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Research paper

Occurrence of blood parasites in seabirds admitted for rehabilitation in the Western Cape, South Africa, 2001–2013

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ABSTRACT

Blood parasites are generally uncommon in seabirds, and knowledge on their epidemiology is further limited by the fact that they often inhabit remote locations that are logistically difficult or expensive to study. We present a long term data set of blood smear examinations of 1909 seabirds belonging to 27 species that were admitted to a rehabilitation centre in Cape Town (Western Cape, South Africa) between 2001 and 2013. Blood parasites were detected in 59% of species (16/27) and 29% of individuals examined (551/1909). The following blood parasites were recorded: *Babesia ugwidiensis*, *Babesia peircei*, *Babesia* sp., *Plasmodium* sp., *Leucocytozoon ugwidi*, *Hepatozoon albatrossi*, *Haemoproteus skuae* and Spirochaetales. Several of the records are novel host-parasite associations, demonstrating the potential of rehabilitation centres for parasite and disease surveillance, particularly for species infrequently sampled from which no host-specific parasites have been described.

1. Introduction

Global seabird populations are under threat and have been declining rapidly in recent decades (Croxall et al., 2012), with disease becoming more important as a threat to species with an increase in the International Union for Conservation of Nature (IUCN) status category (Heard et al., 2013). Diseases are infrequently the single threat leading to the decline of seabird populations, but instead act in synergy with other ecological factors (Heard et al., 2013). Blood parasites can affect survival, reproductive success, plumage colouration and changes in community structure (Valkiūnas, 2005; Quillfeldt et al., 2011), and even though most studies on blood parasites in wild bird populations have found little to no evidence of these parasites as the cause of mortality, they were not designed to test for pathogenicity (Bennett et al., 1993). Furthermore, most studies on Antarctic seabirds are limited to the description of diseases and parasites rather than on their prevalence and pathogenicity (Barbosa and Palacios, 2009).

A number of parasites have been described in the blood of seabirds, including intracellular (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*, *Hepatozoon* and *Babesia*) and extracellular protozoans (*Trypanosoma*) (Jones and Shellam, 1999; Peirce, 2005; Quillfeldt et al., 2011), spirochaete bacteria (*Borrelia*) (Lobato et al., 2011) and microfilariae of nematode worms (Onchorcercidae) (Hoberg, 1986; Siers et al., 2010). In comparison to terrestrial birds, however, blood parasites are remarkably uncommon in seabirds. The main hypothesis proposed to explain this pattern is that coastal and marine environments inhabited by these species are not favourable to the arthropod vectors responsible for their transmission (Jones and Shellam, 1999; Quillfeldt et al., 2011). However, the causes may be more complex and include other factors such as the lack of the correct host-parasite assemblages or the immunological capabilities of the hosts (Martínez-Abraín et al., 2004).

Another factor limiting our understanding of the blood parasites of seabirds is the fact that they often inhabit remote locations that are logistically difficult or expensive to reach, limiting the sampling effort for disease surveillance. With the characteristically low

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prevalence of blood parasites in seabirds (Quillfeldt et al., 2011) and the endangered status of a large fraction of seabird species regularly handled (Croxall et al., 2012), which limits the sample sizes that can be obtained, it is not surprising that data on the blood parasites of these species is scarce. In this context, rehabilitation centres may provide a unique opportunity for disease surveillance among multiple seabird species, performing structured non-random surveillance (OIE 2010, 2014). In fact, because birds admitted to such facilities often present signs of illness or debilitation, they may serve as valuable sentinels for pathogens that are otherwise uncommon in the species' general population.

In this study we evaluate a 13-year dataset on the presence of blood parasites from 27 seabird species admitted for rehabilitation at the Cape Town facility of the South African Foundation for the Conservation of Coastal Birds (SANCCOB).

2. Materials and methods

2.1. Admission and bleeding

Seabirds brought to the SANCCOB Cape Town rehabilitation centre from 1 January 2001–31 December 2013 were admitted and treated using the methods described by Parsons and Underhill (2005). African penguins (*Spheniscus demersus*), the most frequently received species at this facility, were not included in this study. All species examined are included in Table 1. The vast majority of these seabirds were found along the coast of the Western Cape, South Africa, however occasionally some individuals were transferred from the Eastern Cape, South Africa. All birds were dusted with an insecticide powder (Karbadust (carbaryl (carbamate) 50 g/kg, Efekto™, Agro-Serve (Pty) Ltd, Benmore, South Africa) on admission to remove all ectoparasites present; this was repeated if necessary. Birds were subjected to varying blood collection schedules depending on veterinary assessment of their clinical condition and logistical constraints; most individuals were bled on admission and then weekly until they were released. Blood samples were obtained from the dorsal metatarsal vein (penguins) or from the medial tibiotarsal vein (other birds). Depending on the size of the bird, a 0.50 or 0.65 mm-thick needle was used to collect a drop of blood that was directly transferred into a heparinised capillary tube. If a bird died or was admitted as dead on arrival, a necropsy was performed and blood was collected from the subcutaneous veins.

2.2. Blood smear preparation and analysis

A thin blood smear was prepared immediately after blood collection. The slides were fixed with methanol and stained with a modified Wright-Giemsa stain (Kyro-Quick stain set, Kyron Laboratories (Pty) Ltd, Benrose, South Africa). Smears were examined by light microscopy for approximately 10 min each, using 50X oil immersion objective lens (*c.* 600 erythrocytes per field). Experienced veterinary and laboratory personnel examined the smears and the presence of any blood parasites was recorded. Parasites were identified on the basis of morphology alone. Blood smears of poor quality (too thick, dirty or incorrectly stained) were excluded from the dataset due to a low confidence in their negative results.

2.3. Statistical analysis

Individual bird information and blood smear results were recorded and then entered into a centralised database. 'Age class' was classified as chick (C), juvenile (J) or adult (A) on the basis of plumage. 'Time to first positive' was calculated as the number of days from the admission date to the first date in which a blood smear was found to be positive. 'Duration of stay' was calculated as the number of days from admission to outcome (release or death). 'Release rate' was calculated as the percentage of individuals admitted to the facility that survived until release back into the wild.

Additional analyses were conducted for the most prevalent parasite (*Babesia*) in the two seabird species with more than a hundred individuals sampled (Cape cormorants and Cape gannets). Fisher's exact tests were used to compare *Babesia* infections (present or absent) in relation to the rehabilitation outcome (released or died) and by age class. When data was not normally distributed (according to an Anderson-Darling test), the first quartile (Q_1), median (Q_2), and third quartile (Q_3) were used to describe the distribution. Mann-Whitney tests were used to compare time to first positive and duration of stay between *Babesia*-positive and *Babesia*-negative individuals in each age class. Only juveniles or adults were included in these analyses, due to small sample size for chicks. Additionally, Chi-square tests were used to compare the release rate between individuals that were positive or negative to *Plasmodium*.

3. Results

3.1. Detection and characterisation of blood parasites

A total of 4825 blood smears from 1909 individuals belonging to 27 species of seabirds were examined (Table 1), with an average 2.5 blood smears examined per individual. Five groups of blood parasites were detected (Figs. 1 and 2, Tables 2 and 3), with 551 infected individuals (28.7%). Multiple infections were recorded in 25 cases: *Babesia* and *Plasmodium* (16 Cape cormorants, one crowned cormorant, one rockhopper penguin), *Babesia* and *Leucocytozoon* (six Cape cormorants), *Plasmodium* and Spirochaetales (one rockhopper penguin).

Three parasite species were described in previous publications based on the same dataset: *Haemoproteus skua* in a Subantarctic skua, *Leucocytozoon ugwidi* in a Cape cormorant (Parsons et al., 2010), and *Babesia ugwidiensis* in four species of cormorants (Peirce and Parsons, 2012). All leucocytozooids in the blood smears of Cape, crowned and reed cormorants were considered morphologically consistent with *L. ugwidi* as described in Parsons et al. (2010). Even though not all blood smears of *Babesia* in cormorants were thoroughly morphologically characterized, they were found to be generally consistent with *B. ugwidiensis*. *Babesia peircei* was identified in a king penguin and details will be provided in a future publication. Spirochaetes were not morphologically or genetically characterized, but their morphology was generally consistent with the Relapsing Fever *Borrelia* previously reported in African penguins at the same facility (Yabsley et al., 2012). The several cases of *Plasmodium* spp. were not morphologically characterized, but the occasional presence of large round gametocytes displacing the nucleus (characteristic to the subgenus *Haemamoeba*) and elongated gametocytes that did or did not displace the nucleus (compatible with the subgenera *Novyella*, *Huffia*, *Giovannolaia*) indicates that more than one species were present.

The only case of *Hepatozoon* was morphologically consistent with *Hepatozoon albatrossi* as described by Peirce and Prince (1980). A colour plate was prepared to assist its identification in future studies (Fig. 1). Measurements of 50 gametocytes averaged 10.71 ± 0.59 μm in length (range: 8.52–11.23 μm) and 4.97 ± 0.43 μm in width (range: 3.87–5.74 μm).

3.2. Epidemiology of blood parasites of cape cormorants and cape gannets

For Cape cormorants, *B. ugwidiensis* infections were more frequent in juveniles (59.1%) than in adults (50.6%) ($p = 0.05$). The release rate of *Babesia*-positive juveniles was significantly lower

Table 1
Seabirds examined for blood parasites following admission at SANCCOB Cape Town rehabilitation centre, 2001–2013. Information on the status of the species was obtained from Sinclair and Ryan (2009).

Family	Species (common name)	Species (Latin name)	Status in South Africa	Individuals examined	Blood smears examined
Phalacrocoracidae	Bank cormorant	*Phalacrocorax neglectus*	Breeding endemic	35	415
	Cape cormorant	*Phalacrocorax capensis*	Breeding endemic	785	1752
	White-breasted cormorant	*Phalacrocorax lucidus*	Breeding endemic	73	189
	Crowned cormorant	*Microcarbo coronatus*	Breeding endemic	67	173
	Reed cormorant	*Microcarbo africanus*	Breeding endemic	13	19
Sulidae	Cape gannet	*Morus capensis*	Breeding endemic	746	1829
	Red-footed booby	*Sula sula*	Vagrant	1	26
Procellariidae	Northern giant petrel	*Macronectes halli*	Common visitor	18	29
	Southern giant petrel	*Macronectes giganteus*	Common visitor	27	42
	Unidentified giant petrel	*Macronectes* sp.	Common visitor	3	3
	Cape petrel	*Daption capense*	Common visitor	9	15
	White-chinned petrel	*Procellaria aquinoctialis*	Common visitor	17	27
	White-headed petrel	*Pterodroma lessonii*	Rare visitor	1	1
	Cory's shearwater	*Calonectris diomedea*	Common visitor	6	15
	Great shearwater	*Puffinus gravis*	Common visitor	1	3
	Sooty shearwater	*Puffinus griseus*	Common visitor	3	8
	Unidentified shearwater	*C. diomedea* or *Puffinus* sp.	Common visitor	4	4
	Southern fulmar	*Fulmarus glacialoides*	Rare visitor	1	1
Pelecanidae	Great white pelican	*Pelecanus onocrotalus*	Breeding endemic	44	93
Spheniscidae	King penguin	*Aptenodytes patagonicus*	Vagrant	1	7
	Macaroni penguin	*Eudyptes chrysolophus*	Vagrant	1	17
	Rockhopper penguin	*E. chrysocome* or *E. moseleyi*	Vagrant	11	75
Stercorariidae	Subantarctic skua	*Catharacta antarctica*	Common visitor	11	20
Diomedeidae	Black-browed albatross	*Thalassarche melanophris*	Common visitor	8	13
	Shy albatross	*Thalassarche cauta*	Common visitor	2	2
	Light-mantled albatross	*Phoebetria palpebrata*	Vagrant	1	1
Anhingidae	African darter	*Anhinga rufa*	Breeding endemic	10	21
Haematopodidae	African oystercatcher	*Haematopus moquini*	Breeding endemic	9	24
Phaethonidae	Red-billed tropic bird	*Phaethon aethereus*	Vagrant	1	1
Total				1909	4825

(44.5%) than that of *Babesia*-negative juveniles (70.8%) ($p < 0.01$); no such difference was noted in the survival rate of adults ($p = 0.21$). Median time to first positive was 1 day ($Q_1 = 0$, $Q_3 = 5$), and there was no difference in relation to age class ($W = 22098$, $p = 0.27$). Duration of stay was lower in *Babesia*-positive ($Q_1 = 1$, $Q_2 = 12$, $Q_3 = 25$) than in *Babesia*-negative juveniles ($Q_1 = 4$, $Q_2 = 28$, $Q_3 = 42$) ($W = 94180$, $p < 0.01$). An opposite pattern was noted for adults, with positive individuals ($Q_1 = 4$, $Q_2 = 17$, $Q_3 = 31$) having a longer duration of stay than negative individuals ($Q_1 = 1$, $Q_2 = 6$, $Q_3 = 17$) ($W = 6274$, $p < 0.01$). The number of Cape cormorants at the study facility was highest during summer and autumn (December to May), and the seasonal distribution of first positive blood smears followed a remarkably similar pattern (Fig. 3A).

For Cape gannets, *Babesia* sp. infections were more frequent in juveniles (5.1%) than in adults (2.0%) ($p = 0.03$). The release rate of *Babesia*-positive individuals was similar to that of *Babesia*-negative individuals both in juvenile ($p = 0.62$) and adult Cape gannets ($p > 0.9$). Median time to first positive was 5 days ($Q_1 = 2$, $Q_3 = 10$), and this parameter did not differ between juveniles and adults ($W = 119$, $p = 0.16$). Duration of stay was similar for individuals positive or negative to *Babesia* sp., both in juveniles ($W = 67672$, $p = 0.41$) and adults ($W = 60928$, $p = 0.24$). The number of Cape gannets at the study facility was highest in autumn and winter (March to July), and the seasonal distribution of first positive blood smears followed a generally similar pattern (Fig. 3B).

For Cape cormorants, *Plasmodium*-positive individuals had a higher release rate (79.2%) than those *Plasmodium*-negative (52.8%) ($\chi^2 = 7.00$, $df = 1$, $p = 0.01$); no such difference was noted for Cape gannets ($\chi^2 = 0.05$, $df = 1$, $p = 0.82$). The temporal distribution of *Plasmodium* sp. infections is presented in Fig. 4.

3.3. Clinical and necropsy remarks

B. peircei was determined to have contributed to the death of the only studied king penguin. On the other hand, *Babesia* sp. was successfully treated in a Macaroni penguin through primaquine 1 mg/kg oid PO for 10 days, resulting in complete elimination of parasitaemia; however, the animal was later euthenased due to lack of improvement of a leg injury. All three rockhopper penguins with *Babesia* sp. had been admitted due to arrested moult; all received the same primaquine treatment, and one of them (an adult) died 19 days after admission (10 days after its first positive smear) but death was attributed to avian malaria, and the role of the *Babesia* sp. infection was not clear. None of the deaths of infected Cape cormorants and Cape gannets were attributed to babesiosis, and the role played by *Babesia* spp. in their demise, if any, was not clear, however histopathology was not conducted in most cases.

Seabirds diagnosed with *Plasmodium* spp. infections were treated with chloroquine (10 mg/kg PO at 0 h, then 5 mg/kg PO at 6, 18, 24 h and oid for a further 9 days) and primaquine (1 mg/kg PO oid for 10 days). This treatment successfully eliminated parasitaemia in the *Plasmodium*-infected northern giant petrel and red-footed booby, both of which later died; due to a traumatic injury in the case of the petrel and a *Pasteurella* sp. infection in the case of the booby. The only case of *Plasmodium* sp. in an African oystercatcher was thought to have contributed to its death, with necropsy findings including heart failure and a positive blood smear. Likewise, all five *Plasmodium*-infected rockhopper penguins died as the result of avian malaria despite medical treatment, with necropsy findings including generalised congestion of the carcase, hepatomegaly, splenomegaly, lung congestion/oedema and hydropericardium with positive blood and kidney impression smears. None of the *Plasmodium*-infected Cape gannets and Cape cormorants were thought to have died as a result of avian malaria based on gross necropsy findings, however histopathology was not conducted in most cases.

Leucocytozoon-positive individuals were treated with metronidazole 40 mg/kg PO bid for 10 days. Two *Leucocytozoon*-positive Cape cormorants died, but their deaths were not attributed to the infection; one died from visceral gout and no post-mortem exam-

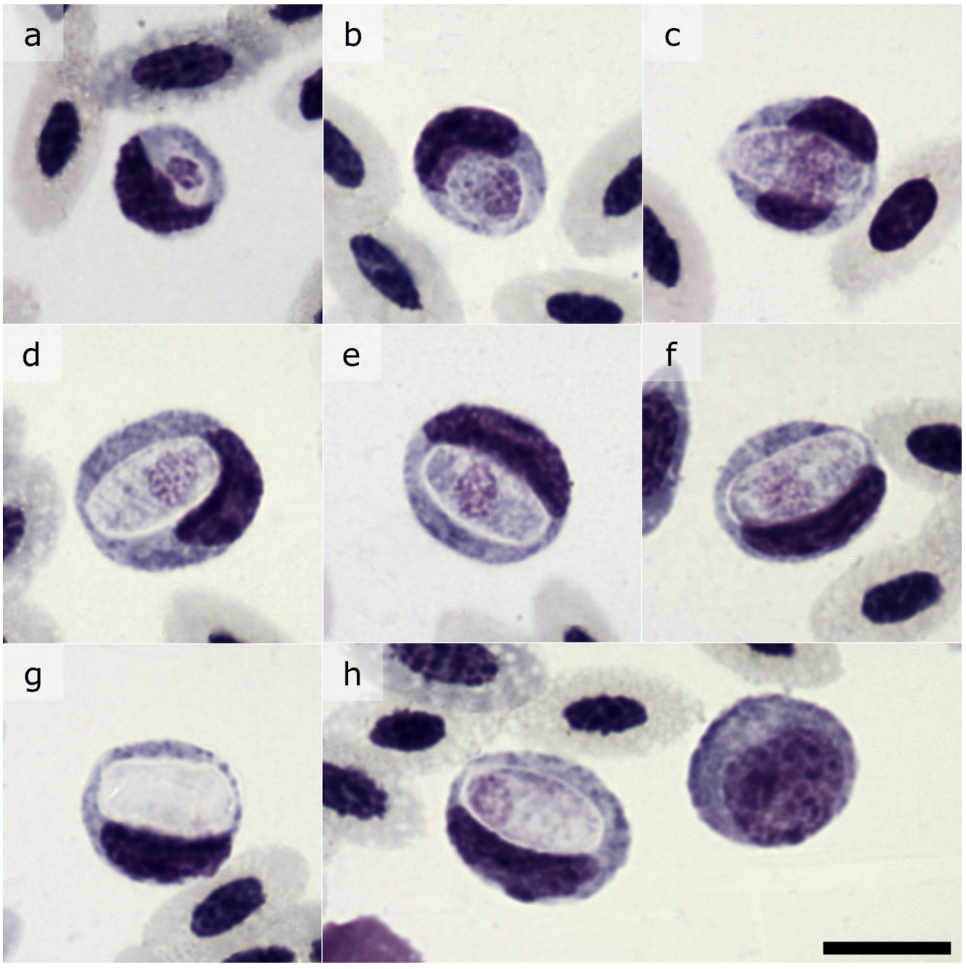

Fig. 1. *Hepatozoon albatrossi* in the lymphocytes of a black-browed albatross. Legend: (a,b) young stages in development, (c-f) adult stages illustrating variations in the texture and positioning of parasite nucleus and in the displacement of host cell nucleus, (g) unstained parasite, (h) side-by-side comparison of parasitized and non-parasitized lymphocytes. Scale bar = 10 μm.

ination was done on the other, both were negative at the time of death. The crowned and reed cormorants had been admitted as chicks for hand-rearing, their deaths were considered to be caused from starvation with an undetermined role played by the *Leucocytozoon* parasite seen on blood smear at the time of death.

The Cape gannet with Spirochaetales presented no signs of illness upon veterinary physical examination, was treated with amoxycillin 200 mg/kg PO tid for 10 days and was negative on release. The rockhopper penguin with a multiple infection of *Plasmodium* sp. and Spirochaetales received the same treatment. This bird had been diagnosed with *Plasmodium* sp. 19 days after its admission and was diagnosed with Spirochaetales one week later. The bird died 5 days later (31 days post-admission), death was concluded to have resulted from avian malaria, and the role played by the spirochaetal infection is unclear. On the other hand, the only Spirochaetales-positive Southern giant petrel was diagnosed within 6 days after its admission, and died two days later; specific cause of death was not determined with necropsy findings including congestion of the carcase and hepatomegaly.

The black-browed albatross with *Hp. albatrossi* and the Subantarctic skua with *H. skuae* showed no clinical signs of illness and were both released approximately a week after the positive diagnosis. The albatross was treated with metronidazole 40 mg/kg PO bid for 7 days however the skua did not receive drug treatment.

4. Discussion

A recent literature review found that 30% of the 60 seabird species for which a minimum sample size was examined (15 or more individuals) had been found to be infected by hemosporidian parasites in the wild (Quillfeldt et al., 2011). In this study, six of the nine (67%) species with the minimum sample size were found to be infected by at least one species of hemosporidian. When all species are considered regardless of sample size, haemosporidian infections were detected in 11 of 27 (41%) species examined in this study; additionally, *Babesia* was recorded in 10 species, Spirochaetales were recorded in three species and *Hepatozoon* was recorded in one species. It is worth considering nonetheless that all diagnostic methods are imperfect, and failure to detect a parasite on a blood smear does not necessarily indicate absence of the parasite (Cooper and Anwar, 2001). Blood smears are known to underestimate the prevalence of blood parasites (Garamszegi, 2010; Quillfeldt et al., 2011), and it is reasonable to assume this occurred to some extent in this study.

Quillfeldt et al. (2011) identified increased haemosporidian prevalence among seabird species that occur at low latitudes, are burrow-nesters and have long fledging periods. When only mainland South African breeding endemic species are considered, eight of the nine (89%) species examined in this study were found to be infected by haemosporidians; their occurrence at low latitudes and long fledging period (Hockey et al., 2005) may explain this high fre-

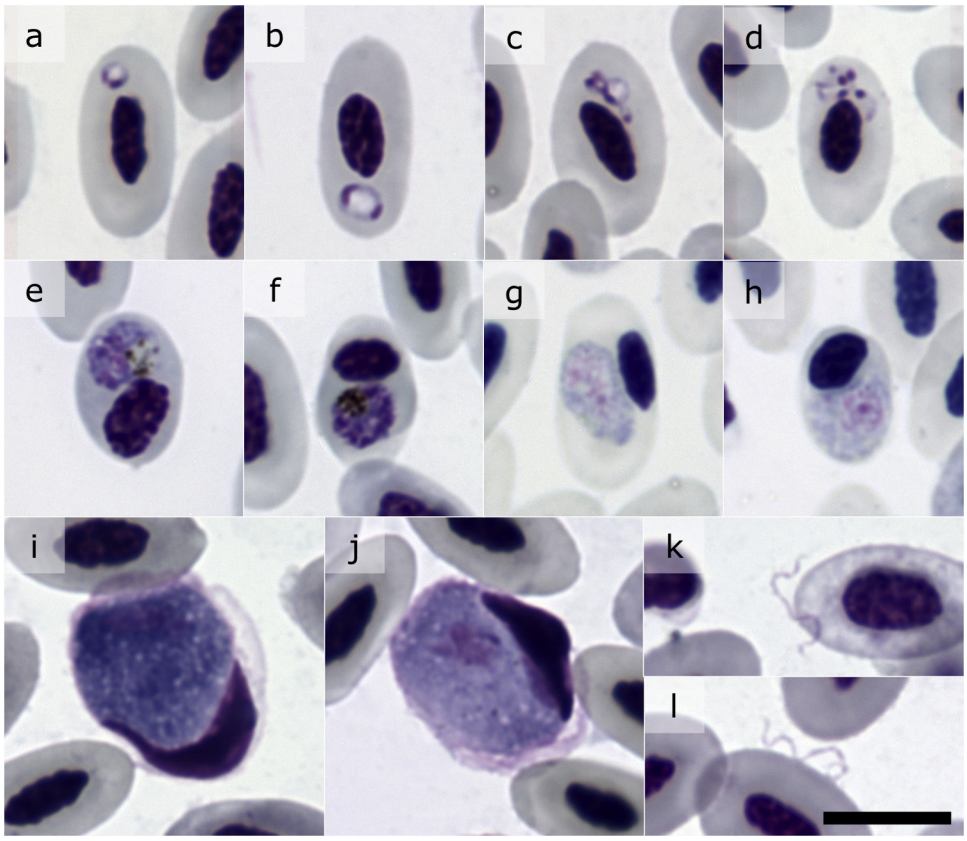

Fig. 2. Examples of the blood parasites identified in seabirds following admission at SANCCOB Cape Town rehabilitation centre, 2001–2013. Legend: (a-d) *Babesia ugwidiensis* in a Cape cormorant, (e,f) *Plasmodium* sp. meronts in a Cape gannet, (g,h) *Plasmodium* sp. gametocytes in a Cape gannet, (i,j) *Leucocytozoon ugwidi* erythrocytic gametocytes in a Cape cormorant, (k,l) Spirochaetales in a Southern giant petrel. Scale bar = 10 μm.

quency, although none are burrow-nesters. More relevant, perhaps, is the fact that sampling was conducted at a rehabilitation centre, as opposed to free-ranging specimens. Seabirds at rehabilitation centres can be expected to present a higher prevalence of blood parasites because: (a) rescued seabirds are often admitted in poor health condition, which may be the result of blood parasite infections and/or may reflect a poor immune status that predisposes them to blood parasite infections; (b) rescued seabirds may have spent a considerable amount of time on shore or close to the shore, during which they may have been exposed to invertebrate vectors to which they would not normally have been exposed; or (c) during their temporary captivity at rehabilitation centres, seabirds may be exposed to invertebrate vectors and/or maintained in close proximity with other species with which they would seldom interact in the wild, favouring their exposure to novel parasites. As a result, the frequency of parasites in this study should not be interpreted as a faithful representation of their prevalence in wild populations.

It is interesting to determine whether the blood parasite infections recorded in this study occurred in the wild or following admission to the rehabilitation centre. The prepatent period of avian haemosporidians can vary considerably depending on the parasite species, but laboratory experiments have found it to be generally within the range of 3–15 days (Valkiūnas, 2005). There is virtually no information on the prepatent period of avian *Babesia* and *Hepatozoon*, but the incubation period is usually within 4–14 days for *Babesia* in mammals (Griffiths, 1978; Chauvin et al., 2009) and up to 28–35 days for *Hepatozoon* in reptiles (Telford, 2009). The prepatent period of *Borrelia anserina* is approximately 4–7 days (Lisbôa et al., 2009). Considering these prepatent periods and the minimum time to first positive in this study (see Table 3), it is clear that at least some of the *Babesia* spp. infections preceded the admission to the rehabilitation centre in all cormorant species, Cape gannets and rockhopper penguins, as did some of the *Plasmodium* sp. infections in the Cape and white-breasted cormorants and Cape gannets, the *L. ugwidi* infections in Cape, crowned and reed cormorants, the *Hp. albatrossi* infection in the black-browed albatross and the *H. skuae* infection in a Subantarctic skua.

4.1. Babesia

Babesia spp. are tick-transmitted parasites with 16 currently recognized species from 15 avian families (Peirce, 2005; Paparini et al., 2014). Although infrequently reported in sub-Saharan birds (Bennett et al., 1992) or in seabirds (Quillfeldt et al., 2011), it was the most common blood parasite in our study. In the case of Cape cormorants and Cape gannets, although infections did have a markedly seasonal distribution, upon closer inspection this seasonal pattern was found to match the time of the year when most juveniles are admitted to the centre, corresponding to the end of their breeding cycles (Berry, 1976; Makhado et al., 2006). *B. ugwidiensis* was found in all five cormorant species studied, corroborating that this parasite is widespread in cormorants admitted to rehabilitation at SANCCOB (Peirce and Parsons, 2012).

B. peircei was described from African penguins in South Africa (Earlé et al., 1993), and *Babesia* sp. has been reported infecting little penguins (*Eudyptula minor*) in Australia (Vanstreels et al., 2015) and chinstrap penguins (*Pygoscelis antarctica*) at the South Shetland Islands (Montero et al., 2016). The case of *B. peircei* in a king penguin in this study is the first record for this host species. Additionally, the cases herein examined are the first records of *Babesia* sp. in Macaroni and rockhopper penguins, supporting the prediction that Subantarctic penguin colonies probably provide

Table 2
Frequency of parasites in the blood smears of seabirds admitted for rehabilitation at SANCCOB Cape Town rehabilitation centre, 2001–2013.

Family	Species	Individuals examined	Percentage of individuals with blood smears positive [n (%)] to					
			Babesia	*Plasmodium*	*Leucocytozoon*	Spirochaetales	*Hepatozoon*	*Haemoproteus*
Phalacrocoracidae	Bank cormorant	35	13 (37%)	1 (2.9%)	0	0	0	0
	Cape cormorant	785	447 (57%)	24 (3.1%)	6 (0.8%)	0	0	0
	White-breasted cormorant	73	17 (23%)	2 (2.7%)	0	0	0	0
	Crowned cormorant	67	2 (3.0%)	1 (1.5%)	1 (1.5%)	0	0	0
	Reed cormorant	13	1 (7.7%)	0	1 (7.7%)	0	0	0
Sulidae	Cape gannet	746	26 (3.5%)	14 (1.9%)	0	1 (0.1%)	0	0
	Red-footed booby	1	0	1 (100%)	0	0	0	0
Procellariidae	Northern giant petrel	18	0	1 (5.6%)	0	0	0	0
	Southern giant petrel	27	0	0	0	1 (3.7%)	0	0
	Unidentified giant petrel	3	0	0	0	0	0	0
	Cape petrel	9	0	0	0	0	0	0
	White-chinned petrel	17	0	0	0	0	0	0
	White-headed petrel	1	0	0	0	0	0	0
	Cory's shearwater	6	0	0	0	0	0	0
	Great shearwater	1	0	0	0	0	0	0
	Sooty shearwater	3	0	0	0	0	0	0
	Unidentified shearwater	4	0	0	0	0	0	0
	Southern fulmar	1	0	0	0	0	0	0
Pelecanidae	Great white pelican	44	0	0	0	0	0	0
Spheniscidae	King penguin	1	1 (100%)	0	0	0	0	0
	Macaroni penguin	1	1 (100%)	0	0	0	0	0
	Rockhopper penguin	11	3 (27%)	5 (45%)	0	1 (9.1%)	0	0
Stercorariidae	Subantarctic skua	11	0	0	0	0	0	1 (9.1%)
Diomedeidae	Black-browed albatross	8	0	0	0	0	1 (13%)	0
	Shy albatross	2	0	0	0	0	0	0
	Light-mantled albatross	1	0	0	0	0	0	0
Anhingidae	African darter	10	2 (20%)	0	0	0	0	0
Haematopodidae	African oystercatcher	9	0	1 (11.1%)	0	0	0	0
Phaethonidae	Red-billed tropic bird	1	0	0	0	0	0	0

environmental conditions and host-parasite assemblages adequate for the transmission of these parasites (Vanstreels et al., 2016).

The cases of *Babesia* sp. in Cape gannets also represent the first records for this host. Considering that most species of avian *Babesia* are specific to the family level (Peirce, 2000), it seems likely this parasite corresponds to *Babesia poelea*, the only species known to infect Sulidae. *B. poelea* was described from brown boobies (*Sula leucogaster*) in Hawaii (Work and Rameyer, 1997) and was also found infecting brown boobies and masked boobies (*Sula dactylatra*) in Brazil (Quillfeldt et al., 2014). Unfortunately, as is often the case in chronic infections (Vanstreels et al., 2015), we were not able to evaluate the morphology of the ameboid tetrads and cruciform schizonts as is necessary to differentiate *Babesia* species. Future studies are warranted to employ molecular characterization and/or to sample wild juvenile Cape gannets to examine acute infections that allow for morphological characterization.

Babesia spp. infection in birds does not always result in clinical signs of illness but may contribute to the overall morbidity and mortality, particularly when concomitant infections are present and the individuals are otherwise compromised (Brossy et al., 1999; Peirce and Parsons, 2012). In penguins, there is evidence to indicate that *Babesia* infection may lead to mild anaemia, leucocytosis and impairment of hepatic function (Sergent et al., 2004; Parsons et al., 2016). For both Cape cormorants and Cape gannets, *Babesia* sp. infections were more frequent in juveniles than in adults; however a significant increased mortality was noted only in juvenile Cape cormorants. A possible explanation is that juvenile Cape cormorants tend to develop more acute infections, which often are more pathogenic and are more easily detected by blood smears than chronic infections (Garamszegi, 2010; Quillfeldt et al., 2014). An alternative hypothesis is that *Babesia* sp. could be more prevalent in juveniles that are already otherwise compromised, without significantly contributing to the increased mortality. On the other hand, babesiosis was considered to have played a key role in the death of the king penguin herein studied, representing the first case of *Babesia*-related mortality in penguins.

4.2. *Plasmodium*

In this study *Plasmodium* sp. was recorded in seabirds from five families, which is consistent with the fact that avian-infecting plasmodia are considered generalist parasites with a broad host range (Valkiūnas, 2005). *Plasmodium* spp. is known to occur with relatively high prevalence in wild birds and mosquitoes on the South African coast (Schultz and Whittington, 2005; Okanga et al., 2013a, 2013b). Our results corroborate that malarial infection occurs in wild Cape cormorants, white-breasted cormorants and Cape gannets, indicating that coastal environments can also provide

Table 3
Detailed parameters of seabird species found to be infected by blood parasites after having been admitted at SANCCOB Cape Town rehabilitation centre, 2001–2013. Age class determined as chick (C), juvenile (J) or adult (A).

Parasite	Species	Individuals bled	Age class of positive individuals	Minimum time to first positive result (days)	Release rate	
					Positive individuals	Negative individuals
Babesia ugwidiensis	Bank cormorant	35	5C, 4J, 4A	0	6/13 (46%)	13/22 (59%)
	Cape cormorant	785	2C, 357J, 88A	0	206/447 (46%)	215/338 (64%)
	White breasted cormorant	73	3C, 5J, 9A	0	12/17 (71%)	37/56 (66%)
	Crowned cormorant	67	2J	1	1/2 (50%)	38/65 (58%)
	Reed cormorant	13	1J	1	Died	5/12 (42%)
Babesia peircei	King penguin	1	1A	67	Died	–
Babesia sp.	Cape gannet	746	19J, 7A	0	16/26 (62%)	496/720 (69%)
	Macaroni penguin	1	1A	119	Died	–
	Rockhopper penguin	11	1J, 2A	0	2/3 (67%)	3/8 (38%)
Plasmodium sp.	Bank cormorant	35	1J	394	Released	18/34 (53%)
	Cape cormorant	785	21J, 3A	0	19/24 (79%)	402/761 (53%)
	White breasted cormorant	73	2A	0	1/2 (50%)	48/71 (68%)
	Crowned cormorant	67	1J	26	Released	38/66 (58%)
	Cape gannet	746	1J, 13A	0	10/14 (71%)	502/732 (69%)
	Red-footed booby	1	1J	201	Died	–
	Northern giant petrel	18	1J	19	Died	6/17 (35%)
	Rockhopper penguin	11	1C, 1J, 3A	16	0/5 (0%)	5/6 (83%)
	African oystercatcher	9	1A	6	Died	6/8 (75%)
Leucocytozoon ugwidi	Cape cormorant	785	5J, 1A	4	4/6 (67%)	417/779 (54%)
	Crowned cormorant	67	1C	1	Died	39/66 (59%)
	Reed cormorant	13	1C	2	Died	5/12 (42%)
Spirochaetales	Cape gannet	746	1J	4	Released	511/745 (69%)
	Southern giant petrel	27	1J	6	Died	13/26 (50%)
	Rockhopper penguin	11	1A	26	Died	5/10 (50%)
Hepatozoon albatrossi	Black-browed albatross	8	1J	3	Released	6/7 (86%)
Haemoproteus skuae	Subantarctic skua	11	1J	3	Released	9/10 (90%)

adequate environment for mosquito proliferation and *Plasmodium* sp. transmission (see also Fantham and Porter, 1944).

For the other species in this study, however, the long interval between admission and the first positive blood smear suggests that infection occurred while birds were in rehabilitation. There was no clear seasonal distribution of the cases of malaria over the year, however the time series revealed that the number of *Plasmodium* sp. infections paralleled the number of birds admitted to the rehabilitation centre in a given year. This pattern was interrupted in 2008, when the entire rehabilitation centre was covered in new shade-cloth netting. It is therefore clear that although a few seabirds were admitted with *Plasmodium* infections from the wild, most infections occurred while the birds were under care. Netting was probably the most effective strategy, however other measures may also have contributed in the prevention of avian malaria: (a) nocturnal use of mosquito repellent electric mats within the pens (allethrin 40 mg/mL, piperonyl butoxide 9 mg/mL), and (b) daily application of an oil-free mosquito repellent gel stick (diethyltoluamide 35%) to the top of the head feathers of penguins.

Plasmodium is renowned for its potential to cause morbidity and mortality to birds, especially to young and otherwise immunologically naive birds (Atkinson and LaPointe, 2009). In this study, most *Plasmodium*-positive individuals were juveniles. Similarly to *B. ugwidiensis*, this could indicate a higher prevalence of infection in this age group, or it could reflect that younger birds tend to develop more acute infections with a higher probability of detection in blood smears (Garamszegi, 2010; Quillfeldt et al., 2014). Unfortunately the small sample size for most species precluded an analysis on whether or not *Plasmodium* sp. infections led to reduced survival/release rates. Surprisingly, the release rate of *Plasmodium*-positive Cape cormorants was actually higher than that of *Plasmodium*-negative individuals, which is probably related to unaccounted-for variables concomitantly affecting survival and exposure to *Plasmodium* sp. In contrast, all five *Plasmodium*-positive rockhopper penguins died as a result of avian malaria, corroborating this species' high susceptibility to the parasite (Sladen et al., 1979; Dinhopl et al., 2011).

4.3. Other parasites

One black-browed albatross was found to be infected by *Hp. albatrossi*. This parasite was first described in three species of albatrosses (including black-browed albatrosses) at South Georgia Island (Peirce and Prince, 1980), and to our knowledge, has only been seen once since then, in southern royal albatrosses (*Diomedea epomophora*) sampled at Campbell Island in 2005 (unpublished data, MA Peirce). It is the only known blood parasite of Diomedeidae (Quillfeldt et al., 2011). In this study, we detected the parasite in a vagrant individual on the Western Cape of South Africa, approximately 4800 km from South Georgia Island and 2800 km from the nearest breeding colony of black-browed albatrosses (Crozet Islands), which is not surprising in face of the circumpolar distribution of albatrosses (Lindsey, 2008). The most probable vector of this parasite is the seabird tick *Ixodes uriae* (Peirce and Prince, 1980), which is common in most Subantarctic islands (Mun˜oz-Leal and Gonzaílez-Acun˜a, 2015); it thus seems reasonable to suspect that *Hp. albatrossi* is widely distributed in albatrosses in the Subantarctic region.

The Subantarctic skua in this study is the same individual that was evaluated in the first description of *H. skuae* (Parsons et al.,

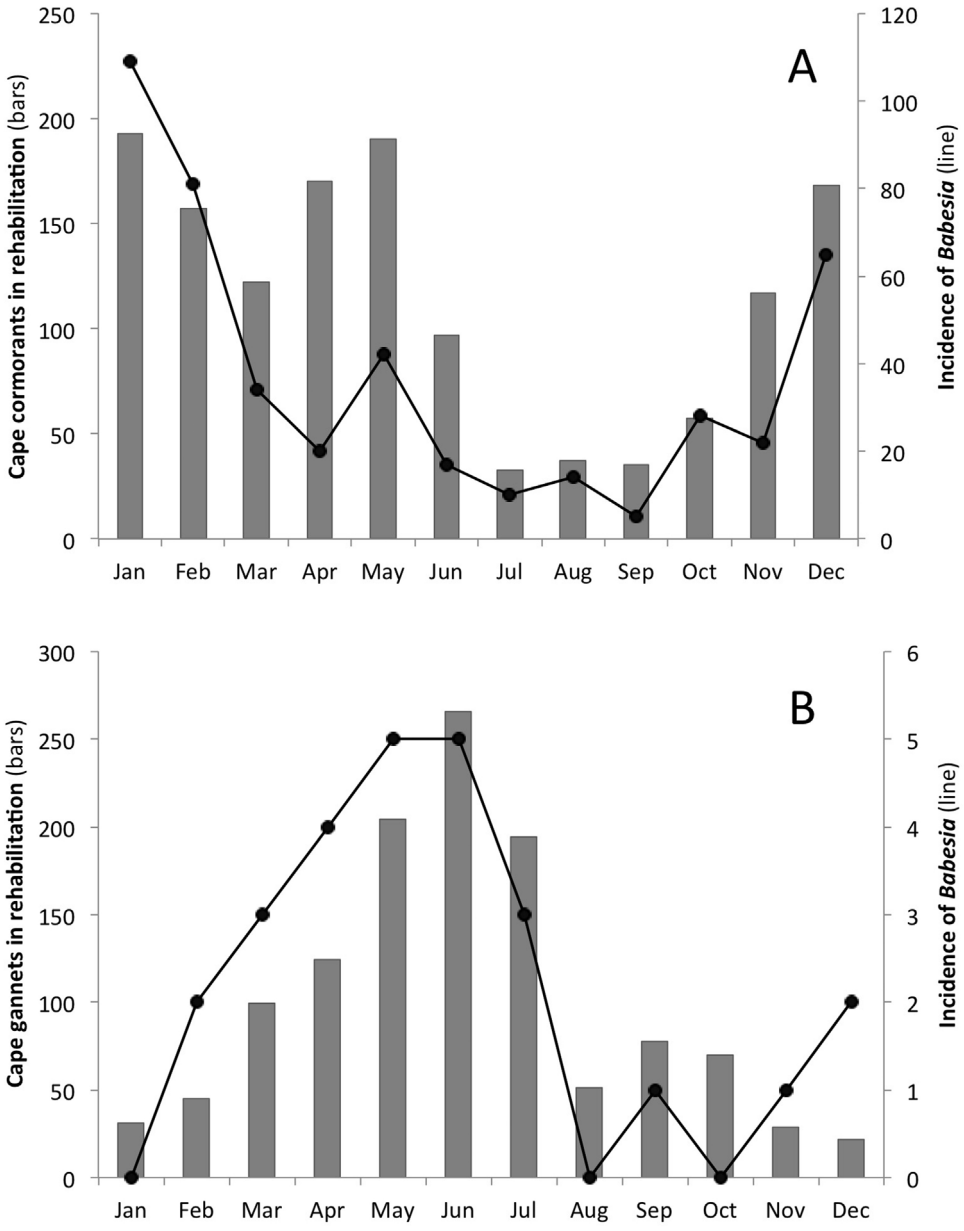

Fig. 3. Seasonal distribution of the susceptible population (number of individuals present at the rehabilitation centre in each month) and the incidence of *Babesia* infections (number of individuals that were first diagnosed as positive in each month) for Cape cormorants (A) and Cape gannets (B) admitted at SANCCOB Cape Town rehabilitation centre, 2001–2013.

2010). Despite an additional 10 individuals of the same species having been sampled in this study, no other blood parasite infections were detected and that individual remains the only record of a blood parasite in Stercorariidae (Parsons et al., 2010; Quillfeldt et al., 2011). Unfortunately molecular characterization cannot be conducted until additional positive individuals are identified, and it is therefore not known to which subgenus this species belongs to. Future studies will hopefully shed light on whether this parasite belongs to subgenus *Haemoproteus*, which are vectored by Hippoboscidae, or *Parahaemoproteus*, which are vectored by Ceratopogonidae (see Valkiūnas, 2005).

L. ugwidi was described based on three Cape cormorants from the same dataset (Parsons et al., 2010). Besides those individuals, in this study *L. ugwidi* was detected in another three Cape cormorants, one crowned cormorant and one reed cormorant, expanding the known host range of this parasite. This parasite does not appear to be prominently pathogenic to cormorants, since no obvious signs of morbidity or mortality caused by leucocytozooid infections were noted.

To our knowledge this study provides the first record of spirochaetal infection in Cape gannets, rockhopper penguins and southern giant petrels. The spirochaetes observed in this study could not be conclusively identified on the basis of morphology, however they were found to be generally consistent with *Borrelia* sp. Avian-infecting *Borrelia* are traditionally classified in three groups: Lyme disease *Borrelia* (LDB), relapsing fever *Borrelia* (RFB) and animal spirochaetosis *Borrelia* (ASB) (Barbour and Hayes, 1986; McDowell et al., 2003; Olsen, 2007). Considering the evidence of pathogenicity that was observed in this study, the existence of past studies demonstrating infection by RFB in African penguins at SANCCOB (Yabsley et al., 2012), and the fact that the spirochaetes observed were morphologically consistent with those previously described at the same facility, we suspect the spirochaetal infections in this study corresponded to RFB.

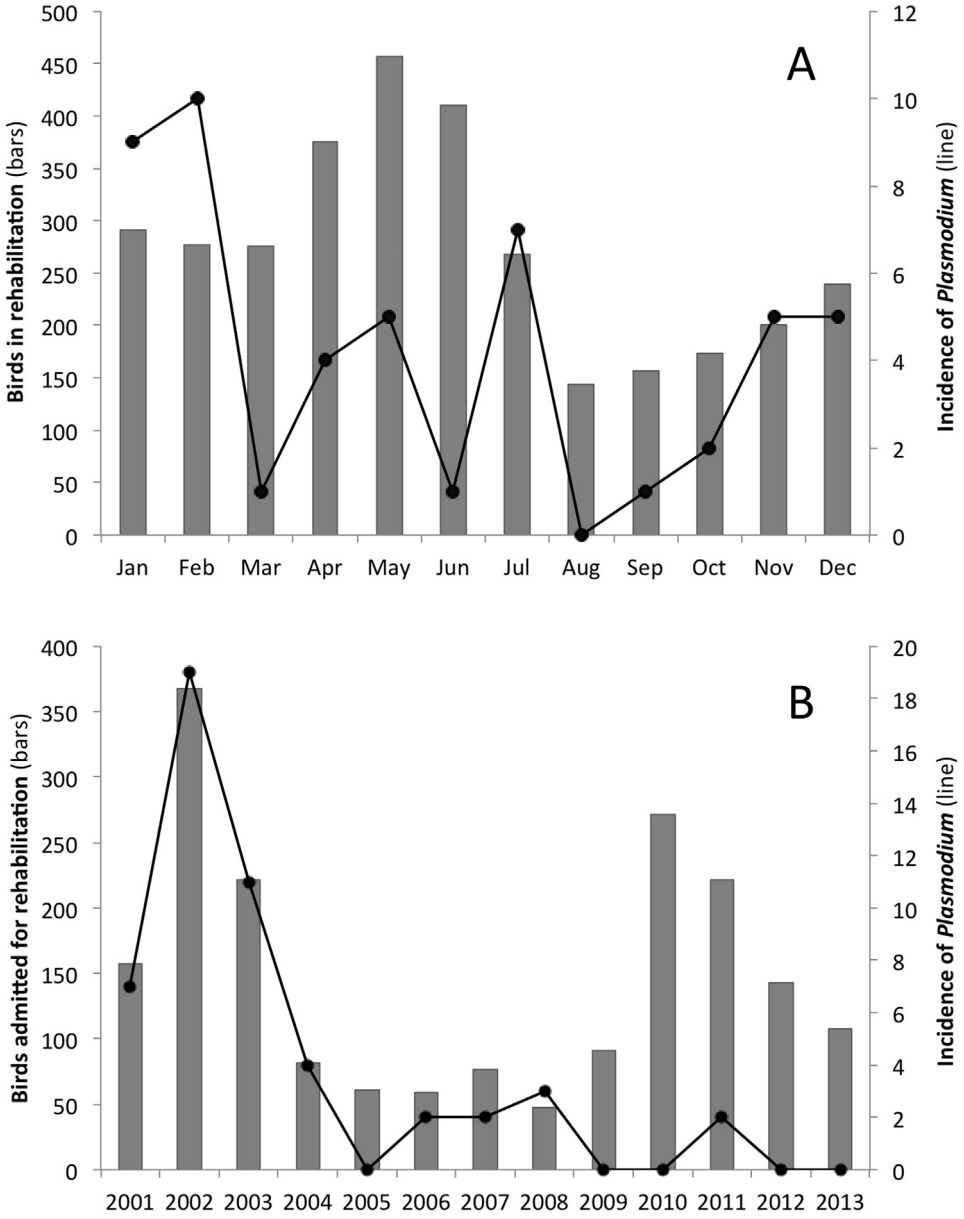

Fig. 4. Seasonal distribution (A) and historical series (B) of the incidence of *Plasmodium* infections (number of individuals that were first diagnosed as positive in each month) in seabirds admitted at SANCCOB Cape Town rehabilitation centre, 2001–2013.

4.4. Conclusion

Although the interpretation of epidemiological studies based on data from rehabilitation centres may be limited by the opportunistic nature of the sampling and by the unpredictability of the factors modulating seabird admission (e.g. uneven beach survey effort, human and bird population density along the coastline, differences in individual health and behaviour, among others), they can be instrumental for the identification of novel host-parasite associations. Moreover, such studies can provide valuable insight on the pathology of parasites that are otherwise uncommon or from hosts that are infrequently sampled in the wild. In a changing climate in which the prevalence and distribution of pathogens and parasites is expected to shift (Marcogliese, 2008; Lafferty, 2009; Garamszegi, 2011), marine animal rehabilitation centres must use the opportunity to collect data and conduct research that will contribute to our understanding of how the health of marine fauna is affected by the anthropogenic (and non-anthropogenic) pressures and how this may affect their conservation.

Conflict of interest statement

All authors declare no conflict of interest in this research.

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