

© Copyright 2015

Olivia Jessica Woods (Kane)

Disease in *Spheniscus* penguins: Feather-loss disorder and Avian Pox

Olivia Jessica Woods (Kane)

A dissertation

submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington

2015

Supervisory Committee:

P. Dee Boersma, Chair

Jennifer Ruesink

Carl Bergstrom

Gordon Orions

Program Authorized to Offer Degree:

Biology

University of Washington

University of Washington

Abstract

Disease in *Spheniscus* penguins: Feather-loss disorder and Avian Pox

Olivia Jessica Woods (Kane)

Chair of the Supervisory Committee:

Dr. Dee Boersma

Wadsworth Endowed Chair, Department of Biology

Avian pox and a previously undiscovered feather-loss disorder affect *Spheniscus* penguins. Avian pox is an enveloped doublestranded DNA virus that is mechanically transmitted via arthropod vectors or mucosal membrane contact with infectious particles or birds. Avian pox outbreaks primarily affected chicks, often resulted in death, and were not associated with handling, rainfall, or temperature. The *Avipoxvirus* identified in Magellanic Penguins (*Spheniscus magellanicus*) in Argentina was phylogenetically similar to an *Avipoxvirus* found in Black-browed Albatross (*Thalassarche melanophrys*) from the Falkland Islands in 1987. This phylogentic proximity suggests a long-term circulation of seabird *Avipoxviruses* in the southwest Atlantic.

The feather-loss disorder in captive African Penguin (*Spheniscus demersus*) chicks and wild Magellanic Penguin chicks is new, rare, and more common in a rehabilitation center in Africa than in the wild. The cause of the feather loss is unknown, but the disorder results in slower growth, smaller fledglings, and appears to increase mortality in Magellanic Penguin chicks in the wild.

TABLE OF CONTENTS

Chapter 1. Feather-loss disorder in African and Magellanic Penguins

Chapter 2. Avian Pox in Magellanic Penguins (*Spheniscus magellanicus*)

ACKNOWLEDGEMENTS

I thank Dr. Dee Boersma for the enormous amount of support and encouragement she provided during my graduate education. I will be forever grateful for the countless hours of mentoring, the number of life-changing opportunities she provided, and for believing in me every step of the way. Thank you Dee. I also thank my committee members, Dr. Jennifer Ruesink, Dr. Carl Bergstrom, Dr. Gordon Orions and Dr. Miles Logsdon, for their advice and inspiration. Lastly, I thank all Penguin Sentinel volunteers that collected data since 1983, of which, I am specifically indebted to Dr. Ginger Rebstock, Clay Gravelle, Jeffrey Smith, Dr. Jack Cerchiara, and Laura Koehn for teaching me new skills and sharing their advice and ideas.

DEDICATION

I dedicate this thesis to my parents, Mike and Cheryl Kane and Shirley and Steve Aiken for making me believe that I can do anything and for all their sacrifices that let me try. I also thank my grandma Thelma Kane for her genuine excitement and curiosity about the natural world and specifically my studies.

Feather-loss Disorder in African and Magellanic Penguins

OLIVIA J. KANE^{1,*}, JEFFREY R. SMITH¹, P. DEE BOERSMA¹, NOLA J. PARSONS², VENESSA STRAUSS²,
PABLO GARCIA-BORBOROGLU³ AND CECILIA VILLANUEVA³

¹Department of Biology, University of Washington and the Wildlife Conservation Society, Seattle, WA, 98195, USA

²Southern African Foundation for the Conservation of Coastal Birds, P.O. Box 11116, Bloubaerg, 7443, South Africa

³Centro Nacional Patagónico CONICET, Blvd. Brown 2825 Puerto Madryn 9120, Chubut, Argentina

*Corresponding Author; E-mail: oliviaj@u.washington.edu

Abstract.—A feather-loss disorder, first observed in captive African Penguin (*Spheniscus demersus*) chicks in a South African rehabilitation center in 2006, was found one year later in wild Magellanic Penguin (*Spheniscus magellanicus*) chicks in four colonies in Argentina. Two years later, it was found in African Penguin chicks in the wild. The featherless African Penguin chicks in the rehabilitation center (N = 176) lost their down and emerging juvenile feathers, remaining featherless for several weeks until they died (N = 31) or grew juvenile (N = 3) or adult plumage (N = 145) before being released. The featherless African Penguin chicks took 16 days longer to reach the rehabilitation center's standards for release than feathered chicks ($t_{176} = -8.8$, $P < 0.00001$). Likewise, the featherless wild Magellanic Penguin chicks (N = 13) lost their second coat of down, remaining featherless for several weeks; but those that survived to fledging all grew normal juvenile plumage (N = 4). Featherless Magellanic Penguin chicks grew more slowly and were smaller at fledging age than most feathered chicks. The disorder in Africa and Argentina is new, rare, and more common in a rehabilitation center in Africa than in the wild. The cause of the feather loss is unknown, but the disorder results in slower growth, smaller fledglings, and appears to increase mortality in Magellanic Penguin chicks in the wild. Received 21 January 2009, accepted 31 March 2010.

Key words.—African Penguin, feather loss, Magellanic Penguin, *Spheniscus demersus*, *Spheniscus magellanicus*.

Waterbirds 33(3): 415-421, 2010

Feather-loss disorders are uncommon in most bird species and rarely reported in wild birds. We report a new feather-loss disorder that exposes bare skin in two species of *Spheniscus* penguin chicks: one in South Africa (but see van Heezik and Seddon (1992)) and the other in Argentina (Fig. 1).

Since 1968, the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB, Cape Town, South Africa) has rehabilitated thousands of African Penguins (*Spheniscus demersus*) and developed a baseline for normal feathering. Likewise, for more than 25 years the Penguin Project (the Project) has studied Magellanic Penguins (*Spheniscus magellanicus*) at Punta Tombo, Argentina (Boersma *et al.* 1990; Boersma 2008), determining the normal feathering pattern.

Normal feathering for *Spheniscus* penguins includes a hatching down, a second layer of down that replaces the hatching down, and juvenile plumage that replaces the second down (Boswall and MacIver

1975). In the following year, the juvenile molts into adult plumage (Williams 1995). A normally-feathered penguin chick has down or feathers covering the body except for the feet and bill.

SANCCOB first observed feather loss in African Penguin chicks in 2006 in the rehabilitation center and in the following year, 2007, the Project found featherless Magellanic Penguin chicks for the first time in Argentina. Here we report the number and severity of feather loss cases in African and Magellanic Penguins.

STUDY AREAS AND METHODS

African Penguin Chicks

SANCCOB began rehabilitating African Penguins in 1968 (Morant *et al.* 1981, Adams 1994; Parsons and Underhill 2005) and treats several hundred African Penguins each year from colonies along the coast of South Africa. These include: Dyer Island (approximately 2,000 breeding pairs; 34°40.6'S, 19°25.0'E), Robben Island (approximately 7,000 breeding pairs; 33°48.3'S, 18°21.6'E) and Betty's Bay (approximately 200 breeding pairs; 34°21.4'S, 18°57.6'E) (Underhill *et al.* 2006).

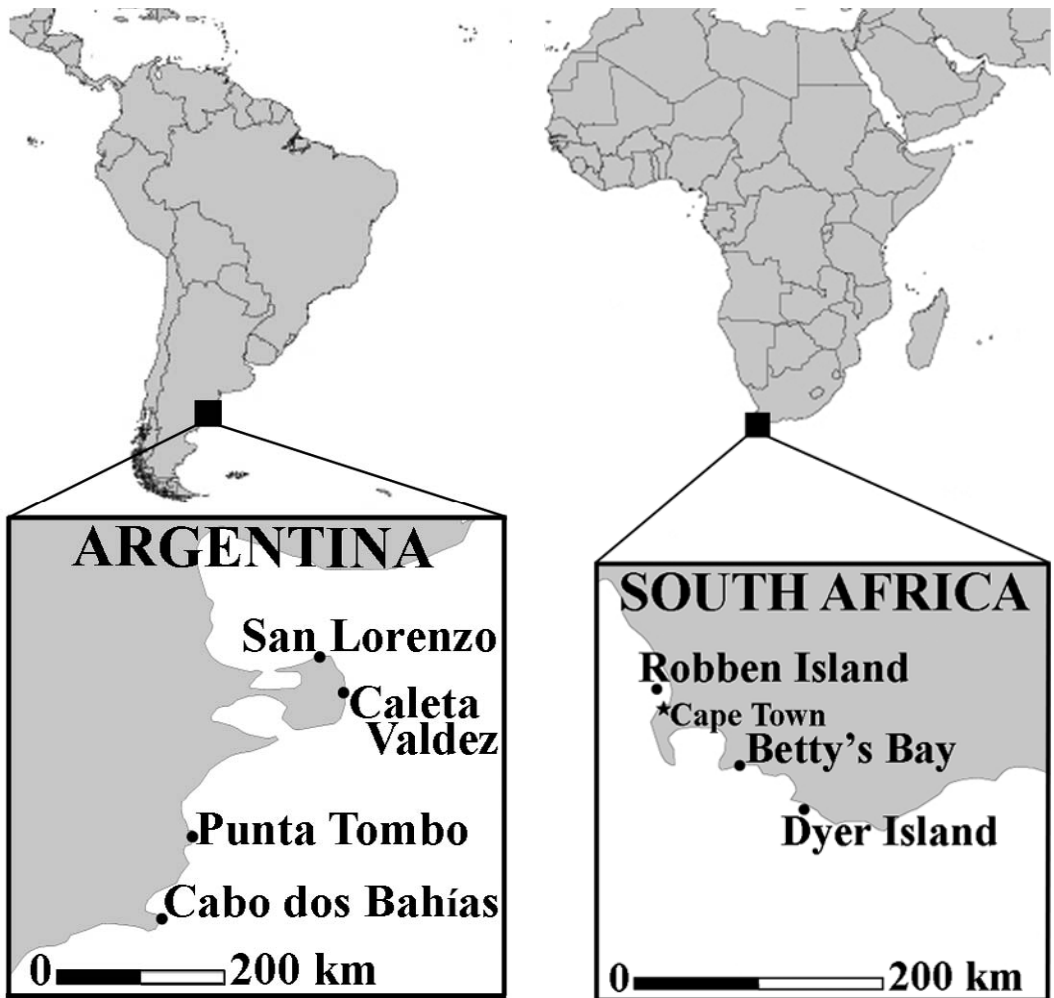


Figure 1. Location of African (*Spheniscus demersus*) and Magellanic Penguin (*Spheniscus magellanicus*) colonies with featherless chicks in Argentina and South Africa.

Upon admission, SANCCOB temporarily bands, deworms, and sprays penguins with Newcastle Disease vaccine (La Sota Strain) and injects supplemental vitamins and iron. SANCCOB archives a record for each penguin, including a weekly plumage evaluation (Parsons and Underhill 2005).

In 2006, 2007 and 2008, SANCCOB admitted 854, 538 and 181 African Penguin chicks, respectively. As in other years, chicks had normal feathering at admission. In 2006 and 2007, SANCCOB removed the majority of these chicks (841 and 481, respectively) from the wild after breeding adults began molting in large numbers and could no longer feed their young. SANCCOB admitted the remaining chicks because they were in poor body condition. Some chicks lost feathers and we tested differences in duration of stay in the rehabilitation center between feathered and featherless chicks using a two-sample *t*-test assuming unequal variances (EXCEL SP3).

Magellanic Penguin Chicks

The Penguin Project handles hundreds of Magellanic Penguins at Punta Tombo, Argentina each year (44°2.7'S, 65°13.4'W) and often visits other colonies, including San Lorenzo (approximately 57,000 breeding pairs; 42°5.0'S, 63°51.8'W), Caleta Valdes (approximately 10,000 breeding pairs; 42°29.3'S, 63°36.4'W), and Cabo Dos Bahías (approximately 9,000 breeding pairs; 45°0.5'S, 65°37.2'W) (Boersma *et al.* 1990; Schiavini *et al.* 2005). Punta Tombo is the largest Magellanic Penguin colony in the world with approximately 200,000 breeding pairs (Boersma 2008).

The Project checks hundreds of nests each year at Punta Tombo to record breeding success, egg laying and chick hatching dates, and to measure known-age chicks every ten days until they die or fledge (Boersma *et al.* 1990; Boersma 2008). We used these data to compare the growth rate of one Magellanic featherless chick

that fledged in 2007 and one Magellanic featherless chick that went missing before 33 days of age in 2008 with the growth of Magellanic second-hatched chicks that went missing before 33 days of age or fledged in 2007 and 2008. Second chicks are generally smaller and grow more slowly than chicks that hatch first (Boersma and Stokes 1995). Additionally, the Project measured the growth of two featherless Magellanic Penguin chicks of unknown age: one at San Lorenzo in 2007 and one at Punta Tombo in 2008. Lastly, in December 2008, when Magellanic Penguin chicks were mainly in their second layer of down, the Project checked more than 17,000 nests and approximately 20,000 Magellanic Penguin chicks at San Lorenzo.

RESULTS

African Penguin Chicks

In 2006, African Penguin chicks developed large featherless patches after being admitted to the rehabilitation center; the disorder was not found in the wild in South Africa until 2008. In 2006, 2007 and 2008, 59 (7% of 854 admitted), 97 (18% of 538) and 20 (11% of 181) chicks lost feathers in the rehabilitation center, respectively (Table 1, Fig. 2). Although those chicks appeared normal at admission to the rehabilitation center, they began losing their feathers and had large bald patches 18 days after admission (SD = 7 days). At about 34 days after admission (SD = 5 days) the chicks began growing new feathers. Chicks that had already begun growing juvenile feathers before feather loss grew adult plumage (N = 145) and chicks that were in their second layer of down before feather loss grew juvenile plumage (N = 3). Only chicks that were fully or partially down-covered lost their down and had bare patches of skin; no chicks in full juvenile plumage lost feathers.

Mortality in the rehabilitation center was similar for featherless and feathered African Penguin chicks in 2006 (8 and 9%, respectively) and 2007 (26 and 28%, respectively). In 2008, mortality of African penguin chicks was lower for featherless chicks than feathered chicks (5% and 12%, respectively). Chicks that lost their feathers took significantly longer (\bar{x} = 59 days; SD = 20) to reach the standards for release (Parsons and Underhill 2005) than feathered chicks (\bar{x} = 43 days; SD = 18; t_{176} = -8.8, $P < 0.00001$).

Additionally, in the wild, in 2008, five African Penguin chicks from Dyer Island, two chicks from Robben Island, and one chick from Dassen Island had large featherless patches (L. Waller, CapeNature, pers. obs.; N. Parsons, pers. obs.). Many additional African Penguin chicks had small featherless patches on their shoulders, a pattern seen in the wild due to pecking or plucking by adult penguins; these chicks were not considered featherless.

Magellanic Penguins Chicks

The Project first documented featherlessness in Magellanic Penguin chicks in 2007 when ten chicks lost their feathers, exposing bare skin. Four of those chicks were at Punta Tombo, three were at San Lorenzo, two were at Caleta Valdes and one was at Cabo Dos Bahías. In 2008, the Project found three featherless chicks: two at Punta Tombo and one at San Lorenzo (Table 1, Fig. 3). The Project found most of these chicks opportunistically; only one of 660 and one of 630 Magellanic Penguin chicks were in Project study nests in 2007 and 2008, respectively. The

Table 1. Year, species, number of African Penguin chicks SANCCOB admitted [total admitted: feathered (N), and featherless (F)], and number of African and Magellanic featherless chicks in the wild.

Year	Species	Total:	# Admitted		# Wild
			N (% Died)	F (%Died)	F (%Died)
2006	<i>S. demersus</i>	854	795 (9)	59 (8)	0
	<i>S. magellanicus</i>	0	—	—	0
2007	<i>S. demersus</i>	538	441 (28)	97 (26)	0
	<i>S. magellanicus</i>	0	—	—	10 (40)
2008	<i>S. demersus</i>	181	161 (12)	20 (5)	6 (—)
	<i>S. magellanicus</i>	0	—	—	3 (33)

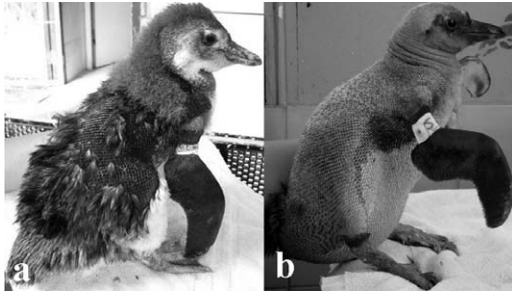


Figure 2. Featherless African Penguin (*Spheniscus demersus*) chicks: (a) African chick losing juvenile feathers and down and (b) adult plumage emerging on an African chick.

2008 featherless San Lorenzo chick was the only featherless chick out of approximately 20,000 chicks checked.

Affected Magellanic Penguin chicks had few or no down feathers on their backs and abdomens, no signs of lesions or inflamma-

tion of the feather follicles and no evidence of feather loss due to plucking or pecking by adult penguins. They were covered with down at hatching, but when they molted their hatching down they lost or failed to grow in their second layer of down. Dorsal skin of three chicks was blue in color and one chick was hot to the touch. Featherless chicks basked in the sun on a hot day when most feathered chicks were in the shade. Five featherless chicks died from unknown causes, four grew juvenile plumage and fledged, and we did not revisit the remaining four chicks. Four of the featherless chicks had siblings, but only one of the siblings had feather loss. One of the featherless study chick's parents had five chicks since 2004, but none had feather loss. The other featherless study chick's parents were newly mated and it was the first time the male had bred.

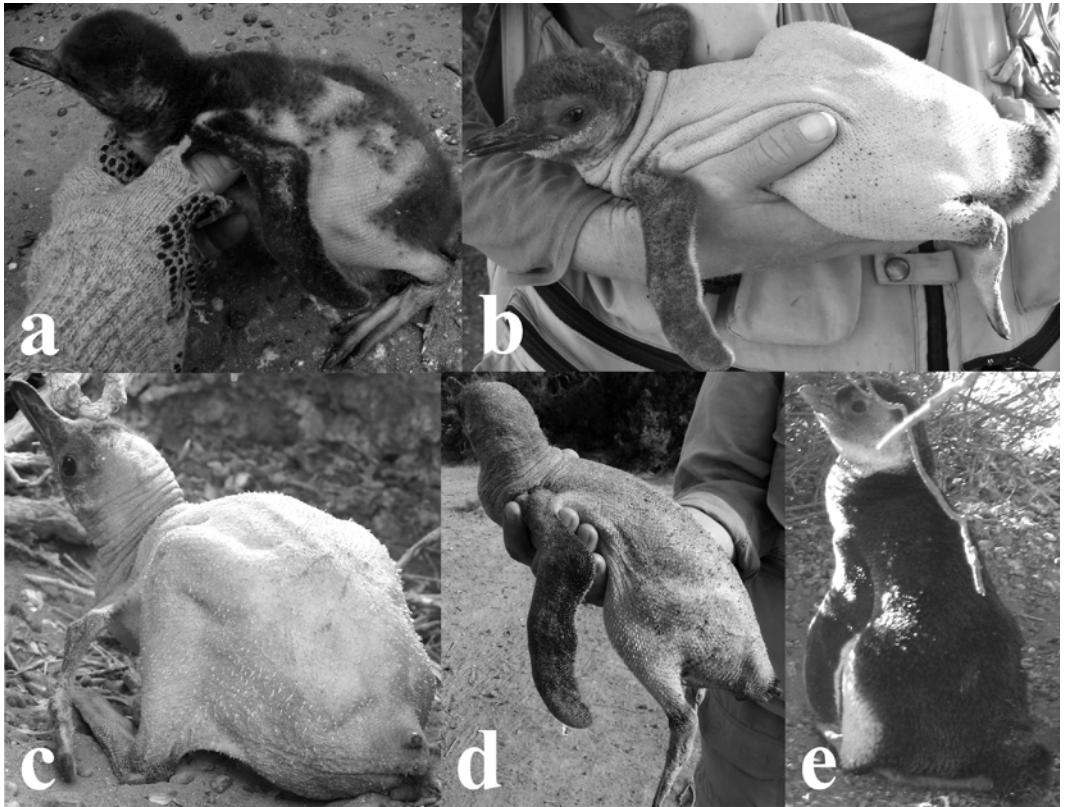


Figure 3. Progression of feather loss in Magellanic Penguin chicks (different chicks shown at each stage): (a) beginning to lose down on abdomen and sides; (b) almost naked, but down remaining on head, flippers and tail; (c) naked with the exception of down near eyes; (d) starting to grow in juvenile plumage; and (e) juvenile plumage fully grown in, healthy but smaller than feathered chicks.

The two Magellanic Penguin featherless study chicks from Punta Tombo (Fig. 4), a featherless non-study chick from Punta Tombo and the featherless chick from San Lorenzo in 2007 were smaller than feathered chicks. The 2008 study chick was feathered from 23 November to 4 December, became featherless by 14 December, and remained featherless until it went missing ten days later when it was 33 days of age. The chick's measurements, prior to losing all its feathers, were similar to feathered chicks. Within ten days of losing all its feathers, its growth slowed and it was smaller than feathered chicks. The chick was in good condition when it disappeared.

The featherless non-study chick's flipper measurements were small compared to feathered Magellanic Penguin chicks (11.3 cm on 17 January 2009 compared to = 15.1 cm, SD = 0.6, N = 27 on 16-18 January 2009); the featherless chick from San Lorenzo had a flipper length of 11.5 cm before fledging,

similar to the featherless chicks from Punta Tombo.

DISCUSSION

Feather loss in penguin chicks first appeared in a South African rehabilitation center in 2006, but did not appear in the wild in South Africa until 2008. In Argentina, the disorder first occurred in four colonies simultaneously the year after its emergence in the South African rehabilitation center. The disorder was more common in the African rehabilitation center than in the wild, suggesting that close contact and/or enclosed spaces facilitated the disorder. The cause of the disorder has yet to be identified and it is unknown whether feather loss in African and Magellanic Penguin chicks is related.

Feather loss in *Spheniscus* penguins has been documented once before, in South Africa in 1989 (van Heezik and Seddon 1992). In that instance, feather loss in African Pen-

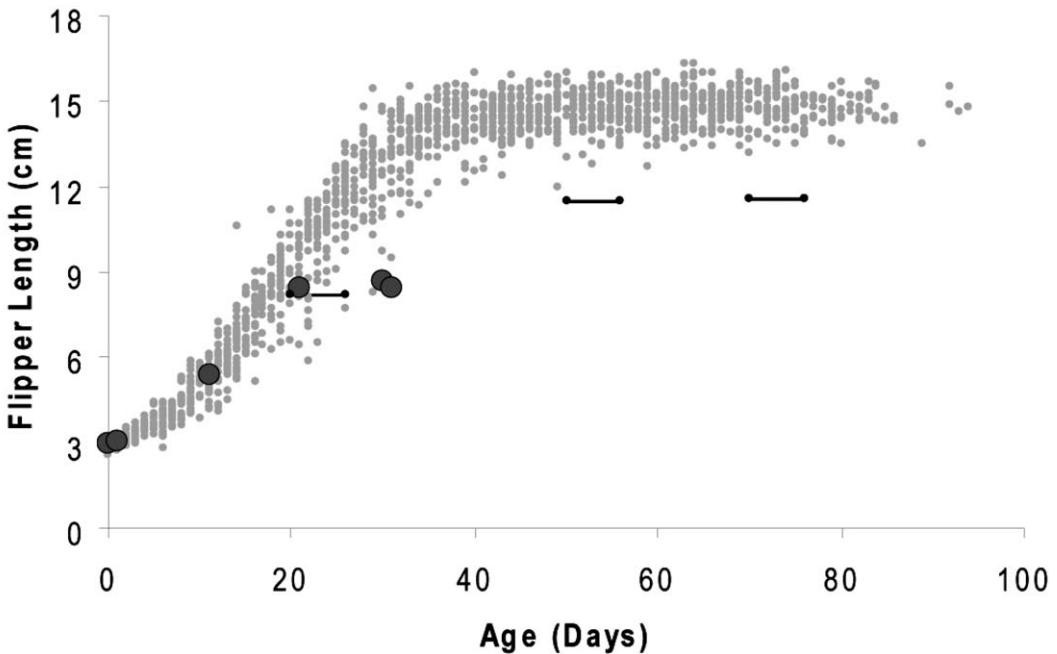


Figure 4. Magellanic Penguin Flipper length (cm) by age (days) of second-hatched feathered Magellanic Penguin chicks that either went missing before 33 days of age or fledged in 2007 or 2008 (N = 221 chicks; label ●), a known-age second-hatched Magellanic featherless chick that went missing at 33 days of age (N = 1; label ●), and a Magellanic featherless chick of age known within a six-day range and of unknown hatch order (N = 1; label —•; the bar shows the six-day range of possible ages). Second-hatched Magellanic Penguin chicks are smaller than first-hatched Magellanic Penguin chicks (Boersma and Stokes 1995) and therefore a conservative comparison.

guin chicks in the wild was apparently caused by malnutrition, and thus appears to be different from the feather disorder we report. The feather loss since 2006 has affected chicks in a rehabilitation center, where food and supplemental vitamins are provided, as well as wild Magellanic Penguin chicks in good body condition that later grew juvenile plumage and fledged. Malnutrition, therefore, seems an unlikely cause of this feather-loss disorder.

The disorder disrupted feather growth in both species, resulting in chicks with bare skin for several weeks. Feather loss caused most African Penguin chicks to grow adult instead of juvenile plumage. In contrast, Magellanic Penguin chicks grew juvenile plumage, the same as chicks without the disorder. The phase of feather development at which feather loss occurs may explain this disparity. African Penguin chicks lost feathers after beginning to grow in their juvenile plumage, but Magellanic penguins lost their second coat of down before the onset of juvenile feather development. After becoming featherless, the chicks appeared to start the next developmental phase of feather growth.

Featherless chicks are poorly insulated and lose heat, so they should, therefore, have to use more energy to maintain their body temperature than feathered chicks. Increased energy expenditure on thermoregulation should decrease growth. African featherless chicks took 16 days longer to reach release weight than feathered chicks. Likewise, Magellanic featherless chicks grew more slowly and were smaller than featherless chicks. Both the Magellanic featherless chicks grew more slowly and were smaller at the time of their death or when they fledged than Magellanic feathered, second-hatched chicks suggesting the lack of feathers usurps energy usually dedicated to growth.

In the African rehabilitation center, mortality rates were similar for featherless and feathered chicks in 2006 and 2007, but higher for feathered chicks in 2008. In 2008, SANCCOB admitted a higher percentage of young chicks that died before they were old enough to lose their hatching down, which explains the higher mortality rate of feath-

ered chicks in 2008. In the center, chicks had unlimited food and could stay until they were in good condition, which likely contributed to featherless chick survival. Feather loss likely increases mortality in the wild because of the higher energy needs of the chick and their longer fledgling period.

The discovery of featherless chicks in 2006 in Africa and in 2007 in Argentina suggests the disorder is new. Feather loss was more common in the rehabilitation center than in the wild, indicating the disorder is more likely to occur in close contact and enclosed spaces. The disorder appears to reduce chick growth in both species and is likely to increase chick and fledgling mortality in African and Magellanic Penguins in the wild. Feather-loss disorders are uncommon in most bird species, but they can be caused by pathogens, thyroid disorders, nutrient imbalances, pollution or genetics (Bernier *et al.* 1981; Arends 1997; Johne and Müller 1998; Hoffman 2002; Leeson and Walsh 2004; Bert *et al.* 2005; Gartrell *et al.* 2005; Lennox 2007). Further study is needed to determine the cause of the disorder and whether it is spreading to other penguin species.

ACKNOWLEDGMENTS

The Wildlife Conservation Society, Friends of the Penguins and Wadsworth Endowed Chair in Conservation Science fund research at Punta Tombo, Argentina, which is made possible by the Province of Chubut Office of tourism and the estancia La Regina. SANCCOB is supported by the International Fund for Animal Welfare, the Norway South Africa Fisheries Agreement, the National Research Foundation, the University of Cape Town Research Committee and the Earthwatch Institute.

LITERATURE CITED

- Adams, N. J. 1994. Patterns and impacts of oiling of African Penguins *Spheniscus demersus*: 1981-1991. *Biological Conservation* 68: 35-41.
- Arends, J. J. 1997. External parasites and poultry pests. Pages 804-805 *in* *Diseases of Poultry* 10th edition (B. W. Calnek, Ed.). Iowa State University Press, Iowa.
- Bernier, G., M. Morin and G. Marsolais. 1981. A generalized inclusion body disease in the budgerigar (*Melopsittacus undulatus*) caused by a papovavirus-like agent. *Avian Diseases* 25: 1083-1092.
- Bert, E., L. Tomassone, C. Peccati, M. G. Navarrete and S. C. Sola. 2005. Detection of beak and feather disease virus (BFDV) and avian polyomavirus (APV)

- DNA in psittacine birds in Italy. *Journal of Veterinary Medicine* 52: 64-68.
- Boersma, P. D., D. L. Stokes and P. Yorio. 1990. Reproductive variability and historical change of Magellanic Penguins (*Spheniscus magellanicus*) at Punta Tombo, Argentina. Pages 15-43 in *Penguin Biology* (L. Davis and J. Darby, Eds.). Academic Press, Inc., San Diego, California.
- Boersma, P. D. and D. L. Stokes. 1995. Mortality patterns, hatching asynchrony, and size asymmetry in Magellanic Penguin *Spheniscus magellanicus* chicks. In *The Penguins: Ecology and Management* (P. Dann, I. Norman and P. Reilly, Eds.). Surrey Beatty & Sons Pty Limited, NSW, Australia.
- Boersma, P. D. 2008. Penguins as marine sentinels. *BioScience* 58: 597-607.
- Boswall, J. and D. MacIver. 1975. The Magellanic Penguin *Spheniscus magellanicus*. In *The Biology of Penguins* (Bernard Stonehouse, Ed). The Macmillan Press Limited, Bristol, UK.
- Gartrell, B. D., L. Rogers and M. R. Alley. 2005. Eosinophilic dermatitis associated with *Trichosporon asahii* in a cockatiel (*Nymphicus hollandicus*). *Journal of Avian Medicine and Surgery* 19: 25-29.
- Hoffman, D. J. 2002. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquatic Toxicology* 57: 11-26.
- Johne, R. and H. Müller. 1998. Avian polyomavirus in wild birds: genome analysis of isolates from *Falconiformes* and *Psittaciformes*. *Archives of Virology* 143: 1501-1512.
- Leeson, S. and T. Walsh. 2004. Feathering in commercial poultry—II. Factors influencing feather growth and feather loss. *Worlds Poultry Science Journal* 60: 52-63.
- Lennox, A. M. 2007. Mycobacteriosis in companion psittacine birds: a review. *Journal of Avian Medicine and Surgery* 21: 181-187.
- Morant, P. D., J. Cooper and R. M. Randall. 1981. The rehabilitation of Jackass Penguins *Spheniscus demersus* 1970-1980. In *Proceedings of the Symposium on Birds of the Sea and Shore*. (J. Cooper, Ed.). Cape Town: African Seabird Group: 267-301.
- Parsons, N. J. and L. G. Underhill. 2005. Oiled and injured African Penguins *Spheniscus demersus* and other seabirds admitted for rehabilitation in the Western Cape, South Africa, 2001 and 2002. *African Journal of Marine Science* 27: 289-296.
- Schiavini, A., P. Yorio, P. Gandini, A. R. Rey and P. D. Boersma. 2005. Los pingüinos de las costas Argentinas: estado poblacional y conservación. *Hornero* 20: 5-23.
- Underhill, L. G., R. J. M. Crawford, A. C. Wolfaardt, P. A. Whittington, B. M. Dyer, T. M. Leshoro, M. Ruthenberg, L. Upfold and J. Visagie. 2006. Regionally coherent trends in colonies of African penguins *Spheniscus demersus* in the Western Cape, South Africa, 1987-2005. *African Journal of Marine Science* 28: 697-704.
- van Heezik, Y. M. and P. J. Seddon. 1992. Starvation and delayed plumage development in Jackass Penguin chicks. *Ostrich* 63: 129-130.
- Williams, T. D. 1995. Magellanic Penguin. In *The Penguins* (C. M. Perrins, W. J. Bock, and J. Kikkawa, Eds). Oxford University Press Inc., New York, New York.

Avian Pox in Magellanic Penguins (*Spheniscus magellanicus*)

Olivia J. Kane,^{1,2,6} Marcela M. Uhart,³ Virginia Rago,³ Ariel J. Pereda,⁴ Jeffrey R. Smith,^{1,2} Amy Van Buren,^{1,2} J. Alan Clark,^{1,2,5} and P. Dee Boersma^{1,2,1} Department of Biology, University of Washington, 24 Kincaid Hall, Seattle, Washington 98195, USA; ² Wildlife Conservation Society, 2300 Southern Blvd., Bronx, New York 10460, USA; ³ Global Health Program, Wildlife Conservation Society, CC 1N19, Puerto Madryn (9120), Chubut, Argentina; ⁴ Instituto de Virología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), C.C. 25, Castelar (1712), Buenos Aires, Argentina; ⁵ Current address: Department of Biological Sciences, Fordham University, 441 East Fordham Rd., Bronx, New York 10458, USA; ⁶ Corresponding author (email: olivaj@u.washington.edu)

ABSTRACT: Avian pox is an enveloped double-stranded DNA virus that is mechanically transmitted via arthropod vectors or mucosal membrane contact with infectious particles or birds. Magellanic Penguins (*Spheniscus magellanicus*) from two colonies (Punta Tombo and Cabo Dos Bahías) in Argentina showed sporadic, nonepidemic signs of avian pox during five and two of 29 breeding seasons (1982–2010), respectively. In Magellanic Penguins, avian pox expresses externally as wart-like lesions around the beak, flippers, cloaca, feet, and eyes. Fleas (*Parapsyllus longicornis*) are the most likely arthropod vectors at these colonies. Three chicks with cutaneous pox-like lesions were positive for *Avipoxvirus* and revealed phylogenetic proximity with an *Avipoxvirus* found in Black-browed Albatross (*Thalassarche melanophrys*) from the Falkland Islands in 1987. This proximity suggests a long-term circulation of seabird *Avipoxviruses* in the southwest Atlantic. Avian pox outbreaks in these colonies primarily affected chicks, often resulted in death, and were not associated with handling, rainfall, or temperature.

Key words: Argentina, avian pox, Magellanic penguin, *Spheniscus magellanicus*.

Avian pox, caused by an *Avipoxvirus* in the *Poxviridae* family, is an enveloped double-stranded DNA virus that is mechanically transmitted via arthropod vectors or mucosal membrane, broken skin, or abraded skin contact with infected particles or individuals (Hansen, 1999). Poxvirus infection has been reported for at least 278 of the approximately 9,800 bird species (Van Riper and Forrester, 2007), but all avian species are likely susceptible (Karstad, 1971).

In the most common form, cutaneous, wart-like growths occur around the eyes, beak, or unfeathered skin (Hansen, 1999). Infected birds are often emaciated, and

although pox infection itself is rarely fatal, secondary bacterial or fungal infections are common and cause mortality (Hansen, 1999). The rarer form, diphtheritic pox, has a higher case fatality ratio and manifests as raised, yellow plaques in the mouth, throat, trachea, and lungs, causing difficulty breathing or swallowing (Hansen, 1999). Mortality from avian pox depends on infection type, species infected, and location of growths (Hansen, 1999; Van Riper et al., 2002). Avian pox prevalence often coincides with weather-induced increases in vector populations. For example, Hawaiian bird populations have higher infection rates during seasons of high precipitation when breeding conditions for mosquitoes are better (Van Riper et al., 2002; Young and VanderWerf, 2008).

The most commonly reported forms of *Avipoxvirus* might be fowlpox and canarypox viruses (Stannard et al., 1998); however, the actual number of strains remains unknown (Smits et al., 2005). Avian pox harms many wild bird populations and continues to be discovered in new species (Bolte et al., 1999). Little is known about the prevalence of the disease in wild seabird populations (Young and VanderWerf, 2008; Parker, 2009).

Avipoxvirus has been documented in three penguin species—African (*Spheniscus demersus*; Stannard et al., 1998), Humboldt (*Spheniscus humboldti*; Landowska-Plazewska and Plazewski, 1968 as cited by Bolte et al., 1999), and Gentoo (*Pygoscelis papua*; Munro, 2007), but more species are affected. In 2006 an avian pox outbreak affected five Gentoo Penguin colonies in the Falkland Islands (Islas Malvinas), but

nearby Magellanic Penguin (*Spheniscus magellanicus*) colonies showed no signs of infection (Munro, 2007). However, in 2000 and 2001 a few Rockhopper Penguin (*Eudyptes chrysocome*) chicks and one Magellanic Penguin chick (in separate colonies in the Falkland Islands) had wart-like growths characteristic of avian pox (Van Buren, unpubl. data). Additionally, Galapagos Penguins (*Spheniscus mendiculus*) in the El Niño years of 1972 and 1998 had pox-like lesions on their beaks with juveniles more frequently infected than adults (Boersma, 1977, unpubl. data).

Here, we document Magellanic Penguins with pox-like lesions at two colonies in Chubut, Argentina: Punta Tombo (44°2.7'S, 65°13.4'W) and Cabo Dos Bahías (45°0.5'S, 65°37.2'W, Fig. 1). The colonies are in a temperate desert where the penguins nest under bushes or in burrows. We have studied Magellanic Penguins at Punta Tombo since 1982, handling 200–4,000 chicks, up to 530 juveniles, and 200–3,000 adults yearly. We visited Cabo Dos Bahías one to seven times per year handling up to 77 birds annually. To track noninfected chicks (all years) and infected chicks (starting in 1997), we marked individuals' flippers with tape until their feet were greater than 9 cm long. We then pierced foot webbings with numbered small-animal ear tags (No. 1005–3, 2×10 mm; National Band and Tag Company, Newport, Kentucky, USA). We banded chicks that reached fledging age and weight (>1.8 kg) with numbered stainless-steel flipper bands (Lambournes-Porzana, East Sussex, United Kingdom). We washed our hands and disinfected equipment, clothing, and boots in 10% bleach for 20 min after handling infected chicks.

With the use of simple linear regression, we investigated presence or absence of presumptive avian pox from 1983 to 2009 at Punta Tombo with annual temperature (average minimum and average maximum temperature [C]) and total precipitation (mm) from October through January (chick hatching through fledging), as they are known factors that affect ectoparasite



FIGURE 1. Location of two Magellanic Penguin (*Spheniscus magellanicus*) colonies in Argentina (Punta Tombo and Cabo Dos Bahías) where we observed pox-like infections. Also, the Falkland Islands (Islas Malvinas) location of pox-infected Black-browed Albatross (*Dyomedea melanophrys*) from 1987.

abundance and thus may correlate with avian pox prevalence (Krasnov et al., 2001). We looked for observational bias with the use of simple linear regression to model presumptive avian pox prevalence as a function of number of chicks handled per year. We calculated prevalence at Punta Tombo as the number of infected chicks divided by total number handled per year. For all chicks in a bounded area in 1997, we compared 1) percent of infected to percent of noninfected chicks that fledged and 2) adult resighting of those fledged chicks with the use of a χ^2 goodness-of-fit test. All statistical tests were done with R version 2.13.0.

We collected tissue samples from three necropsied chicks with pox-like lesions at Cabo Dos Bahías in 2007. We preserved tissue samples in 10% formalin for histopathologic examination and stored a subset of samples in liquid nitrogen for culture, viral isolation, and molecular diagnosis. Centro Diagnostico Veterinario (a

private diagnostic laboratory, Conde 4799, Buenos Aires, Argentina) analyzed the samples histopathologically and Wildlife Conservation Society pathologist, Denise McAloose (Bronx Zoo, New York), did confirmatory image analysis.

The National Institute of Agricultural Technology (Castelar, Argentina) used ionic interchange columns to extract nucleic acids from a maceration of lesion scabs for molecular diagnosis (QIAamp DNA Mini Kit, QIAGEN, Valencia, California, USA). Polymerase chain reaction (PCR) reactions and primers for the pox virus 4b core protein were identical to those described by Luschow et al. (2004) and Lee and Lee (1997). We used DNA from an *Avipoxvirus* isolated from turkeys as a positive control and DNA from a nonrelated avian virus (chicken anemia virus) as a negative control. We sequenced directly from PCR fragments with the use of the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) following the manufacturer's instructions. We generated the consensus nucleotide sequences of the viruses with the use of Megalign (DNASTAR, Madison, Wisconsin, USA) and then performed phylogenetic analyses with additional virus sequence data available in GenBank. We then assembled sequences and edited with Lasergene 8.1 (DNASTAR) and used BioEdit 7 for alignment and residue analysis. We constructed neighbor-joining (NJ) trees with the use of PAUP* 4.0. We calculated estimates of the phylogenies by performing 1,000 NJ bootstrap replicates and used TREEVIEW to create the phylogenetic trees.

We encountered 108 Magellanic Penguins with pox-like lesions in five and two of the last 29 breeding seasons (1982–2010) at Punta Tombo (1983, 1984, 1985, 1997, and 2007) and Cabo Dos Bahías (2004 and 2007), respectively. Mean prevalence was 0.002 (SE=0.001, $n=28$ yr) infected chicks to every handled chick. We did not find any observational bias (i.e.,

there was no correlation between infected chicks and number of chicks handled yearly ($F_{(1,25)}=2.54$, $P=0.12$). Birds with suspected avian pox had nodular lesions in feathered and unfeathered areas on flippers, feet, neck, eyes, beak, and surrounding area, under and on the tongue, and around and inside the cloaca. Chicks were often in poor condition (Fig. 2). Fifteen of 77 (20%) infected chicks developed lesions in their eyes. Of those, seven (47%) became blind in at least one eye. None of the infected chicks' parents exhibited symptoms, however; one 3-yr-old female had bare patches with wart-like lesions at the base of each flipper.

Of the 107 chicks with pox-like lesions, 18 died, the fates of 87 are unknown, and we humanely euthanized one severely infected chick. Thirty-four percent (30 of 87) of the unknown chicks likely fledged; however, survival at sea is unknown and infected fledglings are likely more susceptible to starvation and predation. The remaining 57 chicks were not revisited or disappeared at a time or body condition not conducive to survival. Whether the 18 chicks who died on land succumbed to *Avipoxvirus* infection, secondary infections, or unrelated causes is unknown.

Histopathology of tissues from two of the three sampled chicks showed unspecific inflammatory lesions in the lungs, kidneys, heart, spleen, and digestive tract. All sampled chicks had dermatitis, epidermal hyperplasia, and eosinophilic intracytoplasmic inclusions consistent with an *Avipoxvirus* infection. We positively identified poxviruses by PCR from the collected samples ($n=3$, JN615018 GenBank accession number). Phylogenetic analysis from these three sample amplicons revealed they were identical and are highly similar to an *Avipoxvirus* from a Black-browed Albatross (*Thalassarche melanophrys*) in the Falkland Islands in 1987 (AM050392 GenBank accession number).

In 1997, the outbreak was in a bounded area where we observed and measured all

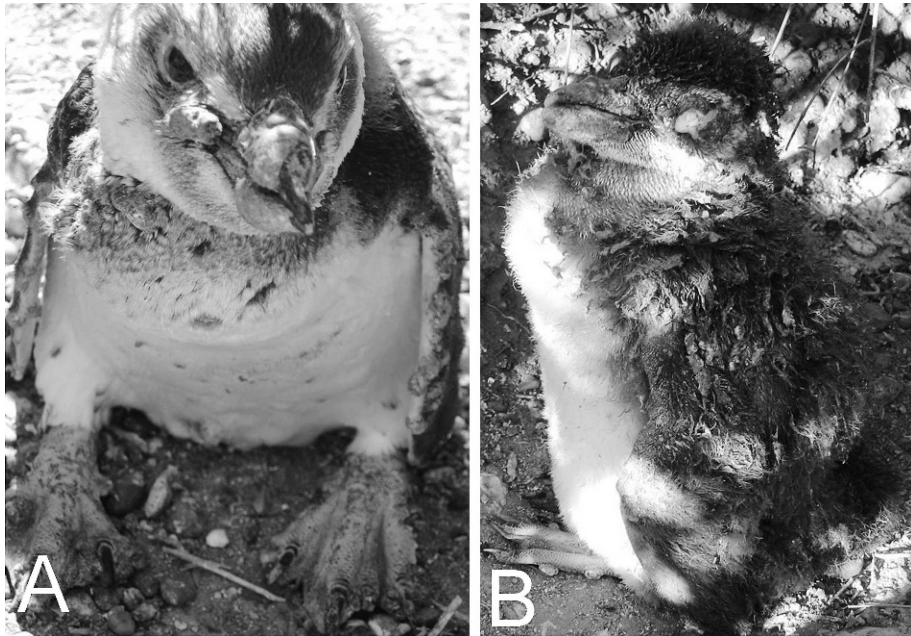


FIGURE 2. Two Magellanic Penguin (*Spheniscus magellanicus*) chicks with pox-like lesions: (a) chick's bill double the normal size with large lesions over most of body and (b) chick blinded by lesions and subsequent secondary infection in both eyes.

chicks from hatching until fledging or death. Of 130 chicks that hatched in this area, 10 showed evidence of poxvirus infection. The percent of chicks that died or went missing was similar for infected (67%) and noninfected (62%) chicks ($\chi^2=0.05$, $df=1$, $P=0.81$). Our adult resight rate was similar for infected (0 resighted of 3 fledged) and noninfected (2 of 48) chicks that fledged with flipper bands ($\chi^2=1.37$, $df=1$, $P=0.24$). We have not resighted any of the seven banded infected chicks that fledged in 2007. In 2008, 2009, and 2010 the adult female that previously exhibited pox-like symptoms returned to the colony in healthy condition.

Many infected chicks occurred in clusters, while some were isolated cases. One cluster occurred near a tourist walkway and in close proximity to many other penguins, suggesting the possibility of transmission between colonies by tourists. Chicks huddle together, allopreen, and feed competitively allowing for direct transmission of the virus; however,

isolated infected individuals and distance between clusters suggests vector transmission may occur.

Two species of ectoparasites affect Magellanic Penguins. The most common is the flea (*Parapsyllus longicornis*, Carnegie Museum B-98582, B-98581, B-99243), which infests most adults, chicks, and nests and may be a mechanical vector (Smits et al., 2005). The second ectoparasite, the mosquito, is rarely observed. *Parapsyllus longicornis* are confined to birds and are most commonly found on seabirds, including Black-browed Albatross (Murray et al., 2003). Flea survival and abundance is affected by humidity and temperature, but unlike mosquitoes, fleas do not need standing water for reproduction (Krasnov et al., 2001). We did not collect data on ectoparasite abundance, so we investigated the effect of weather condition on infection presence. For all years, average minimum (9.9 C, SE=0.15) and maximum temperature (23.7 C, SE=0.33) showed little variation

while total precipitation (78.3 mm, SE=9.93) did vary. We found annual average minimum ($F_{(1,25)}=2.39$, $P=0.13$) and maximum ($F_{(1,25)}=0.53$, $P=0.47$) temperature and total annual precipitation ($F_{(1,24)}=3.28$, $P=0.08$) for all years were not significant covariates in explaining infection presence.

Avian pox in Magellanic Penguins is spatially and temporally variable. Infection is more common in chicks than adults suggesting that adults may have acquired immunity or a stronger immune system. Phylogenetic proximity of the *Avipoxvirus* found in Magellanic Penguins at Cabo Dos Bahías with that found in Black-browed Albatross suggests long-term circulation of seabird avipoxviruses in the Southwest Atlantic. Further study is needed to understand the transmission mechanism and the unpredictable nature of outbreaks.

We thank the Wadsworth Endowed Chair in Conservation Science, the Wildlife Conservation Society, and Friends of the Penguins for funding research. We thank the Province of Chubut Office of Tourism and the estancia La Regina for access to the research site, and the Chubut Wildlife Agency for sample collection. We thank D. McAloose, V. Ruoppolo, C. Poleschi, S. Harris, R. Cook, G. Munro, Z. Luxton, and A. Woods for reporting cases, collecting or analyzing samples, and information, and J. Rawlins and B. Lewis for flea identification.

LITERATURE CITED

- BOERSMA, P. 1977. An ecological and behavioral study of the Galapagos Penguin. *Living Bird* 15: 43–93.
- BOLTE, A., J. MEURER, AND E. KALETA. 1999. Avian host spectrum of avipoxviruses. *Avian Pathology* 28: 415–432.
- HANSEN, W. 1999. Avian pox. In *Field manual of wildlife diseases*, M. Friend and J. C. Franson (eds.). USGS, National Wildlife Health Center, Madison, Wisconsin, pp. 163–169.
- KARSTAD, L. 1971. Pox. In *Infectious and parasitic diseases of wild birds*, J. W. Davis, R. C. Anderson, L. Karstad and D. O. Trainer (eds.). Iowa State University Press, Ames, Iowa, pp. 34–41.
- KRASNOV, B., I. KHOKHLOVA, L. FIELDEN, AND N. BURDELOVA. 2001. Effect of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). *Journal of Medical Entomology* 38: 629–637.
- LEE, L. H., AND K. H. LEE. 1997. Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. *Journal of Virology Methods* 63: 113–119.
- LUSCHOW, D., T. HOFFMANN, AND H. HAFEZ. 2004. Differentiation of avian poxvirus strains on the basis of nucleotide sequences of 4b gene fragment. *Avian Diseases* 48: 453–462.
- MUNRO, G. 2007. Outbreak of avian pox virus in Gentoos Penguins in the Falklands, February 2006. Falklands Conservation, Falkland Islands.
- MURRAY, M. D., R. L. PALMA, R. L. C. PILGRIM, AND M. SHAW. 2003. Ectoparasites of Australian, New Zealand and Antarctic birds. In *Handbook of Australian, New Zealand and Antarctic birds*. 6th Edition, S. Marchant and P. J. Higgins (eds.). Oxford University Press, Melbourne, Australia, pp. 1215–1217.
- PARKER, P. 2009. Parasites and pathogens: Threats to native birds. In *Galapagos: Preserving Darwin's legacy*, T. Roi (ed.). Firefly Books, Ontario, California, pp. 177–183.
- SMITS, J., J. TELLA, M. CARRETE, D. SERRANO, AND G. LÓPEZ. 2005. An epizootic of avian pox in endemic Short-toed Larks (*Calandrella rufescens*) and Berthelot's Pipits (*Anthus berthelotti*) in the Canary Islands, Spain. *Veterinary Pathology* 42: 59–65.
- STANNARD, L., D. MARAIS, D. KOW, AND K. DUMBELL. 1998. Evidence for incomplete replication of a penguin poxvirus in cells of mammalian origin. *Journal of General Virology* 79: 1637–1646.
- VAN RIPER, C., III, AND D. J. FORRESTER. 2007. Avian Pox. In *Infectious and parasitic diseases of wild birds*, N. Thomas, B. Hunter and C. A. Atkinson (eds.). Blackwell Publishing Professional, Ames, Iowa, pp. 131–176.
- VAN RIPER, C., III, S. VAN RIPER, AND W. HANSEN. 2002. Epizootiology and effect of avian pox on Hawaiian forest birds. *Auk* 119: 929–942.
- YOUNG, L., AND E. VANDERWERF. 2008. Prevalence of avian pox virus and effect on the fledging success of Laysan Albatross. *Journal of Field Ornithology* 79: 93–98.

Submitted for publication 27 May 2011.

Accepted 3 February 2012.