as supplementary material.

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