

Parasite diversity associated with African penguins (*Spheniscus demersus*) and the effect of host and environmental factors

Research Article

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Abstract

The African penguin (*Spheniscus demersus*) is a critically endangered species endemic to southern Africa. Limited information is available on the parasite diversity associated with the species in natural settings. This study explores the diversity and incidence of parasites associated with African penguins and their nests, and records the effect of host and environmental factors on parasite infestation. Ecto-, haemo- and helminth parasites were recorded from 210 adult birds, 583 chicks and 628 nests across five colonies (two mainland and three islands) along the south-western coast of South Africa, in 2016 and 2017. Mean nest density (total and active nests) and climate variables (temperature and precipitation) were obtained for each colony. *Parapsyllus humboldti* was the most abundant and prevalent ectoparasite on penguins and in nests (69.10 and 57.80%, respectively), while Piroplasmorida/Haemospororida (33.51%) and *Cardiocephaloides* spp. (56.17%) were the most prevalent haemo- and helminth parasites of penguins, respectively. In general parasite abundance and prevalence was significantly affected by penguin age (chicks vs adults), location (mainland vs islands), nest density (total and active nests) and season (spring vs autumn/winter). It is concluded that parasite infestations are structured and that penguin chicks at mainland colonies are more susceptible to parasite infestations during spring.

Introduction

Seabirds occupy diverse habitats that include marine and terrestrial ecosystems where they find resources to feed, nest, breed, find shelter and moult (Schreiber and Burger, 2001). In this dual environment, seabirds are exposed to parasites through their diet and eating habits (e.g. helminths present in fish species) (Randall and Bray, 1983; Brandão *et al.*, 2014), nesting sites (e.g. ectoparasites in the nest material and soil) (Daturi, 1986) and through their interaction with other co-occurring congeneric and confamilial birds (e.g. bird-specific ectoparasites and pathogens such as viruses and bacteria) (McCoy *et al.*, 2002).

There are several factors that can cause among colony variation in parasite diversity and level of infestations in seabirds (Jones and Shellam, 1999). Most seabird species form large colonies comprising up to thousands of breeding pairs (Schreiber and Burger, 2001) and therefore colony size and especially the nest density can facilitate high infestations of both host (lice) and nest parasites (fleas and ticks) (Rivera-Parra *et al.*, 2014; Ramos and Drummond, 2017). For example, tick infestation has been found to be higher in Peruvian Boobies (*Sula variegata*), a seabird that nest in large groups than in blue-footed boobies (*Sula nebouxi*), which tend to cluster at relatively lower densities (Duffy and Campos de Duffy, 1986). The presence of co-occurring congeneric and confamilial bird can further increase host and nest density, which can result in higher abundances and transmission of bird-specific parasites (Duffy, 1988). The level of parasite infestation in prey species can also vary spatially and may influence the risk of infection for seabirds that act as definitive hosts (Van der lingen *et al.*, 2015; Levsen *et al.*, 2018). A large scale study by Levsen *et al.* (2018) recorded regional difference in parasite infestations of Anisakid nematodes in sardine (*Sardina pilchardus*) and other commercial fish species within the European fishing grounds. Several of these nematodes require seabirds as definitive hosts (Anderson, 2000). Climatic conditions on land and in the water can also affect parasite distribution. Nidicolous ectoparasite species (spend time in nests and shelters) are susceptible to mean temperature and precipitation (Marshall, 1981a; Sonenshine, 1993) while water temperature and salinity can effect most marine endoparasites in fish (Möller, 1978). In addition, bird age and immune status are also important factors (De Lope *et al.*, 1998; Van Rensburg, 2010). For example, a study on the seabird Kittiwake (*Rissa tridactyla*) recorded higher tick infestations on intermediate age chicks compared with younger and older chicks. The authors surmised that this may be due to the fact that intermediate aged chicks spend

more time in the nests and are therefore more exposed to ticks (Boulinier and Danchin, 1996). Knowledge of the factors that drive parasite infestation is important as parasites can directly (cause stress, anaemia and reduced fitness) (Johnson and Clayton, 2003; Bitam *et al.*, 2010) and indirectly (transmit disease causing microbes such as protozoa, bacteria and viruses) affect the condition and survival of their host (Nuttall, 1984). Although several studies have been conducted on parasites of seabird at the terrestrial-marine interface (e.g. Gauthier-Clerc *et al.*, 1999; Frenot *et al.*, 2001; Carrera-Játiva *et al.*, 2014; Rivera-Parra *et al.*, 2014), little is known about the factors that influence parasite loads on seabirds that naturally occur along the southern African coastline (Daturi, 1986; Duffy and Daturi, 1987).

The African penguin (*Spheniscus demersus*) is endemic to the Benguela Upwelling Ecosystem (Crawford *et al.*, 2011) and the only penguin species that breeds in Africa (Shelton *et al.*, 1984). The species breeds in 28 colonies (24 islands and four mainland) (BirdLife International, 2016) distributed from central Namibia to the east coast of South Africa (Crawford *et al.*, 2011). The suitability for breeding sites has been linked to the distribution and abundance of their primary prey: the Cape anchovy (*Engraulis encrasicolus*) and the South African sardine (*Sardinops sagax*) (Crawford *et al.*, 2006). During the breeding season (in South Africa normally extended from February to September/October; Crawford *et al.*, 1995; Crawford *et al.*, 2006), the adults spend most of the day catching fish at sea and return to the colonies in the evenings to feed the chicks and relieve their mate (Cooper, 1980). They lay two eggs that are incubated for about 40 days. After hatching, the chicks are under parental care and become independent after ca. 80 days (Williams and Cooper, 1984). Historically, African penguins were common on nearshore islands, but more recently the species also started to occupy mainland areas. It is surmised that this movement may have been due to excessive harvesting of eggs (for human consumption) and guano (for fertilizer) on islands (Rand, 1969; Whittington *et al.*, 1996), although a reduction in food resources along certain islands may have also contributed (Shelton *et al.*, 1984; Crawford *et al.*, 2011). Consequently, the species has suffered severe (>50% over three generations) and rapid population decline mainly on island colonies, and its conservation status is listed as endangered (BirdLife International, 2016). African penguins are parasitized by a diverse range of parasite taxa that include ectoparasites (soft ticks, lice and fleas), helminths (nematodes, cestodes and trematodes) and haemoparasites (Brandão *et al.*, 2014; Parsons and Vanstreels, 2016). To date, most studies on the parasite of African penguins have been conducted on animals admitted to rehabilitation centres (e.g. Horne *et al.*, 2011; Yabsley *et al.*, 2012; Parsons and Vanstreels, 2016) or have focused on parasites in nests at a single island colony (Daturi, 1986; Duffy and Daturi, 1987). As yet, there is no empirical data on the parasites associated with African penguins and their nests across multiple colonies in South Africa. In addition, little is known with regards to the factors that drive among and within colony parasite infestations on African penguins and in their nests.

The aims of the study were: (1) to record the diversity and prevalence of parasites associated with African penguins and their nests at multiple colonies along the south-western coast of South Africa, and (2) to establish the effect of various host and environmental factors on parasite infestation patterns. We predict that penguin chicks will have higher parasite infestations compared with adults. This may be due to a combination of factors such as lower immunity and closer association with nests (and nest parasites) in chicks. We further predict that parasite abundance and prevalence will be positively related to nest density. Colonies with higher nest densities provide more resources (food and shelter) that can facilitate higher parasite infestations.

Materials and methods

Study site and design

The study was conducted at five African penguin colonies: three islands: Dassen-, Dyer- and Robben Island and two mainlands: Stony Point and Simon's Town (previously known as Boulders Beach) along the south-western coast of South Africa during 2016 and 2017 (Fig. 1; Table 1). Penguins (adults and chicks: 20 day-old and older) and their respective nests were randomly selected and sampled at the peak of the first breeding season between May and July (autumn/winter, i.e. cold and rainy season) each year. In addition, penguins and their nests were sampled at one colony (Stony Point) during a second breeding season in October–November (spring, i.e. warm and dry season) during 2016. Sixty penguins (20 adults and 40 chicks) and 40 nests were sampled at each colony in autumn/winter each year. At Stony Point, 105 penguins (22 adults and 83 chicks) and 109 nests were sampled in autumn/winter 2016, 103 penguins (eight adults and 95 chicks) and 81 nests were sampled in spring 2016 and 105 penguins (20 adults and 85 chicks) and 118 nests were sampled in autumn/winter 2017 (Table 1). Sampling was conducted during the day starting at 9:00 and ending at 16:00.

Parasite collection from penguins and nests

Each penguin (adult and chick) was examined for 8 min. Ectoparasites (fleas, lice and ticks) were collected by systematically brushing the plumage for 1 min around the pelvic area using a soft brush. Ectoparasites that occur on the face of the animals were also removed using forceps. Parasites were stored in 70% ethanol. A new brush and clean tweezers were used for each animal. A blood smear was made from a drop of blood collected from the dorsal aspect of the foot using a mechanical pipette attached to a 23-gauge needle. The blood smear was air-dried and fixed with methanol. Penguin chicks naturally defecate when handled. This allowed the collection of fresh faecal material, which were fixed in 10% formalin and kept cool until examination in the laboratory. Body mass (kg) was recorded for each penguin with a handheld electronic scale (25 kg/50 lb Sensation). Penguin nests were sampled for parasites by collecting 200 mL nest material (including soil) from the centre of the nest. Nest material was stored in plastic jars sealed with a lid and kept cool until further processing.

Parasite recovery and identification

Ectoparasites were extracted from the nest material using a modified Berlese funnel method (Southwood, 1978). In a sealed unit naphthalene moth balls (100 g) were used as a repellent and hung above the nest material for 24 h (Daturi, 1986). Thereafter, each nest material sample was systematically examined using a dissecting microscope. The latter method was included due to the ineffectivity of the extraction method to remove all parasites. Parasites recorded by the two methods were combined. Ectoparasites were identified morphologically using taxonomic reference keys (Jordan, 1942; Von Keler, 1952; Arthur, 1963; Kohls *et al.*, 1965; Segerman, 1995; Banks and Palma, 2003) and counted. Ectoparasite species were identified to species level and the life stage and sex was recorded. Thin blood smears were stained using an Eosin-Methylene Blue stain (RapiDiff kit) and examined by detecting presence of haemoparasites 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 (Piroplasmorida/Haemospororida and Spirochaetales) based on morphological characters (Campbell and Ellis, 2007; Peirce and

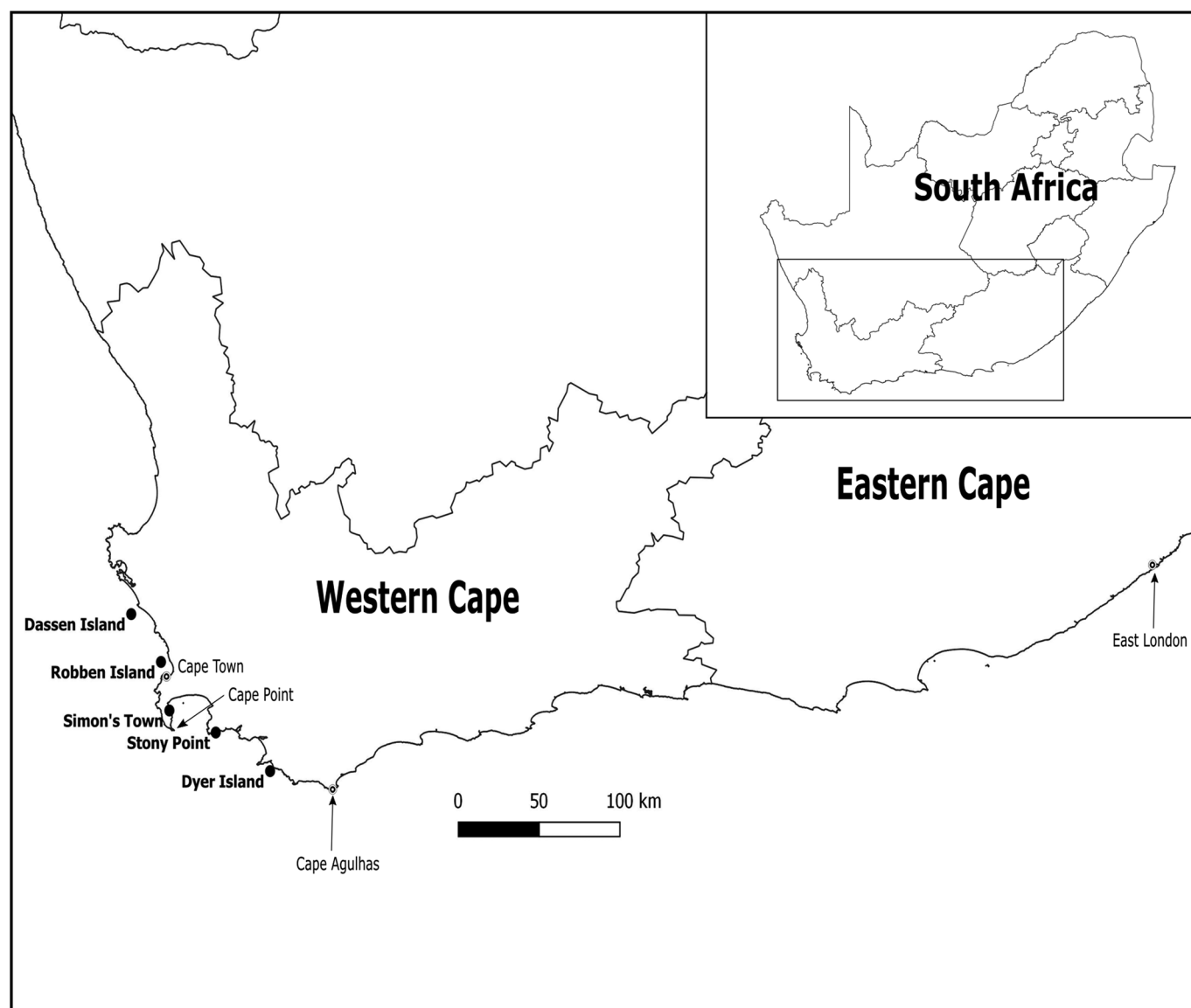


Fig. 1. Map of the selected African penguin colonies along the south-western coast of South Africa. Two mainland (Simon's Town and Stony Point) and three island colonies (Dassen-, Dyer- and Robben Island). Areas were plotted using GPS coordinates and QGIS open source Geographic Information System (<http://qgis.osgeo.org>).

Table 1. Locality, date of sampling, sample size, season and nest density at five African penguin colonies along the south-western coast of South Africa during 2016 and 2017

Locality	Coordinates	Sampling date		Sample size (adult:chicks)	Season	Mean nest density (average per m ²) (total:active)
		2016	2017			
Island-based colonies						
Dassen Island	33.423647S, 18.086542E	12 May–14 May	08 May–12 May	120 birds (40:80) and 80 nests	Autumn/winter	0.02:0.012
Dyer Island	34.684075S, 19.414769E	30 May–01 June	29 July–31 July	120 birds (40:80) and 80 nests	Autumn/winter	0.08:0.06
Robben Island	33.807607S, 18.371231E	07 June–26 June	29 May–02 June	120 birds (40:80) and 80 nests	Autumn/winter	0.05:0.007
Land-based colonies						
Stony Point	34.374151S, 18.895248E	29 June–13 July	19 June–12 July	210 birds (42:168) and 227 nests	Autumn/winter	0.28:0.13
			24 October–07 November	103 birds (8:95) and 81 nests	Spring	
Simon's Town	34.197220S, 18.451285E	13 June–20 June	26 June–03 July	120 birds (40:80) and 80 nests	Autumn/winter	0.22:0.14

Parsons, 2012; Vanstreels *et al.*, 2016). Faecal material (1 g) was examined for helminth eggs using qualitative techniques. Nematode and cestodes eggs were detected with the modified Wisconsin sugar flotation method (Nolan, 2006) (specific gravity of sugar solution >1.14). The sedimentation technique described by Hansen and Perry (1994) was used to detect trematodes, acanthocephalans and any eggs that did not float with the flotation technique. The helminth parasites were identified to genus level (Horne *et al.*, 2011; Carrera-Játiva *et al.*, 2014).

Nest density

Nest density was recorded by counting the total number of nests (non-active and active nests) and active nests only (nests containing eggs, chicks and/or adults) in a 15 × 15 m quadrant during the autumn/winter season each year. Five quadrants were randomly selected each year at all colonies apart for Stony Point. At the latter colony, 12 quadrants were selected each year.

Climate data

Data on the annual mean temperature (°C) and annual precipitation (mm) was obtained for each colony from WorldClim (Global Climate Data) using the function `getData` in the 'raster' package in R (Hijmans and van Etten, 2012). Remote sensed data were selected due to the lack of local weather data at all the colonies.

Data analysis

To assess the effect of different parameters on parasite infestations, we considered the total number of parasites (i.e. parasites at all life stages) and combined the two flea species found in this study into one group. Since flea larvae only occur in the nest and adult fleas are found in the nest and on the host, we considered analysis at each life stage only for this ectoparasite in order to record differences. Morphological differentiation between flea species at the larval stage is notoriously difficult (Krasnov, 2008) and as such the larvae of *P. humboldti* and *E. gallinacea* (recorded only at Dassen Island) could not be distinguished. Consequently, data on flea larvae from Dassen Island were not considered in the calculation of abundance and prevalence of total fleas (i.e. no available data).

The effect of penguin age (adult and chick), colony location (mainland and island) and colony (Dassen-, Dyer-, and Robben Island, Stony Point and Simon's Town) on parasite loads during the autumn/winter season were assessed primarily using generalized linear models (GLMs). Where needed the effect of penguin body mass (kg) and year (2016 and 2017) were corrected for in the models. Since parasite data were highly skewed with an excess of zeros, parasite data were first modified by adding the value of 1, then log transformed and rounded (Changyong *et al.*, 2014), followed by testing for overdispersion (GLM 'quasipoisson'). To model data on parasite abundance, we used zero-inflated regression with a negative binomial residual distribution to correct for data overdispersion, using the `zeroinfl` function from the 'pscl' R package (Jackman, 2017). Whenever the model did not fit the data we transformed the abundance data into presence/absence format and used GLM with a binomial distribution [function `glm()`]. Parasite prevalence was assessed by GLM with a binomial distribution. Since we aimed at assessing the effect of different factors on parasite infections, we presented the full models with all independent variables in the main text. However, we also performed backward model selection based on Akaike information criterion (AIC), using the function `step()` in R, and compared the selected models with the corresponding full models using a χ^2 test. To compare cross-colony parasite mean abundance/

prevalence in relation to nest density (total nest density and active nest density) and climatic factors (air temperature and precipitation) we used analysis of variance and Tukey HSD tests. The effect of nest density and climatic factors on parasite mean abundance (i.e. number of parasites of a particular species divided by the total number of hosts examined; Bush *et al.*, 1997) and prevalence (i.e. number of infected hosts by a particular parasite species divided by the total number of hosts examined; Bush *et al.*, 1997) was assessed using Pearson and Spearman correlation tests. Statistical analysis included Wilcoxon rank sum test and proportion test to compare parasite abundance and prevalence, respectively, between sampling seasons (autumn/winter and spring) at Stony Point. Seasonal differences in parasite prevalence were assessed using parasites from chicks because chicks were mainly sampled in the spring season at Stony Point and helminth parasites were only recorded for chicks. All statistical tests and plot design were conducted in R 3.4.3 (R Core Team, 2017).

Results

Three parasitic groups (ectoparasites, haemoparasites and helminth parasites) were recorded from 793 African penguins and 628 penguin nests at five colonies along the south-western coast of South Africa (Fig. 1). Ectoparasites comprised of two fleas (*Parapsyllus humboldti* and *Echidnophaga gallinacea*), a louse (*Austrogoniodes demersus*) and a soft tick (*Ornithodoros capensis* s. s.). Haemoparasites were morphologically consistent with the orders Piroplasmorida/Haemospororida and Spirochaetales. Four helminth genera were detected in chicks (*Cardiocephaloides* spp., *Renicola* spp., *Contracaecum* spp. and *Cyathostoma* spp.). Prevalence, mean abundance, mean intensity (i.e. average number of parasites of a particular species divided by the number of infected hosts; Bush *et al.*, 1997) and sex ratios of parasites associated with penguins, across the selected colonies, are provided in Table 2. Although mites (Acari) were recorded, they were mainly found in penguin nests and in high abundance. Mites are very specious and were not included in this study due to the taxonomic difficulty in distinguishing between parasitic and non-parasitic soil mites (Proctor and Owens, 2000). However, the importance of mite data is recognized and will be included in follow-up studies.

Ectoparasites on penguins

The most abundant and prevalent ectoparasite on penguins was *P. humboldti* (4.57 ± 0.2 ; 69.10%), while the second most abundant ectoparasite was *O. capensis* (s. s.) (0.51 ± 0.07 ; 16.65%) of which larvae were mainly collected (Table 2). This life stage was the most abundant on penguins at three of the five colonies (Supplementary Table S1). *Parapsyllus humboldti* also exhibited the highest mean intensity on penguins (6.61 ± 0.25), followed by *E. gallinacea* (4.95 ± 0.60). The infestation level of *A. demersus* was in general very low, and apart from its absence at Simon's Town, no pattern was evident. The sex ratios of the individual parasite taxa varied: *P. humboldti* recorded an equal sex ratio, *E. gallinacea* and *A. demersus* recorded a female-biased ratio, while only female *O. capensis* (s. s.) (three females infested three penguins) were recorded from penguins (Table 2).

Haemoparasites and helminth parasites of penguins

Piroplasmorida/Haemospororida was the most prevalent haemoparasite group (33.51%) compared with Spirochaetales (2.59%) in penguins. The helminth *Cardiocephaloides* spp. was the most prevalent genus (56.17%) in penguin chicks followed by *Contracaecum* spp. (12.83%) (Table 2).

Table 2. Ectoparasites, haemoparasites and helminths recorded from African penguins at five colonies along the south-western coast of South Africa during 2016 and 2017

Parasite species	Total prevalence (%)	Mean abundance (\pm S.E.)				Mean intensity (\pm S.E.)				Sex ratio (δ : ♀)
		Larvae	Nymphs	Adults	Total	Larvae	Nymphs	Adults	Total	
Fleas										
<i>P. humboldti</i>	69.10 ^a	0.001 ^b (\pm 0.001)	-	4.57 (\pm 0.20)	4.57 ^a (\pm 0.20)	1 ^b	-	6.61 (\pm 0.25)	6.61 ^a (\pm 0.25)	1.08:1
<i>E. gallinacea</i>	5.30	-	-	0.26 (\pm 0.05)	0.26 (\pm 0.05)	-	-	4.95 (\pm 0.60)	4.95 (\pm 0.60)	0.16:1
Lice										
<i>A. demersus</i>	1.01	-	-	0.01 (\pm 0.006)	0.01 (\pm 0.006)	-	-	1.38 (\pm 0.38)	1.38 (\pm 0.38)	0.38:1
Ticks										
<i>O. capensis</i>	16.65	0.43 (\pm 0.06)	0.08 (\pm 0.03)	0.004 (\pm 0.002)	0.51 (\pm 0.07)	2.97 (\pm 0.30)	2.25 (\pm 0.75)	1	3.09 (\pm 0.31)	Only female
Haemoparasites										
Piroplasmorida/Haemospororida	33.51	-	-	-	-	-	-	-	-	-
Spirochaetales	2.59	-	-	-	-	-	-	-	-	-
Helminths										
<i>Cardiocephaloides</i> spp.	56.17	-	-	-	-	-	-	-	-	-
<i>Renicola</i> spp.	0.73	-	-	-	-	-	-	-	-	-
<i>Contracaecum</i> spp.	12.83	-	-	-	-	-	-	-	-	-
<i>Cyathostoma</i> spp.	3.87	-	-	-	-	-	-	-	-	-

Sample sizes $N = 793$ (ectoparasites), 734 (haemoparasites) and 413 (helminths).

^aTotal prevalence, mean abundance and mean intensity of fleas excluded flea larvae from Dassen Island.

^bMean abundance and mean intensity of flea larvae excluded flea larvae from Dassen Island.

Ectoparasites in nests

The prevalence, mean abundance, mean intensity and sex ratios of parasites recorded from penguin nests across the selected colonies are provided in Table 3. Only ectoparasites were recorded in nests, therefore the results are presented only for fleas and ticks. More than half of the nests were infested with *P. humboldti* (57.80%), of which the larval stage was the most abundant and prevalent (13.60 ± 1.49; 47.29%). The second most abundant and prevalent parasite was *O. capensis* (s. s.) (6.37 ± 1.90; 54.30%), of which nymphs were the most abundant and prevalent (2.5 ± 0.94; 39.81%) (Supplementary Table S2). *Parapsyllus humboldti* also recorded the highest mean intensity, in infested nests, (25.30 ± 2.52) of which larvae recorded the highest mean intensity (28.55 ± 2.90), followed by *O. capensis* (s. s.) (11.74 ± 3.48) of which tick larvae recorded the highest mean intensity (9.08 ± 3.02) (Table 3). Ectoparasite taxa in nests exhibited different sex ratios: *P. humboldti* recorded a female-biased ratio, *E. gallinacea* recorded an equal number of males and females, and *O. capensis* (s. s.) recorded a male-biased ratio in nests (Table 3).

Factors that influence parasite infestations

The outcome of regression models showed a strong effect of penguin age, colony location and colony (Table 4). The majority of the full models used in the analysis did not show significant differences from the best models selected according to the AIC (Supplementary Table S3). In particular, abundance of total ectoparasites, fleas (*P. humboldti* and *E. gallinacea* combined) and *O. capensis* (s. s.), and prevalence of Piroplasmorida/Haemospororida were significantly higher in chicks compared with adult penguins (Table 4). Interspecific variation in parasite infestations, on penguins, was recorded in mainland compared with island colonies. Penguins at mainland colonies recorded significantly higher abundances for total ectoparasites and fleas on penguins than island colonies. In addition, Piroplasmids/Haemospororida and *Cardiocephaloides* spp. were significantly more prevalent in penguins at mainland colonies compared with islands. A similar pattern was recorded in penguin nests with significantly higher abundance recorded for total ectoparasites, fleas (both life stages combined), adult fleas and flea larvae at mainland colonies compared with islands.

Parasite infestations also varied between colonies with Stony Point and Simon’s Town generally harbouring significantly more parasites on penguins and in nests. In particular, total ectoparasite abundance was significantly higher on penguins at Stony Point and Simon’s Town than most of the other colonies (Table 4). In addition, at Stony Point a significantly higher *O. capensis* (s. s.) abundance and higher Piroplasmorida/Haemospororida prevalence were recorded for penguins compared with most or all other colonies. Penguin chicks at Stony Point also recorded a significantly higher prevalence of *Cardiocephaloides* spp. compared with other colonies, though when compared with Simon’s Town the difference in prevalence was not significant. Abundance of *O. capensis* (s. s.) in nests were also generally higher at Stony Point compared with the other colonies and significantly so for Simon’s Town and Dassen Island. In contrast, penguins at Simon’s Town recorded a significantly higher abundance of fleas on penguins compared with other colonies. In addition, this colony recorded significantly higher infestations of total ectoparasites, total fleas (both life stages combined), adult fleas and flea larvae in nests compared with the other colonies.

Cross-colony comparison of mean abundance and prevalence of parasites in autumn/winter in relation to nest density (total and active nest density) and climatic factors (air temperature and

Table 3. Ectoparasites recorded from nests of African penguins (N = 628) along the south-western coast of South Africa during 2016 and 2017

Parasite species	Prevalence (%)			Mean abundance (±s.e.)			Mean intensity (±s.e.)			Sex ratio (♂:♀)			
	Larvae	Nymphs	Adults	Total	Larvae	Nymphs	Adults	Total	Larvae		Nymphs	Adults	Total
Fleas													
<i>P. humboldti</i>	47.29 ^a	-	33.44	57.80 ^b	13.60 ^b (±1.49)	-	1.03 (±0.10)	14.62 ^b (±1.54)	28.55 ^a (±2.90)	-	3.08 (±0.26)	25.30 ^b (±2.52)	0.7:1
<i>E. gallinacea</i>	-	-	0.64	0.64	-	-	0.01 (±0.005)	0.01 (±0.005)	-	-	1.5 (±0.29)	1.5 (±0.29)	1:1
Ticks													
<i>O. capensis</i>	21.34	39.81	30.57	54.30	1.94 (±0.66)	2.5 (±0.94)	1.94 (±0.67)	6.37 (±1.90)	9.08 (±3.02)	6.27 (±2.34)	6.35 (±2.16)	11.74 (±3.48)	1.63:1

^aPrevalence, mean abundance and mean intensity of flea larvae excluded flea larvae from Dassen Island.
^bTotal prevalence, mean abundance and mean intensity of fleas excluded flea larvae from Dassen Island.

Table 4. Effect of colony location (mainland and island), colony (Stony Point, Simon's Town, Dassen-, Dyer- and Robben Island) and penguin age (adult and chick) on parasite infestation of African penguins and their nests during in the autumn/winter season (2016 and 2017)

Type of analysis	Dependent variable	Predictor	Intercept	Independent variables	Estimate	s.e.	z value	P value
Ectoparasites on penguins								
ZINB	Total Ectoparasites	Location	Islands	Mainland	0.12738	0.032324	3.941	***
		Year	2016	2017	0.087586	0.032304	2.711	**
		Age	Adult	Chick	0.436554	0.05141	8.492	***
ZINB	Total Ectoparasites	Colony	Stony Point	Dassen Island	-0.104378	0.047471	-2.199	*
			Stony Point	Dyer Island	-0.327557	0.058797	-5.571	***
		Year	2016	2017	0.099295	0.031331	3.169	**
		Age	Adult	Chick	0.444792	0.050382	8.828	***
ZINB	Fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Location	Islands	Mainland	0.117148	0.033258	3.522	***
		Year	2016	2017	0.068947	0.033196	2.077	*
		Age	Adult	Chick	0.42249	0.052495	8.048	***
ZINB	Fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Colony	Stony Point	Simon's Town	0.090054	0.04393	2.05	*
			Stony Point	Dyer Island	-0.314059	0.060882	-5.158	***
		Year	2016	2017	0.082524	0.032126	2.569	*
		Age	Adult	Chick	0.431918	0.051295	8.42	***
glm 'binomial'	Ticks (<i>O. capensis</i> s. s.)	Year	2016	2017	0.6829	0.2899	2.355	*
		Age	Adult	Chick	2.2412	0.7432	3.016	**
		Body mass			-0.4595	0.2194	-2.094	*
glm 'binomial'	Ticks (<i>O. capensis</i> s. s.)	Colony	Stony Point	Simon's Town	-2.436	0.747	-3.261	**
			Stony Point	Dassen Island	-1.0322	0.4444	-2.323	*
			Stony Point	Dyer Island	-1.527	0.512	-2.982	**
		Year	2016	2017	0.6398	0.2956	2.165	*
		Age	Adult	Chick	2.0568	0.7473	2.752	**
		Body mass			-0.6267	0.2304	-2.72	**
Haemoparasites								
glm 'binomial'	Piroplasmids/Haemospororida	Location	Islands	Mainland	1.9782	0.2148	9.211	***
		Age	Adult	Chick	2.3298	0.3198	7.286	***
		Body mass			0.396	0.1639	2.415	*
glm 'binomial'	Piroplasmids/Haemospororida	Colony	Stony Point	Simon's Town	-0.5709	0.275	-2.076	*
			Stony Point	Dassen Island	-3.0786	0.4518	-6.815	***
			Stony Point	Dyer Island	-1.2171	0.2873	-4.236	***
			Stony Point	Robben Island	-2.7051	0.3871	-6.987	***
		Age	Adult	Chick	2.3439	0.3273	7.161	***
		Body mass			0.4331	0.169	2.563	**
Helminths								
glm 'binomial'	<i>Cardiocephaloides</i> spp.	Location	Islands	Mainland	0.7119	0.2317	3.073	**

(Continued)

Table 4. (Continued.)

Type of analysis	Dependent variable	Predictor	Intercept	Independent variables	Estimate	s.e.	z value	P value
		Year	2016	2017	-1.4495	0.2351	-6.166	***
		Body mass			0.5537	0.1777	3.115	**
glm 'binomial'	<i>Cardiocephaloides</i> spp.	Colony	Stony Point	Dassen Island	-0.8585	0.3538	-2.426	*
			Stony Point	Dyer Island	-0.7548	0.3519	-2.145	*
			Stony Point	Robben Island	-0.8319	0.3531	-2.356	*
		Year	2016	2017	-1.4688	0.2382	-6.167	***
		Body mass			0.5373	0.1823	2.947	**
Ectoparasites in nests								
ZINB	Total Ectoparasites	Location	Islands	Mainland	0.15051	0.055	2.737	**
		Year	2016	2017	0.21289	0.05421	3.927	***
ZINB	Total Ectoparasites	Colony	Stony Point	Simon's Town	0.21364	0.07566	2.824	**
		Year	2016	2017	0.22431	0.05424	4.136	***
ZINB	Total fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Location	Islands	Mainland	0.16791	0.06119	2.744	**
		Year	2016	2017	0.15607	0.06157	2.535	*
ZINB	Total fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Colony	Stony Point	Simon's Town	0.23235	0.08304	2.798	**
			2016	2017	0.17289	0.06185	2.795	**
ZINB	Adult fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Location	Islands	Mainland	0.159537	0.074776	2.134	*
ZINB	Adult fleas (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Colony	Stony Point	Simon's Town	0.39834	0.08065	4.939	***
ZINB	Flea larvae (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Location	Islands	Mainland	0.18582	0.06615	2.809	**
		Year	2016	2017	0.13789	0.06697	2.059	*
ZINB	Flea larvae (<i>P. humboldti</i> and <i>E. gallinacea</i>)	Colony	Stony Point	Simon's Town	0.21678	0.08927	2.428	*
		Year	ear 2016	2017	0.14373	0.06776	2.121	*
ZINB	Ticks (<i>O. capensis</i> s. s.)	Colony	Stony Point	Simon's Town	-0.22204	0.09968	-2.228	*
			Stony Point	Dassen Island	-0.4778	0.14262	-3.35	***

Type of analysis: regression model ZINB (zero-inflated negative binomial), glm 'binomial' and proportion test. Significant values: ***<0.001, **0.001-0.01, *0.01-0.05.

precipitation) revealed significant differences between colonies for some parasitic groups (Piroplasmids/Haemospororida $F=39.73$, $P<0.001$; *Cardiocephaloides* spp. $F=2.69$, $P<0.05$; total parasites in nest $F=4.12$, $P<0.01$; fleas in nest $F=9.605$, $P<0.001$; total parasites on penguins $F=14.27$, $P<0.001$; fleas on penguins $F=15.15$, $P<0.001$). The infestation levels of several parasite taxa correlated with nest density (total and active). In particular, the prevalence of Piroplasmids/Haemospororida in penguins was significantly positively correlated with total and active nest density ($r=0.97$, $P<0.01$ and $r=0.92$, $P<0.05$, respectively) (total nest density as example Fig. 2A). The prevalence of *Cardiocephaloides* spp. in penguin chicks was significantly positively correlated with total nest density (Fig. 2B) ($r=0.98$, $P<0.01$). Likewise, mean abundance of total

ectoparasite and *O. capensis* (s. s.) in nests was significantly positively correlated with the density of active nests (Fig. 2C) ($r_{\text{Spearman}}=0.9$, $P<0.05$) and total nests (Fig. 2D) ($r_{\text{Spearman}}=0.9$, $P<0.05$), respectively. Flea abundance in nests follow a similar pattern as the total ectoparasites in nests, however the response was not significant. The infestation levels of two parasite taxa correlate with climate. In particular, mean abundance of *A. demersus* on penguins was significantly negatively correlated with annual mean ambient temperature and annual precipitation ($r=-0.95$, $P<0.05$, and $r=-0.92$, $P<0.05$, respectively), while prevalence of *Contraecum* spp. in chicks was significantly positively correlated with annual mean ambient temperature and annual precipitation ($r=0.97$, $P<0.01$, and $r=0.94$, $P<0.05$, respectively).

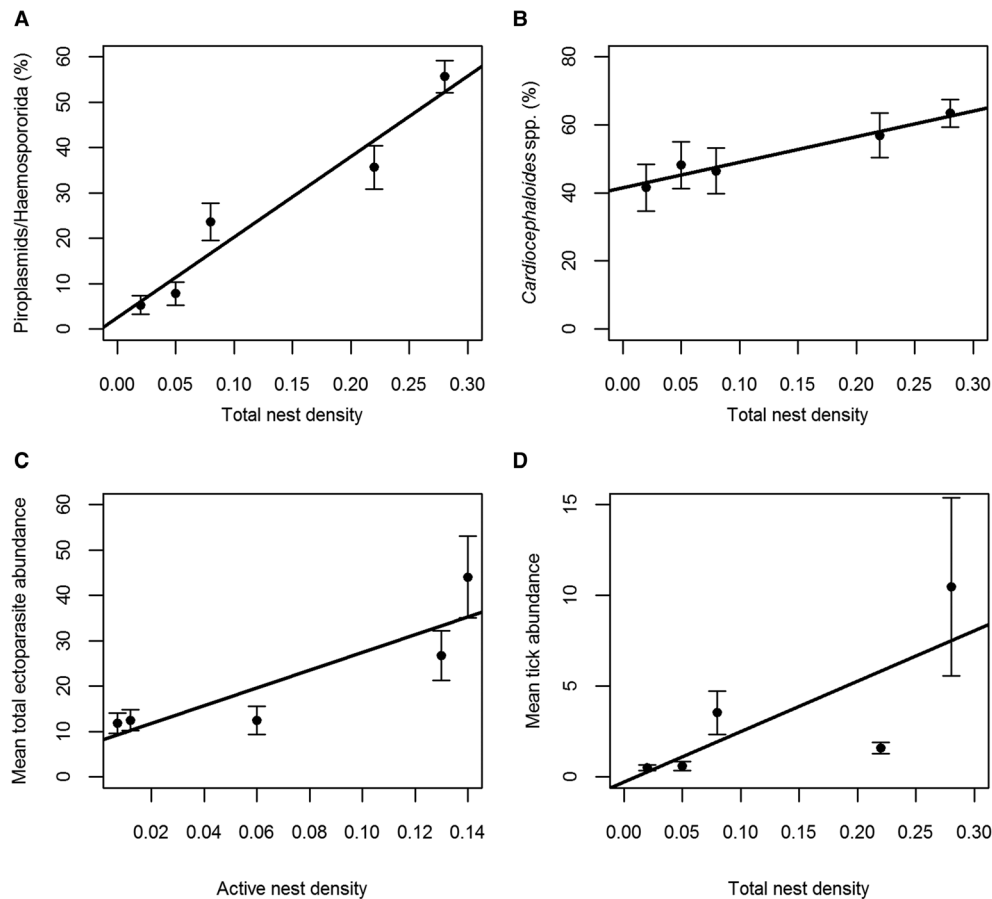


Fig. 2. Pearson correlation between (A) Piroplasmids/Haemospororida prevalence and total nest density, and (B) *Cardiocephaloides* spp. prevalence and total nest density. Spearman correlation between (C) mean total nest ectoparasites and active nest density and (D) mean nest ticks (*O. capensis* s. s.) and total nest density of African penguins.

Several parasite taxa exhibited seasonal variation in infestations on penguin chicks at Stony Point during 2016. In particular, *P. humboldtii* was significantly more prevalent (91.6 and 77.1% respectively, $P < 0.05$) and abundant (8.1 ± 0.7 and 4.6 ± 0.5 respectively, $W = 2619.5$, $P < 0.001$) on chicks in spring compared with autumn/winter. A similar, but stronger pattern was recorded for *O. capensis* (s. s.) prevalence (75.8 and 9.6% respectively, $P < 0.001$) and abundance (2.8 ± 0.4 and 0.1 ± 0.05 respectively, $W = 1181.5$, $P < 0.001$) on chicks [data for *P. humboldtii* and *O. capensis* (s. s.) abundance not shown] (Fig. 3A). Spirochaetales were also more prevalent in penguins in spring compared with autumn/winter, but only marginally significant (11.6% spring and 2.5% autumn/winter, $P = 0.05$) (Fig. 3B). Helminth parasite infestations in chicks were not affected by season (Fig. 3C). Prevalence of ectoparasites in penguin nests also varied significantly across seasons. *Parapsyllus humboldtii* was significantly more prevalent (68.0 and 42.2% respectively, $P < 0.001$) and abundant (8.14 ± 0.7 and 4.6 ± 0.5 respectively, $W = 0.373$, P value < 0.001) in nests during spring compared with autumn/winter. Likewise, *O. capensis* (s. s.) was significantly more prevalent (95.1 and 56.0% respectively, $P < 0.001$) and abundant (13.9 ± 5 and 1.7 ± 0.3 , respectively, $P < 0.05$) in nests in during spring compared with autumn/winter (Fig. 4).

Discussion

Parasite diversity and abundance associated with penguins and their nests

In this study, *P. humboldtii* was the most prevalent and abundant parasite on penguins and in their nests. Most of the *P. humboldtii*

found on penguins were adults, while the larval stage dominated the nests. This is consistent with the life cycle of fleas, given that adult fleas mainly attach to the host for a blood meal while the larvae remain in the nest where they feed on organic matter (Bitam *et al.*, 2010). The sex ratio of *P. humboldtii* was equal on penguins and female-biased in nests (0.7:1). This pattern has previously been observed in nests of other avian species (e.g. passerines) and is consistent with the fact that female fleas live longer than males in natural populations and are thus more prevalent (Rothschild and Clay, 1952; Shutler *et al.*, 2003). The genus *Parapsyllus* seems to be specifically targeting penguins (Clarke and Kerry, 1993) with some species, such as *P. longicornis*, occurring on multiple penguin species (Murray *et al.*, 1990). In the case of *P. humboldtii* the flea has been found on Humboldt penguins (*Spheniscus humboldtii*) and in their nests in Chile and Peru (Segerman, 1995) and is frequently collected from African penguins in rehabilitation centres in South Africa (Parsons and Vanstreels, 2016). It is currently the only species from the genus *Parapsyllus* in southern Africa (Segerman, 1995).

A species of flea not previously reported for African penguins, the sticktight flea (*E. gallinacea*) was attached to the eyelids and body of penguins and recorded in the nests at Dassen Island. *Echidnophaga gallinacea* has a worldwide-distribution (Boughton *et al.*, 2006) and infests an extensive variety of hosts, including poultry, domestic mammals and wildlife (Segerman, 1995; Bitam *et al.*, 2010). The European rabbit (*Oryctolagus cuniculus*) is also a common host and the presence of the rabbit and sharing of burrows on the island would explain the flea's presence on African penguins (Dunnet and Nardon, 1974). Female fleas remain firmly attached on the host for long periods and have a

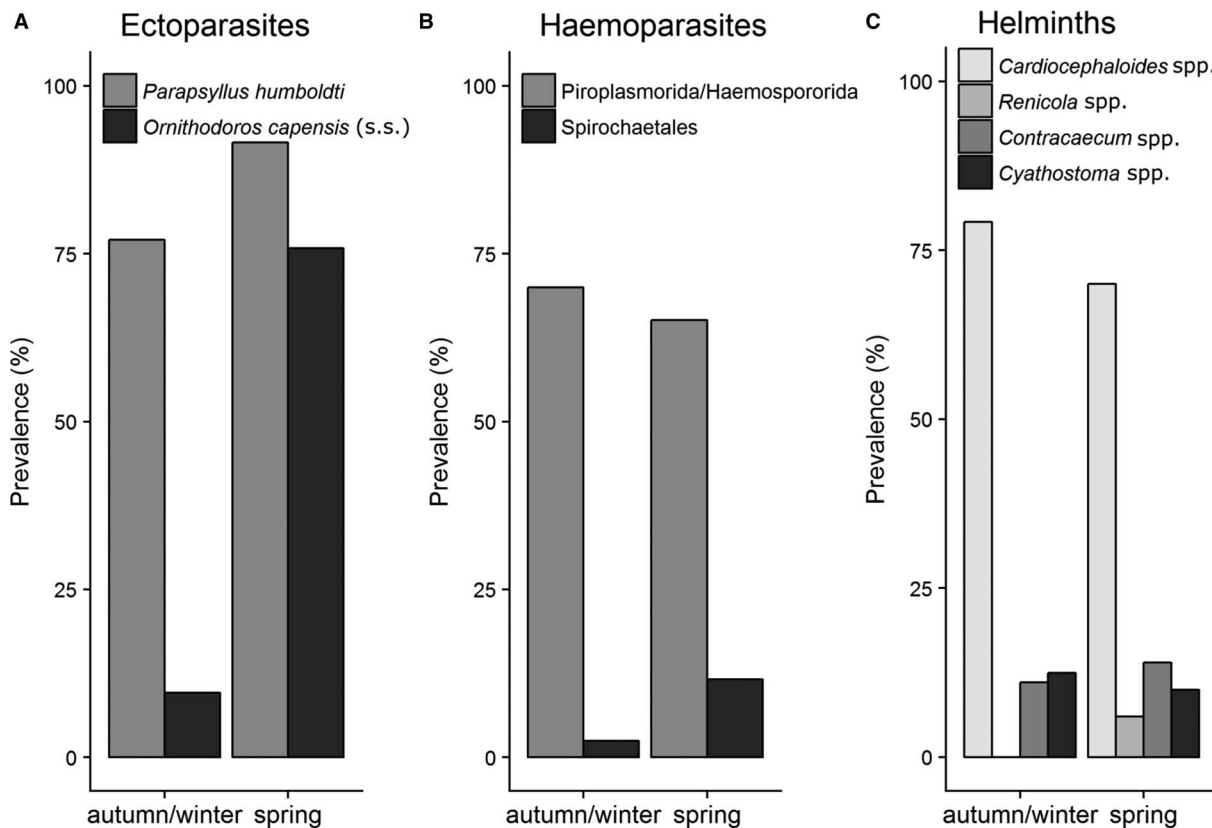


Fig. 3. Prevalence of ectoparasites, haemoparasites and helminth parasites associated with African penguin chicks at Stony Point during two seasons (autumn/winter and spring) in 2016. Sample sizes $N=178$ (ectoparasites), 166 (haemoparasites) and 122 (helminths).

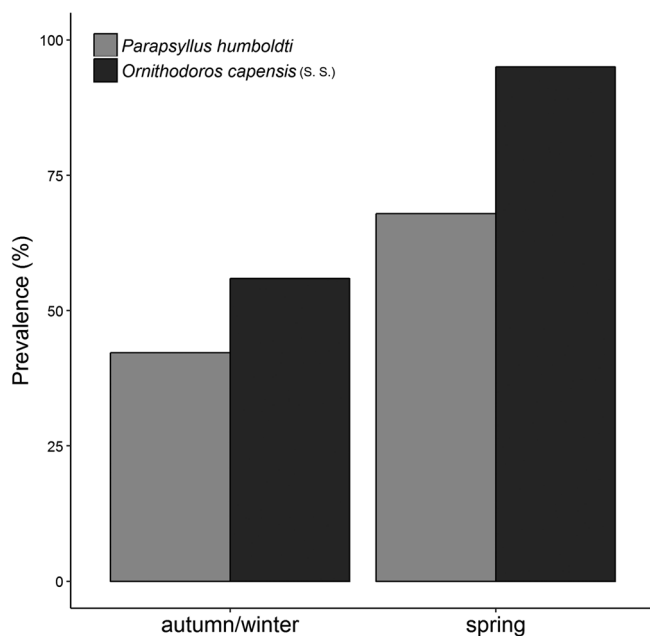


Fig. 4. Prevalence of fleas and soft ticks in the nests of African penguins ($N=190$) at the Stony Point colony during two seasons (autumn/winter and spring) in 2016.

high fecundity (Krasnov, 2008). This would explain the female-biased ratio on the penguins (0.16:1).

As several other *Austrogoniodes* species are associated with penguin species (Pilgrim and Palma, 1982; Clarke and Kerry, 1993), the presence of the chewing louse *A. demersus* on African penguins in the current study is not unexpected (Von Keler, 1952; Banks and Palma, 2003). The louse has also been

recorded on Galápagos penguins (*Spheniscus mendiculus*) in the Galápagos Archipelago (Banks and Palma, 2003). The on-host sex ratio for *A. demersus* was female-biased (0.38:1) and might be the result of the longevity of females and, the smaller size and active lifestyle of males (Marshall, 1981b). Lice transmission occurs by direct body contact between individuals such as between parents to offspring in the nest (Clayton and Tompkins, 1994, 1995) and between older chicks when they group together in the crèche stage (Banks et al., 2006).

The soft tick *O. capensis* sensu stricto (s. s.) infests several seabird species globally (e.g. Keirans et al., 1992; Dupraz et al., 2016). In South Africa, *O. capensis* (s. s.) has been collected from several seabird species such as Cape cormorant (*Phalacrocorax capensis*) (Peirce and Parsons, 2012), great black-backed gull (*Larus marinus*) and African penguins (Theiler, 1959; Daturi, 1986). *Ornithodoros capensis* (s. s.) is nidicolous and is able to live in the host's shelter for long periods of time (with a maximum life span of 25 years) (Sonenshine, 1991, 1993; Vial, 2009). All life stages (larvae, nymphs and adults) attach to the host for short periods (from a few minutes up to an hour) to feed (Oliver, 1989). The colonial lifestyle, repeated use of nests and high population densities that penguins reach, expose them to a greater abundance of ticks (Duffy, 1988; Mangin et al., 2003). In our study, *O. capensis* (s. s.) was the second most abundant ectoparasites found on penguins. The presence of blood in larvae from penguins provides new evidence that larval stages from some *Ornithodoros* spp. do take blood meals. This life stage was also the most abundant on penguins in the majority of the selected colonies. In nests, *O. capensis* (s. s.) was also the second most prevalent and abundant parasite, and exhibited one of the highest mean intensity of infestation. Nymphs were the most prevalent and abundant life stage in all nests, while larvae recorded the highest mean intensity in nests. This agrees with a previous

study on African penguin nests at Marcus Island (Daturi, 1986). In the current study, only female ticks were recorded on penguins, while in nests the tick showed a strong bias towards males. In many nidicolous tick species, males require fewer nymphal stages to emerge as adults (i.e. become adults sooner) compared with females (Sonenshine, 1991). This could explain the presence of more male than female ticks in penguin nests. In fact, it is not unusual to find large numbers of male nidicolous ticks in the host nests (e.g. *Argas arboreus* in nests of cattle egrets (*Bubulcus ibis*); Guirgis, 1971).

In this study, Piroplasmorida/Haemospororida (orders that include *Babesia* spp., *Plasmodium* spp. and *Leucocytozoon* spp.; Levine, 1971; Atkinson, 2008) were more commonly recorded in penguins (33.51%) compared with Spirochaetales (2.59%) (order that includes *Borrelia* spp.; Paster *et al.*, 1991). This pattern supports previous studies and suggests that Piroplasmorida/Haemospororida are more prevalent in penguin species compared with Spirochaetales (Quillfeldt *et al.*, 2011; Yabsley *et al.*, 2012). *Ornithodoros* spp. are known vectors of haemoparasites; it is possible that the high prevalence of *Babesia*-like inclusions in erythrocytes observed in this study is related to the presence of *O. capensis* on penguins and in their nests.

Helminth species from four genera (*Cardiocephaloides*, *Renicola*, *Contraecum* and *Cyathostoma*) were recorded from penguin chicks. Previously, the trematodes *Cardiocephaloides physalis* and *Renicola sloanei*, and the nematodes *Contraecum* sp., *Contraecum variegatum* and *Cyathostoma phenisci* were recorded from African penguins (Horne *et al.*, 2011; Kanarek *et al.*, 2013; Viljoen, 2015). Most of these helminths have been associated with various penguin species, which may be related to their similarity in diet (Brandão *et al.*, 2014). *Cardiocephaloides* spp. was the most prevalent (56.17%) helminth genus recorded in the study followed by *Contraecum* spp. (12.83%) and *Cyathostoma* spp. (3.87%). These results are supported by a previous study on African penguins along the south-western coast of South Africa (Viljoen, 2015). Since the life cycle of the helminth parasites involves fish, squid and krill, it is likely that penguins acquired infection through their diet (Randall and Bray, 1983; Horne *et al.*, 2011; Brandão *et al.*, 2014). The integrity of the immune system, type of diet and behaviour of penguins will however determine the degree of susceptibility to helminth infections (Diaz, 2006; Diaz *et al.*, 2010; Carrera-Játiva *et al.*, 2014).

Factors that influence parasite infestations

Significantly more ectoparasites, and particularly fleas (*P. humboldti* and *E. gallinacea*) and ticks (*O. capensis* s. s.) were recorded on chicks compared with adult penguins. Chicks generally have a less developed immune system and are therefore more susceptible to parasitic infestations compared with adult penguins (van Rensburg, 2010). In addition, chicks spend more time in or close to the nest (Sherley *et al.*, 2014) and are therefore more readily infested by nest-associated ectoparasites (fleas and soft ticks). Since ticks can act as vectors of haemoparasites, the significantly higher incidence of Piroplasmorida/Haemospororida in chicks compared with adult penguins, in the current study, could potentially be a reflection of the pattern observed for *O. capensis* (s. s.) (Peirce, 2000).

Parasite infestations were significantly higher in mainland compared with island colonies. Stony Point and Simon's Town, the two mainland colonies, exhibited the same parasite richness but higher abundance and prevalence of parasites on and in penguins and in their nests than on islands. The most likely explanation for this pattern for ectoparasites is the higher densities of both total and active nests on mainland compared with island

colonies. Large colonies with nests at close proximity allow proliferation and transmission of ectoparasites (Brown and Brown, 1986). Parasite species found in nests vary in host association, and in particular soft ticks are able to remain in nests regardless of the presence of a bird host (Duffy, 1988) while fleas are dependent on the presence of the host (Marshall, 1981a). This was supported by our results, which showed a positive correlation between mean abundance of *O. capensis* (s. s.) in nests and total nest density (active and non-active together), while mean total ectoparasite abundance in nests (of which fleas represented 71.57%) correlated with the density of active nests. The positive correlation between total nest density and Piroplasmids/Haemospororida prevalence in penguins is most probably due to a higher abundance of *O. capensis* (s. s.) in colonies with higher nests densities. Coloniality in birds seems to facilitate elevated haemoparasite richness and prevalence (Tella, 2002). From the current study it appears that higher total nest density can further aggravate the situation.

The trematode *Cardiocephaloides* spp. was significantly more prevalent in chicks at the two mainland colonies compared with islands. The complete life cycle of *C. physalis*, the most likely *Cardiocephaloides* species found in our study, has not been fully described but it is hypothesized that it uses the snail *Burnupena* spp. as its first intermediate host (Ukomadu, 2017). Rock lobsters such as the South Coast (*Palinurus gilchristi*) and West Coast rock lobster (*Jasus lalandii*) are the potential natural predator of *Burnupena* spp. Both lobster species are commercially fished between the west coast (Cape Point) and the southeast coast (East London) of South Africa (Fig. 1). This range includes the two mainland colonies (Stony Point and Simon's Town) and Dyer Island (Department of Environmental Affairs, 2013). This could affect the abundance of rock lobster in the area and facilitate a larger abundance of *Burnupena* spp. Further, the distribution of fish (second intermediate host) and specifically infected fish can also contribute to this pattern. Recently, studies on sardines (food source of penguins) did record higher densities of sardines and specifically higher densities of *Cardiocephaloides* spp. infected sardines along the south-western coast between Cape Point and Cape Agulhas (Mhlongo *et al.*, 2013; Van der lingen *et al.*, 2015). From this evidence, it is possible that the presence of intermediate and definitive hosts facilitates a higher incidence of *Cardiocephaloides* spp. in penguins at the two mainland colonies. Although Dyer island falls within the abovementioned range, it is possible that the inaccessibility of the island to humans may facilitate a healthier lobster population in the immediate area around the island. This may explain why the incidence of *Cardiocephaloides* spp. in penguin chicks was lower compared with the two mainland colonies.

Infestation levels of *O. capensis* on penguins and in their nests were higher at Stony Point compared with other colonies. This pattern explains the higher incidence of Piroplasmids/Haemospororida in penguins from Stony Point. In contrast to other colonies the number of breeding pairs have consistently increased at Stony Point for the last 5 years to reach a size of 2388 in 2016 (2017 counts are yet unconfirmed) (CapeNature, DEA and SANParks, unpublished results). This influx of penguins may explain the higher total nest density (0.28 nests per m²) and *O. capensis* (s. s.) abundance on penguins and in nests at Stony Point compared with the other colonies. Interestingly, the Simon's Town colony had the second highest total nest density, but a higher active nest density (0.14 nests per m²), although only slightly, compared with Stony Point (0.13 nests per m²) (Table 1). The dependence of fleas on hosts (Marshall, 1981a) would explain the higher abundance of total ectoparasites (of which fleas represented >70%) and fleas in active nests at Simon's Town compared with the other colonies.


Very limited deductions can be made from the relationship between the remote sensed climate data and parasite infestation. It appears that there was a significant negative relationship between *A. demersus* infestation on penguins and annual mean temperature and precipitation. Conversely, there was a positive relationship between prevalence of *Contraecaecum* spp. in penguin chicks and annual mean temperature and precipitation. Although there are some evidence that chewing lice are severely affected by temperature and humidity near the host skin (Johnson and Clayton, 2003) and that hatching time of Anisakidae larva (helminth family of *Contraecaecum* spp.) is delayed in colder water temperature (Højgaard, 1998) the sample sizes of the current study are too small to make firm deductions.

Seasonal variation in parasite infestation

Parapsyllus humboldti and *O. capensis* (s. s.) prevalence and abundance on penguin chicks and in nests were higher in spring compared with the colder and wet autumn/winter. Possible drivers of this pattern may be the absence of caring adults (e.g. providing allopreening and food) for the chicks and more favourable climatic conditions for parasites during spring. During the 21-day moult period (spring and summer) in South Africa (Crawford *et al.*, 2006) adults leave chicks unattended and unfed in the nests (Sherley *et al.*, 2014). A lack of food (Obendorf and McColl, 1980) and allopreening (Brooke, 1985) will affect chick condition and parasite infestations. The warm and dry conditions associated with spring are also more favourable for tick development, which would facilitate higher infestation of *O. capensis* (s. s.) in nests in spring (Lees, 1947). The higher incidence of *O. capensis* during spring could explain the higher incidence of Spirochaetales in penguins during spring compared with autumn/winter. Yabsley *et al.* (2012) also recorded a higher prevalence of *Borrelia* spp. in blood smears from African penguins during spring/summer months (October to February) compared with autumn/winter months (March to September) in South Africa.

The current study provides current information on the parasite diversity of natural occurring African penguins and their nests at colonies along the south-western coast of South Africa. In general, penguin chicks are more susceptible to parasite infestations during spring. Further, it is evident that the observed spatial variation in parasite infestations between colonies is driven by several factors. In particular, patterns recorded for ecto- and haemoparasites tend to be facilitated by nest density, while the availability of infected prey influences helminth infestations. Knowing and detecting changes in parasitic diversity and abundance can give insight into the possible intrinsic and extrinsic factors that may threaten the conservation of African penguins in the region.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182018002159>.

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Conflict of interest. None.

Ethical standards. This research project was approved by the Animal Ethics Committee of the University of Stellenbosch (reference number SU-ACUD15-00114) and permit were obtained from the Division of Environmental Affairs (RES2016/95 and RES2017/02), the Threatened or Protected Species (TOPS) of the Biodiversity Act (07962), CapeNature (AAA007-00191-0056) and South African National Parks (CRC/2016-2017/038-2015/V1).

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