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PROGNOSTIC INDICATORS OF IMMATURE REHABILITATED AFRICAN PENGUINS (*SPHENISCUS DEMERSUS*) IN SOUTH AFRICA

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ABSTRACT: The Southern African Foundation for the Conservation of Coastal Birds (Cape Town, South Africa) regularly receives African Penguins (*Spheniscus demersus*) for rehabilitation. The impact of life history and clinical parameters at admission of 3,975 chicks, 1,687 blues (chicks without downy plumage that are about to fledge or have recently fledged), and 850 fledged juveniles admitted over a 12 yr period (2002–13) were assessed in relation to rehabilitation outcomes using multivariate analysis. Younger chicks were more likely to die during rehabilitation compared with older chicks, and chicks admitted because they had been abandoned by their parents were more likely to die than those admitted preemptively (chicks that were removed when rangers considered their nests were in high-risk areas). Blues admitted because of injury and juveniles admitted because of debilitation, injury, and molt were more likely to die or be euthanized relative to the reference category oiling. *Plasmodium* infection contributed to natural death for all age groups and to the decision to euthanize chicks, whereas *Borrelia* infection contributed to both natural death and the decision to euthanize blues. *Babesia* infection was associated with decreased odds of euthanasia among juveniles. Low hematocrit at admission contributed to natural death of chicks and blues and euthanasia of blues. Low total plasma protein, on the other hand, contributed to natural death in chicks, blues, and juveniles, whereas high total plasma protein had a protective effect against natural death in chicks. These results indicate a need to focus on prevention and early intervention among compromised immature penguins in order to increase the rehabilitation success for this endangered species.
Key words: Chick, conservation, juvenile, mortality, rehabilitation, seabird, South Africa, survival.

INTRODUCTION

The population of African Penguins (*Spheniscus demersus*) has collapsed by >95% since the early 20th century, and the species is currently classified as Endangered, with a total population of approximately 25,000 breeding pairs (Crawford et al. 2011; BirdLife International 2016). Rehabilitation of oiled, sick, and injured African Penguins by the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) and other organizations has been an important component of ongoing efforts to halt this species' population decline (Nel and Whittington 2003; Department of Environmental Affairs 2013).

In the last two decades, hand-rearing of African Penguin chicks that were abandoned by their parents because of oil spills or because of the onset of molt has become a valuable strategy to limit mortality and bolster populations at specific colonies (Department of Environmental Affairs 2013). Hand-reared chicks have survival and recruitment rates comparable to those of wild individuals, and reproduce successfully after they reach sexual maturity (Barham et al. 2008; Sherley et al. 2014b). However, limited data exist on the factors that affect the successful rehabilitation and hand-rearing of penguin chicks.

Clinical parameters such as body condition, body mass, hematological values, as well as the severity of clinical signs, can be used as prognostic indicators to predict rehabilitation outcomes, assisting the management of rehabilitation efforts, and guiding the development and improvement of husbandry and medical protocols (Martins et al. 2015; Molina-López et al. 2015; Duerr et al. 2016). In a recent study, we examined data from adult African Penguins at SANCCOB, and found that the outcome of rehabilitation (survived to be released, euthanized, or died while under care) could be predicted by the season of admission, reason for admission, *Plasmodium* infection, and body mass, hematocrit, and total plasma protein at admission (Parsons et al. 2018b). However, young penguins experience distinct health challenges and require a substantially different level of care and investment during rehabilitation. Oiled, sick, and injured marine and coastal birds are frequently recovered and sent to SANCCOB for rehabilitation, where they undergo rehabilitation following standardized protocols with the aim of releasing them back into the wild (Parsons and Underhill 2005; Parsons et al. 2018b). Our objective was to evaluate how individual history and clinical parameters can be used to predict the rehabilitation outcomes of immature African Penguins.

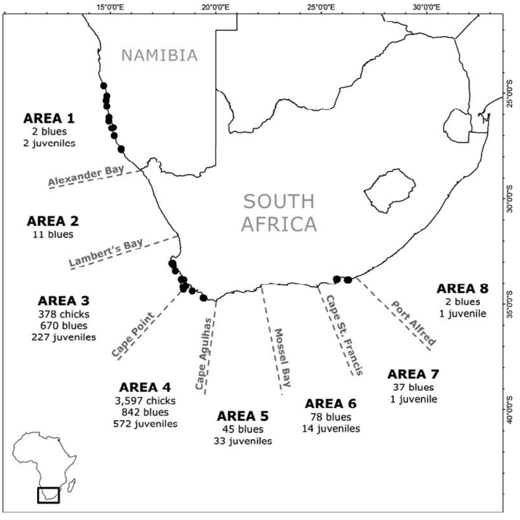

FIGURE 1. Geographic distribution of the number of immature African Penguins (*Spheniscus demersus*) admitted to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013. Black dots represent the species' breeding colonies.

MATERIALS AND METHODS

Data was collected from immature African Penguins admitted to the SANCCOB Cape Town facility (33°50′02″S, 18°29′29″E) in the Western Cape, South Africa, between 2002 and 2013. Immature individuals were distinguished from adults based on the absence of the well-defined black and white bands that are characteristic of adult plumage (Williams 1995). Immature individuals were classified as chicks (nestlings, with downy plumage present), blues (young birds about to fledge or recently fledged, having completely lost their downy plumage and with a shiny gray-blueish plumage; Whittington et al. 2005), or juveniles (young birds whose plumage has an opaque brown tone). For chicks, the age subgroup was also recorded upon admission (Klusener et al. 2018): P0 (newly hatched chick, one or both eyes closed), P1 (small chick, both eyes open, sooty grey down plumage), P2 (medium chick, secondary down plumage fully developed), P3 (large chick, having lost less than 50% of down plumage), and P4 (large chick, having lost more than 50% of down plumage).

The following parameters were recorded for each penguin: date of admission, location of capture, and reason for admittance. The capture location was categorized into eight areas (Fig. 1). The season of admission was categorized according to solstices and equinoxes (Parsons et al. 2018b). The outcome of rehabilitation was categorized as release, natural death, or euthanasia. The date of outcome was also recorded, and was used to calculate the duration of rehabilitation.

The reason for admittance was classified into 10 categories (Table 1). The preemptive category comprised chicks that were removed when park rangers considered they were in high-risk areas, when nests were potentially at risk of flooding, or when they were on private property and the owners requested their removal. The hatched category referred to chicks that were artificially incubated and hatched at SANCCOB after the eggs were preemptively removed from the wild because they were at risk, on private property, or were not being attended to by an adult. The abandonment category referred to chicks that were considered to have been abandoned when the park rangers noted that they were not being attended to by adults for an extended period of time, or when they were sitting on a nest but showed signs of malnutrition, dehydration, or hypothermia. The debilitation category comprised blues and juveniles that were prostrated, lethargic, dehydrated, emaciated, or otherwise weakened but did not have signs of molting, injury, avian pox, or oiling. The avian pox category comprised chicks and blues that were removed because they presented severe wart-like lesions or tumors consistent with cutaneous pox (Kow 1992; Kane et al. 2012). Of note, avian pox was only

TABLE 1. Reason for admittance by age group of 6,512 immature African Penguins (*Spheniscus demersus*) admitted to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013. Chicks are young birds with downy plumage present, blues are young birds about to fledge or recently fledged, having completely lost their downy plumage and with a shiny gray-blueish plumage, and juveniles are young birds whose plumage has an opaque brown tone.

Reason for admittance	% (*n*) Total admissions		
	Chicks	Blues	Juveniles
Hatched	4.6 (182)	0	0
Preemptive	8.1 (323)	0.8 (14)	0
Abandonment	86.4 (3,434)	24.2 (408)	0
Debilitation	0	41.7 (704)	6.8 (58)
Injury	0.1 (2)	9.6 (162)	22.6 (192)
Deformity	0.3 (13)	0.4 (7)	0
Avian pox	0.5 (21)	0.1 (1)	0
Molt	0	0.1 (1)	16.1 (137)
Oiling	0	21.7 (366)	53.8 (457)
Other	0	1.4 (24)	0.7 (6)
Total *n*	3,975	1,687	850

recorded in this study when it was severe enough to be considered a reason for admittance; milder lesions were seen in a number of cases but were not recorded because other conditions were considered as the main reason for admittance or because the lesions developed after admission to the facility. The molt category included juveniles with arrested molt as well as those that were undergoing normal molt but were captured because they were in high-risk areas (e.g., urban areas where they might be attacked by dogs). The other category included miscellaneous uncommon reasons for admittance: healthy penguins (7), exposure to urban pollution (sewage = 13, paint = 1), neurologic symptoms (6), eye infection or blindness (2), and allergic reaction (1). We summarized the distribution of the rehabilitation outcomes in relation to the individual history variables for each age group (Supplementary Material Tables S1–S3).

When feasible, depending on the individual's body size and health status, the following health parameters were obtained within the first week of admission: body mass (kg), hematocrit (%), and total plasma protein (g/dL). Body mass was obtained using a floor scale (accuracy to 0.01 g for individuals $\leq$1 kg, accuracy to 0.02 kg for penguins weighing $>$1 kg). Blood samples were collected from penguins with body mass $>$500 g at admission and then weekly during rehabilitation. Blood was collected from the metatarsal vein to fill a heparinized capillary tube (60–100 μL). This was centrifuged at 10,000 × G for 5 min, and the hematocrit was measured using a Hawksley microhematocrit reader and the total plasma protein concentration was estimated with a temperature-compensated clinical refractometer. From the blood collection, blood smears were prepared, stained with a modified Wright-Giemsa stain (Kyro-Quick, Kyron Laboratories, Benrose, South Africa), and examined for blood parasites under 500× magnification for about 10 min. If, at some point during rehabilitation, a positive diagnosis was made, the individual was recorded as positive for that parasite genus (*Babesia*, *Borrelia*, *Leucocytozoon*, or *Plasmodium*).

Following Anderson-Darling tests for data normality, hematocrit and total plasma protein were compared between categories of reason for admittance using two-sample *t*-tests (chicks) and analysis of variance with Tukey post hoc tests (blues and juveniles). The analysis of variance and Tukey post hoc tests were used to evaluate whether continuous clinical parameters differed among rehabilitation outcomes.

Individuals admitted in the preemptive (chicks) and oiling categories (blues and juveniles) that were negative to blood parasites were used to establish reference values for the hematological parameters. The 20% and 80% percentiles of the hematocrit and total plasma protein of these individuals upon admission were used to establish the thresholds for classification as low, normal, or high (Parsons et al. 2018b). Normal hematocrit was 30–40% for chicks, 37–47% for blues, and 40–50% for juveniles. Normal total plasma protein corresponded to 4.0–5.8 g/dL for chicks, 4.0–6.0 g/dL for blues, and 4.6–6.0 g/dL for juveniles.

As potential predictors of the outcome of rehabilitation, the variables that we examined

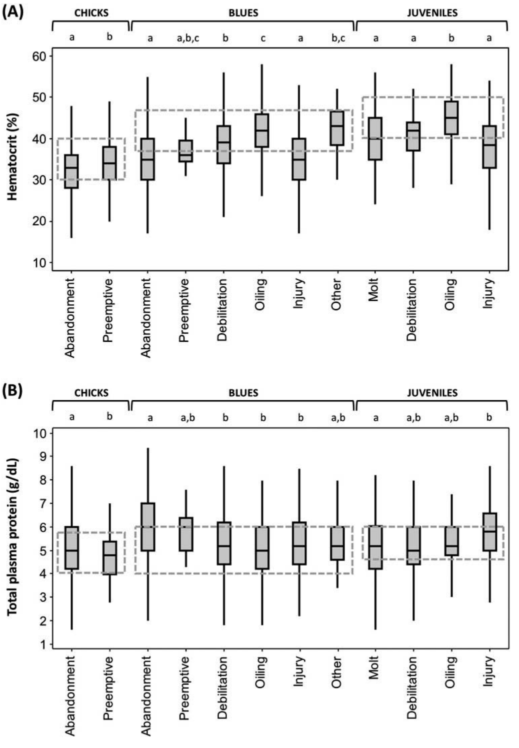

FIGURE 2. Box plots of the (A) hematocrit and (B) total plasma protein at admission of immature African Penguins (*Spheniscus demersus*) for rehabilitation at the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013, by age group and the reason for admittance. Categories with different letters were statistically different within an age group (two-sample *t*-tests or Tukey post hoc tests). Dashed boxes represent the interval of values that were classified as normal for each age group.

included location of capture; season of admission; reason for admittance (only categories representing ≥5% of the individuals within an age group were included); *Babesia*, *Borrelia*, *Leucocytozoon*, or *Plasmodium* infection; age subgroup (chicks only); body mass (blues and juveniles only); hematocrit, and total plasma protein. We tested potential variables affecting outcome for collinearity. We performed forward addition stepwise multinomial logistic regressions to evaluate all significant independent variables together as predictors of the outcome (three categories: release, natural death, euthanasia); this analysis was done separately for each age group, using the release outcome as the reference category. Overall model fit was assessed using McFadden's pseudo-R^2, and only variables that significantly improved the model (threshold for inclusion was $P<0.05$) were included in the final model. Odds ratios were calculated to measure the association between each variable and the rehabilitation outcomes, and may be interpreted as a measurement of the relative probability that an animal belonging to a category will have a natural death or euthanasia outcome when compared with the reference category. Significance level was set at $\alpha=0.05$ for all tests.

RESULTS

A total of 3,975 chicks, 1,687 blues, and 850 juveniles were admitted during the 12-yr study period (Table 1), with an annual mean (±SD) of 331±246 chicks (range: 83–775), 141±37 blues (96–203), and 71±29 juveniles (40–138). The overall release rate in each year was 68.9±11.3% for chicks (55–91%), 76.7±5.8% for blues (65–85%), and 69.9±8.4% for juveniles (54–86%). There were 21 chicks (all P2 stage) and 1 blue admitted due to avian pox in 2013 from the Stony Point colony.

The hematocrit was lower in chicks and blues admitted due to abandonment, and in blues and juveniles admitted due to injury (Fig. 2A). The total plasma protein was lower in chicks admitted due to preemptive removal, blues admitted due to oiling, and juveniles admitted due to debilitation (Fig. 2B). Across all age groups and subgroups, body mass upon admission was consistently higher in released individuals followed by individuals that died because of euthanasia and individuals that died naturally (Table 2). The same pattern was observed for hematocrit upon admission, except for P2 chicks, where the hematocrit value was marginally higher than in released individuals. Across all age groups and subgroups, total plasma protein upon admission was consistently higher in released individuals than in individuals that died naturally.

The variables included in the final regression model for chicks (McFadden's pseudo-R^2=0.124) were age subgroup, reason for admittance, *Plasmodium* infection, hematocrit, and total plasma protein (Table 3). The variables included in the final regression model for blues (McFadden's pseudo-R^2=0.109) were reason for admittance, *Borrelia* or *Plasmodium* infection, hematocrit,

TABLE 2. Clinical parameters at admission compared by rehabilitation outcomes for immature African Penguins (*Spheniscus demersus*) admitted to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013. The *P* values refer to comparisons between outcomes and individual parameters using analysis of variance. Different superscript letters in the same row indicate a significant difference as determined by Tukey post hoc tests. Chicks are young birds with downy plumage present, blues are young birds about to fledge or recently fledged, having completely lost their downy plumage and with a shiny gray-blueish plumage, and juveniles are young birds whose plumage has an opaque brown tone. For chicks, the age subgroup was further categorized as P2 (medium chick, secondary down plumage fully developed), P3 (large chick, having lost less than 50% of down plumage), and P4 (large chick, having lost more than 50% of down plumage).

Variable and age group	Mean (SD) of outcomes						*P* value
	n	Release	*n*	Natural death	*n*	Euthanasia death	
Body mass (kg)							
Chicks (P2)	500	1.19 (0.38)[a]	181	0.95 (0.31)[b]	36	1.00 (0.29)[b]	<0.001
Chicks (P3)	564	1.62 (0.43)[a]	177	1.34 (0.35)[b]	38	1.34 (0.33)[b]	<0.001
Chicks (P4)	876	1.85 (0.46)[a]	178	1.43 (0.37)[b]	36	1.56 (0.41)[b]	<0.001
Blues	1,302	1.89 (0.46)[a]	281	1.55 (0.45)[b]	84	1.77 (0.44)[c]	<0.001
Juveniles	604	2.43 (0.52)[a]	152	2.12 (0.51)[b]	73	2.37 (0.54)[a]	<0.001
Hematocrit (%)							
Chicks (P2)	179	31.9 (5.8)[a]	40	27.2 (6.4)[b]	8	32.9 (9.7)[a]	<0.001
Chicks (P3)	303	32.0 (6.8)[a]	67	28.8 (8.1)[b]	13	30.8 (5.5)[ab]	0.004
Chicks (P4)	460	33.5 (6.7)[a]	53	30.0 (8.6)[b]	18	31.9 (7.2)[ab]	0.002
Blues	1,160	38.3 (7.4)[a]	180	34.9 (9.8)[b]	64	35.0 (7.9)[b]	<0.001
Juveniles	553	42.8 (6.7)[a]	108	39.0 (8.9)[b]	42	40.8 (8.8)[ab]	<0.001
Total plasma protein (g/dL)							
Chicks (P2)	178	4.9 (1.2)[a]	40	4.1 (1.0)[b]	7	4.8 (0.8)[ab]	<0.001
Chicks (P3)	303	5.0 (1.3)[a]	66	4.4 (1.5)[b]	13	4.8 (1.5)[ab]	0.007
Chicks (P4)	456	4.9 (1.2)[a]	53	4.3 (1.2)[b]	18	4.9 (1.2)[ab]	0.005
Blues	1,153	5.5 (1.3)[a]	180	4.8 (1.9)[b]	64	5.4 (1.4)[a]	<0.001
Juveniles	550	5.5 (1.2)[a]	105	4.7 (1.7)[b]	42	5.2 (1.5)[ab]	<0.001

and total plasma protein (Table 4). The variables included in the final regression model for juveniles (McFadden's pseudo-R^2=0.197) were reason for admittance, *Babesia* or *Plasmodium* infection, and total plasma protein (Table 5). For example, a penguin chick that was admitted due to abandonment was 5.67 (95% confidence interval: 1.75–18.38) times more likely to suffer a natural death (instead of being released) than a penguin chick that was admitted due to preemptive removal.

Chicks with low hematocrit (<30%) upon admission were more likely to die compared with chicks with hematocrit in the normal range. For blues, low hematocrit (<37%) was a significant risk factor for both natural death and euthanasia relative to individuals with normal hematocrit. Similarly, low concentrations of plasma protein were a risk factor for natural death in chicks (<4.0 g/dL), blues (<4.0 g/dL), and juveniles (<4.6 g/dL) compared with individuals with normal protein. Additionally, high concentrations of plasma protein (>5.8 g/dL) had a protective effect for natural death in chicks.

The cumulative curve of rehabilitation outcomes varied depending on the age groups and reason for admittance (Fig. 3). With the exception of preemptively removed chicks and molting juveniles, the rehabilitation outcomes followed sigmoidal curves, with natural and euthanasia deaths concentrating in the first 30 d postadmission and most individuals (>98%) reaching an outcome within 120 d.

TABLE 3. Final multinomial logistic regression model of individual history and clinical parameters for two categories of mortality among chicks of African Penguins (*Spheniscus demersus*) admitted for rehabilitation to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013 (n=3,975), using released birds as the reference category in each case. The P values refer to the significance of each variable as a predictor of natural death or euthanasia death. Age subgroup was categorized as P2 (medium chick, secondary down plumage fully developed), P3 (large chick, having lost less than 50% of down plumage), and P4 (large chick, having lost more than 50% of down plumage).

	Natural deaths		Euthanasia deaths	
Variable	Odds ratio (95% confidence interval)	*P* value	Odds ratio (95% confidence interval)	*P* value
Age stage				
P2	1.65 (1.02–2.66)	0.040	1.04 (0.43–2.51)	0.928
P3	1.89 (1.25–2.85)	0.002	1.08 (0.52–2.28)	0.831
P4	1.00	—	1.00	—
Reason for admittance				
Abandonment	5.67 (1.75–18.38)	0.004	4.39 (0.59–32.72)	0.148
Preemptive	1.00	—	1.00	—
Plasmodium infection				
Positive	3.67 (2.15–6.28)	0.001	3.29 (1.27–8.49)	0.014
Negative	1.00	—	1.00	—
Hematocrit				
Low (<30%)	1.89 (1.30–2.77)	0.001	1.14 (0.55–2.36)	0.735
Normal (30–40%)	1.00	—	1.00	—
High (>40%)	1.21 (0.57–2.59)	0.627	1.33 (0.43–4.11)	0.615
Total plasma protein				
Low (<4.0 g/dL)	1.99 (1.32–3.01)	0.001	1.43 (0.62–3.35)	0.404
Normal (4.0–5.8 g/dL)	1.00	—	1.00	—
High (>5.8 g/dL)	0.51 (0.30–0.88)	0.016	1.00 (0.44–2.29)	0.995

DISCUSSION

Reason for admittance

We were not able to determine causes of chick abandonment. Considering that the recent decline of African Penguin populations is linked to changes in prey abundance and distribution (Durant et al. 2010; Sherley et al. 2013), we assumed that shortage of prey was an important factor. Removal of abandoned chicks and blues relied on the park rangers noting the absence of parents at the nest for an extended period of time, or chicks or blues showing signs of malnutrition, dehydration, or hypothermia while sitting on a nest. As a result, abandoned individuals were invariably in poor health upon admission. The poor health of the chicks was reflected by their lower hematocrit and higher plasma protein concentrations, presumably reflecting anemia and the triggering of humoral immune response mechanisms (e.g., positive acute phase proteins, immunoglobulins), and dehydration. Abandoned chicks were far more likely to die while under care than those that were preemptively removed, considering the likely duration of suboptimal nutrition before removal. On the other hand, blues abandoned or preemptively removed did not differ their hematological parameters, and abandonment was not a significant predictor of rehabilitation outcomes for them. Evaluating body condition index (Lubbe et al. 2014; Morten et al. 2017) to remove chicks after abandonment more quickly would likely benefit their survival in rehabilitation.

Because the preemptive removal of penguin chicks was motivated by external factors (e.g., location of the nest in relation to human activities and land use) rather than by their clinical condition, these individuals varied

TABLE 4. Final multinomial logistic regression model of individual history and clinical parameters for two categories of mortality among blues of African Penguins (*Spheniscus demersus*) admitted for rehabilitation to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013 (n=1,687), using released birds as the reference category in each case. The *P* values refer to the significance of each variable as a predictor of natural death or euthanasia death.

Variable	Natural deaths		Euthanasia deaths	
	Odds ratio (95% confidence interval)	*P* value	Odds ratio (95% confidence interval)	*P* value
Reason for admittance				
Abandonment	1.18 (0.67–2.06)	0.574	0.63 (0.22–1.85)	0.403
Debilitation	1.50 (0.95–2.36)	0.083	1.70 (0.75–3.88)	0.204
Injury	2.29 (1.21–4.33)	0.011	4.13 (1.57–10.90)	0.004
Oiling	1.00	—	1.00	—
Borrelia infection				
Positive	6.51 (2.33–18.16)	<0.001	6.01 (1.18–30.71)	0.031
Negative	1.00	—	1.00	—
Plasmodium infection				
Positive	1.85 (1.23–2.77)	0.003	1.62 (0.82–3.19)	0.166
Negative	1.00	—	1.00	—
Hematocrit				
Low (<37%)	1.68 (1.17–2.42)	0.005	2.35 (1.29–4.29)	0.005
Normal (37–47%)	1.00	—	1.00	—
High (>47%)	1.62 (0.86–3.07)	0.136	0.70 (0.16–3.09)	0.637
Total plasma protein				
Low (<4.0 g/dL)	2.92 (1.97–4.34)	<0.001	1.19 (0.61–2.30)	0.610
Normal (4.0–6.0 g/dL)	1.00	—	1.00	—
High (>6.0 g/dL)	1.44 (0.93–2.22)	0.104	1.08 (0.54–2.17)	0.832

considerably in their developmental stage and health status. Chicks in this category often appeared healthy, and this was corroborated by their hematology and high release rate. The cumulative curve of rehabilitation outcomes for preemptively removed chicks followed a relatively linear pattern (rather than a sigmoid curve as observed in other groups), presumably reflective of the fact that this group contained a mixture of younger chicks (that needed more time before they could be released) and older chicks (that could be more quickly released).

Although we lacked adequate sample sizes to include hatched birds in the analyses, chicks in this category had a much lower release rate (25%) than abandoned or preemptively removed chicks (84% and 93%, respectively). Most of the mortality occurring within the first 30 d posthatching (Fig. 3C), which illustrated the difficulty of hand-rearing chicks of altricial species such as African Penguins that are highly vulnerable during the initial stages of development. Since 2012, with refined incubation and chick-rearing practices, release rates of hatched chicks improved to close to 80% (Klusener et al. 2018).

An increased incidence of avian pox was noted in 2013 in wild penguin chicks at the Stony Point colony (Western Cape). The 2013 outbreak was unique because, unlike other years, 22 immature penguins were admitted with severe avian pox lesions.

Individuals admitted in the categories abandonment and debilitation shared the same general pattern of malnourishment, dehydration, and hypothermia, and the differentiation between these categories was primarily related to the context in which the bird was found. Abandoned individuals were still sitting on a nest (i.e., prefledging), whereas debilitated individuals were found

TABLE 5. Final multinomial logistic regression model of individual history and clinical parameters for two categories of mortality among juveniles of African Penguins (*Spheniscus demersus*) admitted for rehabilitation to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013 (n=850), using released birds as the reference category in each case. The *P* values refer to the significance of each variable as a predictor of natural death or euthanasia death.

Variable	Natural deaths		Euthanasia deaths	
	Odds ratio (95% confidence interval)	*P* value	Odds ratio (95% confidence interval)	*P* value
Reason for admittance				
Debilitation	5.04 (2.31–10.98)	<0.001	3.64 (0.74–18.01)	0.113
Injury	3.66 (2.01–6.67)	<0.001	13.94 (6.07–32.03)	<0.001
Molt	3.55 (1.99–6.34)	<0.001	4.26 (1.50–12.12)	0.007
Oiling	1.00	—	1.00	—
Babesia infection				
Positive	0.62 (0.37–1.05)	0.074	0.39 (0.17–0.91)	0.029
Negative	1.00	—	1.00	—
Plasmodium infection				
Positive	2.42 (1.35–4.33)	0.003	0.47 (0.11–2.09)	0.321
Negative	1.00	—	1.00	—
Total plasma protein				
Low (<4.6 g/dL)	2.24 (1.33–3.77)	0.003	2.06 (0.90–4.72)	0.088
Normal (4.6–6.0 g/dL)	1.00	—	1.00	—
High (>6.0 g/dL)	0.84 (0.46–1.52)	0.561	1.27 (0.58–2.80)	0.548

along the waterline near a colony or far from any breeding colonies (i.e., postfledging). Blues admitted because of abandonment had a lower hematocrit and a higher plasma protein than those admitted because of debilitation, which suggested that the suboptimal nutrition experienced by the debilitation category might have been attenuated because they were able to obtain some food at sea.

Oiled juvenile penguins had the highest release rate. Debilitation, injury, and molt were being significant predictors of natural death and euthanasia were consistent with our findings for adult African Penguins (Parsons et al. 2018b). This suggested that the health challenges experienced by juveniles in rehabilitation were qualitatively similar to those experienced by adults, even though juveniles and adults experience differences in survival in the wild (Sherley et al. 2014a).

Chick age subgroups

Chicks of the P2 and P3 stages were more likely to die during rehabilitation than chicks of the P4 stage, consistent with the assumption that younger chicks were inherently more vulnerable to health challenges because of their immature immune systems and physiological coping mechanisms. Chicks of the P0 and P1 stages could not be included in the multivariate analysis because of small sample sizes; however, the lower release rates of P1 and P0 chicks (Supplemental Material Table S1) suggested that this general trend also extended to these younger age groups.

Blood parasites

Plasmodium infection (avian malaria) was a significant risk factor leading to natural death in chicks, blues, and juveniles and for euthanasia of chicks. These parasites can cause substantial morbidity and mortality of penguins (Vanstreels et al. 2016). *Plasmodium* infections were more frequent in chicks (9.1%), blues (14.1%), and juveniles (10.6%; Supplementary Material Tables S1–S3) than in adults (6.5%) at the same facility (Parsons

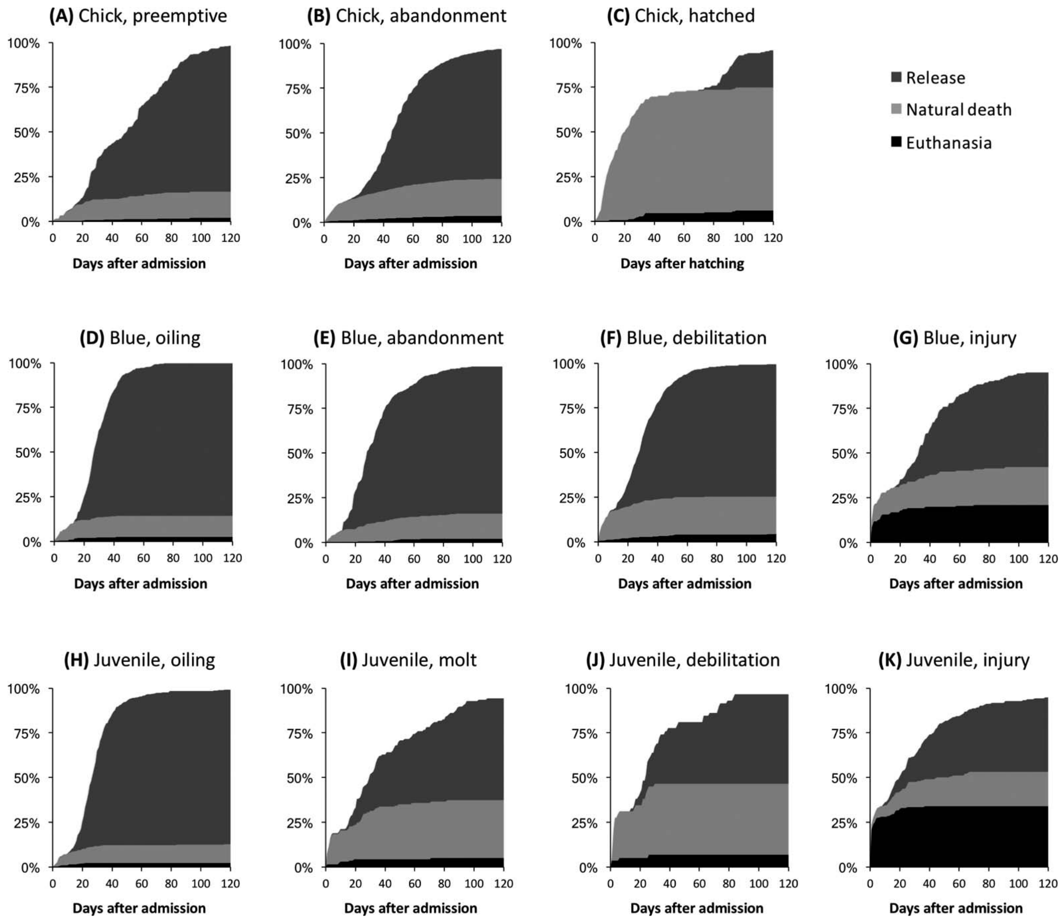

FIGURE 3. Cumulative distribution of the different rehabilitation outcomes during the first 120 d following admission of immature African Penguins (*Spheniscus demersus*) to the Southern African Foundation for the Conservation of Coastal Birds between 2002 and 2013, in relation to the age group upon admission and the reason for admittance.

et al. 2018b), which suggested a higher susceptibility of younger individuals. Most *Plasmodium* infections (95%) were recorded prior to 2008, after which the rehabilitation facilities were enclosed in mosquito netting that drastically decreased the incidence of avian malaria (Botes et al. 2017). Prevention and early treatment of *Plasmodium* infections is important in the rehabilitation of African Penguins regardless of age.

Borreliosis was a significant risk factor for natural death and euthanasia of blues, but not for other age groups, despite this pathogen being less frequently recorded in blues (1.4%) than in chicks (2.7%; Supplemental Material Tables S1, S2), suggesting an age-specific susceptibility to borreliosis. Although not confirmed by molecular methods, their morphology on blood smears was consistent with that of relapsing fever from *Borrelia* infection, as previously documented in African Penguins at the same rehabilitation facility (Yabsley et al. 2011). Our results corroborated the potential lethality of *Borrelia* infections to penguins (Yabsley et al. 2011; Parsons et al. 2016, 2018a). Controlling *Ornithodoros capensis*, the suspected tick vector of relapsing fever from *Borrelia* infection in penguins (Yabsley et al. 2011), through biosafety measures and antiparasitic drug treatment should be of value for the prevention of borreliosis. The early detection and treatment of *Borrelia* infections should also be a priority for the care of African Penguin chicks and blues.

Babesia was the most frequent blood parasite seen (52% of chicks, 49% of blues, 25% of juveniles; Supplemental Material Tables S1–S3), which was consistent with the 61% prevalence of *Babesia* in wild African Penguin chicks in the Western Cape province (Naude 2014). Although not subjected to a thorough characterization, it is likely that all infections corresponded to *Babesia peircei*, as previously documented at the facility (Earlé et al. 1993; Yabsley et al. 2017). The SANCCOB rehabilitation protocols recommended that *Babesia*-positive individuals with high parasitemia (>15 piroplasms per 500× magnification field) or with severe anemia (hematocrit <20%) should receive drug treatment (primaquine at 1 mg/kg once daily orally for 10 d). Despite its high prevalence, this parasite was not a risk factor for natural death or euthanasia in chicks or blues.

Unexpectedly, being diagnosed with a *Babesia* infection actually decreased the odds of a juvenile penguin being euthanized while in rehabilitation. This is surprising, considering that *B. peircei* are potentially lethal to other species of penguins (Parsons et al. 2018a). Possibly, *Babesia* infection had a negligible effect on the health of juvenile African Penguins, but was coincidentally correlated to a variable that was not evaluated in this study (e.g., time since fledging).

Clinical parameters

For most age groups, low hematocrit and low concentrations of plasma protein were risk factors for natural death (and in some cases, euthanasia death) compared with individuals with normal values, confirming that these analytes are valuable indicators of nutrition and health of penguins (Rodrigues et al. 2010; Martins et al. 2015; Parsons et al. 2018b). Hematocrit and total plasma protein varied considerably among age groups, both increasing in older birds. Hematological reference values established for adult penguins (e.g., Parsons et al. 2015) might therefore not be adequate for immature penguins, and our results underscored the need for age group–specific reference values.

Study limitations and future priorities

We classified immature African Penguins into three age groups based on plumage. Chicks have not fledged and rely on their parents to provide food, whereas juveniles have fledged and rely on themselves to obtain food. Blues represented a transition group that may or may not have fledged. We also relied on plumage to differentiate between immature and adult penguins, but not all penguins with adult plumage were sexually mature. Although some subadult individuals could be sexually immature, we did not evaluate them. The development of methods to determine life-history stages accurately could provide better insight into the challenges experienced by penguins during their development. It would be interesting to evaluate whether the timing of the molt into adult plumage (Kemper and Roux 2005) and the stages of head molt that are occasionally observed in juvenile penguins (Ryan et al. 1987) affect their admittance, health status, and rehabilitation outcomes.

We could not evaluate sex as a potential prognostic factor of rehabilitation outcomes because we did not conduct molecular sexing. However, natural mortality of African Penguins is female-biased (Pichegru and Parsons 2014), as is the mortality of juvenile Magellanic Penguins (*Spheniscus magellanicus*) undergoing rehabilitation in South America (Vanstreels et al. 2013).

Developing more effective approaches to mitigate the effects of acute or prolonged malnutrition would be useful to improve the rehabilitation success of the large number of immature African Penguins admitted. This would include improving the criteria for chick removal, employing intensive care procedures (fluid therapy and parenteral nutrition), and the early treatment of blood parasites.

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SUPPLEMENTARY MATERIAL

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