Ardeola 61(1), 2014, 145-152 DOI: 10.13157/arla.61.1.2014.145

LEUCOCYTE LEVELS IN SOME ANTARCTIC AND NON-ANTARCTIC PENGUINS

NIVELES LEUCOCITARIOS EN ALGUNAS ESPECIES DE PINGÜINOS ANTÁRTICOS Y NO ANTÁRTICOS

Verónica L. D'Amico[1] *, Marcelo Bertellotti[1], Julia I. Díaz[2], Néstor Coria[3], Virginia Vidal[4, 5] and Andrés Barbosa[6]

Summary.—We studied leucocyte levels in several Antarctic and non-Antarctic penguins. A total of 107 blood samples of chinstrap *Pygoscelis antarctica*, gentoo *P. papua* and Adélie *P. adeliae* penguins at Isla 25 de Mayo/King George Island (South Shetland Islands, Antarctica) and Magellanic penguins *Spheniscus magellanicus* at Península Valdés (Chubut, Argentina), were collected and analysed during the 2009-2010 breeding season. We observed that chinstrap and Adélie penguins had lower total leucocyte counts than Magellanic and gentoo penguins. We provide some potential explanations for species differences in leucocyte levels.

Resumen.—En este trabajo se estudiaron los niveles de leucocitos como valores de referencia en especies de pingüinos antárticos y no antárticos. Se analizaron 107 muestras de sangre de pingüinos barbijo *Pygoscelis antarctica*, Juanito *P. papua* y de Adelia *P. adeliae* en la isla 25 de Mayo/Rey Jorge (islas Shetland del Sur, Antártida) y de pingüinos magallánicos *Spheniscus magellanicus* en Península Valdés (Chubut, Argentina) durante la temporada de reproducción 2009-2010. Los resultados mostraron bajos conteos de leucocitos en pingüinos barbijo y Adelia en comparación con los pingüinos Juanito y magallánico. Se presentan diferentes explicaciones para las diferencias en los niveles de leucocitos entre las especies de pingüinos.

[1] Centro Nacional Patagónico (CONICET), Bvrd. Brown 2915, 9120, Puerto Madryn, Chubut, Argentina.

[2] Centro de Estudios Parasitológicos y de Vectores (CCT La Plata-CONICET-UNLP), Calle 2 No. 584, 1900, La Plata, Buenos Aires, Argentina.

[3] Instituto Antártico Argentino-Dirección Nacional del Antártico, Cerrito 1248, 1010 Buenos Aires, Argentina.

[4] Dpto. Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain.

[5] Unidad Asociada de Ecología e Inmunología Parasitaria, CSIC-Universidad de Murcia, Murcia, Spain.

[6] Dpto. Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain.

* Corresponding author: damico@cenpat.edu.ar

INTRODUCTION

The study of immune function has acquired great importance for understanding the life histories of organisms (Norris and Evans, 2000) because it is the primary defence against pathogens in animals (Zuk and Stoehr, 2002) and also because immunity can be regulated depending on the availability of energy resources (Norris and Evans, 2000). Variation in immunological parameters in response to different factors (e.g. body condition, Møller and Erritzøe, 2003; sex, Fargallo *et al*., 2002; age, Palacios *et al*., 2007; breeding stage, Dehnhard *et al*., 2011) and environmental characteristics (e.g. geography, Barbosa *et al*., 2007; pathogens and parasites, Horrocks *et al*., 2012) has been reported.

One of the components of the immune system of an organism is cellular immunity, which can be assessed by leucocyte counts (Roitt *et al*., 2001). Leucocytes are classified as phagocytes, that form part of innate immunity, or as lymphocytes, that enable acquired immunity (Roitt *et al*., 2001). Among phagocytes, heterophils are the primary phagocytic leucocytes and proliferate in circulation in response to infections, inflammation and stress or malnutrition (Maxwell and Robertson, 1998). The other phagocytes are eosinophils and monocytes, which are involved in defence against parasites, and basophils whose function is not clearly understood but is thought to be related to inflammatory processes (Campbell, 1995). Lymphocytes are involved in a variety of immunological functions, such as the production of immunoglobulins and modulation of immune defence (Campbell, 1995). The heterophil/lymphocyte ratio (H/L) has been described as a good measure of stress in birds (Davis *et al*., 2008). During chronic stress, such as poor feeding conditions, plasma baseline corticosterone levels remain elevated and lead to adaptive changes in physiology and behaviour, including an increase in the H/L ratio (Davis *et al*., 2008).

Antarctica, and specifically the Antarctic Peninsula, is suffering the effects of climate change with a temperature increase ranging between 0.2ºC and 0.4ºC in the last 60-100 years (Turner *et al*., 2005). This is also the case elsewhere, such as in Patagonia, where temperature increased by 0.5°C and 1°C during the last 52 years, with marked impacts on the environment (Rabassa, 2010). These environmental changes can affect such factors as the distribution, abundance and virulence of parasites and diseases, which in turn can affect the immune system of organisms (Harvell *et al*., 2002). Therefore, it is important to have baseline information on immune parameters of organisms in order to make future comparisons in this changing ecological context.

To date, information on cellular immunity is almost absent for free-living Sphenisciform penguins. One study reports data on leucocyte cell numbers in only one Antarctic penguin species (Vleck *et al*., 2000), a second includes three species but with a small sample size (Zinsmeister and Van Der Heyden, 1987) and a third involves a captive penguin species (Hawkey *et al*., 1985). Moreover, no baseline data on leucocyte profiles have been reported to date in the Magellanic penguin *Spheniscus magellanicus*, the penguin species present in Patagonia. Since penguins have a broad geographical distribution and have variable life histories (Boersma, 2008) they can make good models to study immune investments.

Here, we report for the first time baseline leucocyte numbers under natural conditions in three Antarctic penguins: the chinstrap *Pygoscelis antarctica*, gentoo *P. papua* and Adélie penguins *P. adeliae* and in a non-Antarctic species, the Magellanic penguin.

Material and methods

The study was conducted during the 2009-2010 breeding season. Antarctic penguin species were sampled at Isla 25 de Mayo/King George Island (Stranger Point, 62º 15' S, 58º 37' W, for Gentoo and Adélie penguins and Barton Point, 62º 14' S, 58º 46' W, for Chinstrap penguin) during December 2009 and January 2010. Magellanic penguins were sampled at Estancia San Lorenzo, Península Valdés in the Patagonia region of Argentina (47° S, 63° W) in November 2009.

The sampling occurred during the guard phase of chicks (10-15 days after hatching) to avoid potential variation related to the breeding period. Adults were captured and immediately sampled at the nest from the foot vein. All birds were sampled within 5 minutes of capture to avoid leucocyte production due to stress of handling (Davis *et al*., 2008). We sampled a total of 107 individuals of the four penguin species. Sampled birds showed no external signs of illness or injuries and presented normal body masses (4.5 kg for Adélie, 5.5 kg for gentoo, 4 kg for chinstrap and 4.5 kg for Magellanic penguins; mean body masses reported in Bertellotti, 2014).

Thin blood smears were prepared with a drop of fresh blood from each individual and placed on slides, air-dried, fixed with ethanol for 3 min and stained with Tinción 15 (Biopur). Blood smears were examined with a microscope by scanning fields with similar densities of erythrocytes (Campbell, 1995). The total leucocyte count (TCL) was estimated by counting all leucocytes in 10 consecutive 400x monolayer fields. Leucocyte proportions were obtained as the part of each leucocyte type in a sample of 100 leucocytes scanned at 1000x (oil immersion) (Campbell, 1995). The H/L ratio was calculated from the heterophil and lymphocyte values. For the four penguin species, 10 smears were randomly selected for analysis of repeatability. Three consecutive leucocyte counts were made using the described counting method for each of the 10 smears. Repeatability was calculated following Lessells and Boag (1987) for TCL, heterophils (H), eosinophils (E), lymphocytes (L) and monocytes (M). Basophils (B) were not included because of many zeros in the matrix. All values of repeatability ranked between 74 and 98%. All leucocyte counts were made by VLD.

Data were statistically described and compared among the four species using the nonparametric Kruskal-Wallis H test and nonparametric Multiple Comparisons (STATISTICA version 7.0). To maintain an experiment-wise error rate of 0.05, we used a sequential Bonferroni adjustment (Rice, 1989) of $\alpha = 0.007$ for $N = 7$ parameters compared.

Results and discussion

This study is the first to report leucocyte data for Magellanic penguins, and we also report leucocyte data for three additional species of Antarctic penguins. Our results (table 1) differ from results reported by Zinsmeister and Van Der Heyden (1987) for the percentage of monocytes in the three *Pygoscelis* penguins and this could be explained by the very small sample sizes used in that study (4 Adélies, 6 gentoos and 2 chinstraps). Also our results differ from the percentage of heterophils and H/L ratio reported by Vleck *et al*. (2000) for Adélie penguins in Torgersen Island, Antarctic Peninsula (64º S, 64º W), who reported higher values of heterophils and H/L than ours (50.4 and 0.16-8.09 respectively). Those differences in heterophils and H/L ratio could be explained by differences associated with geographical or temporal differences, since their samples were taken in 1996.

We observed that gentoo and Magellanic penguins had more leucocytes than chinstrap and Adélie penguins (TLC: $H_{3, 107} = 41.8$, Multiple Comparisons, $P < 0.007$, table 1). It has been reported that higher total leucocyte counts generally indicate a higher level of exposure to parasites and/or pathogens (Roitt *et al*., 2001). Among our study species, Magellanic and gentoo penguins carry a greater species diversity of helminth parasites than chinstrap and Adélie penguins (table 2). Differences found for TCL are

TABLE 1

Mean ± standard error for total leucocytes, heterophil/lymphocyte (H/L) ratios and leucocyte types of the four penguin species (N = sample size). Minimum and maximum values are shown in brackets. Significant differences (Multiple Comparisons, $P < 0.007$) among species are denoted by shared letters ($^{a, b, c}$), where "a" is assigned to the highest value for each measure.
[Media ± error estándar del total de leucocitos, relación heterófilos/leucocitos (H/L) y tipos de leucocitos en cuatro especies de pingüinos (N = tamaño muestral). Los valores mínimos y máximos se muestran entre paréntesis. Las diferencias significativas (Comparaciones Múltiples, P < 0,007) entre especies están indicadas por letras compartidas ($^{a, b, c}$), asignándose "a" al valor más alto de cada medida.]

	Non-Antarctic penguin	**Antarctic penguins**		
	Magellanic (*S. magellanicus*) N = 30	Chinstrap (*P. antarctica*) N = 27	Adélie (*P. adeliae*) N = 25	Gentoo (*P. papua*) N = 25
Total leukocytes	32.7 ± 0.3^{a} (10.7-85.3)	22.7 ± 0.2^{b} (8.4-50.4)	15.5 ± 0.09^{b} (7.8-28.5)	32.5 ± 0.1^{a} (21.2-43.9)
H/L	1.1 ± 0.1^{b} (0.3-5)	0.4 ± 0.05^{c} (0.1-1)	1.0 ± 0.1^{b} (0.3-3)	1.6 ± 0.1^{a} (0.7-3)
Lymphocyte %	39.9 ± 1.8^{b} (13-59)	62.3 ± 1.9^{a} (46-82)	40.7 ± 2.1^{b} (23-62)	35.1 ± 1.5^{c} (25-52)
Heterophil %	38.5 ± 1.8^{b} (20-60)	28.7 ± 1.8^{c} (10-45)	38.7 ± 1.9^{b} (20-63)	51.6 ± 1.5^{a} (35-67)
Basophil %	0.3 ± 0.1^{c} (0-2)	1.5 ± 0.3^{b} (0-7)	2.6 ± 0.3^{a} (0-6)	0.4 ± 0.1^{c} (0-2)
Eosinophil %	13.6 ± 1.3^{a} (5-32)	2.3 ± 0.3^{c} (1-6)	13.5 ± 1.3^{a} (6-26)	9.4 ± 1^{b} (2-18)
Monocyte %	7.5 ± 0.6^{a} (2-15)	5.3 ± 0.6^{b} (0-13)	4.3 ± 0.6^{b} (0-13)	3.3 ± 0.6^{b} (0-10)

similar to those reported for immunoglobulin levels among the *Pygoscelis* species, in which the levels were higher in gentoo penguins than in chinstrap and Adélie penguins, and this has been suggested to be related to a higher impact of parasites (Barbosa *et al*., 2007). Therefore, this agreement between both immune parameters could reflect common variation of parasites or diseases affecting both components of the immune response. The higher parasite loads could also explain the heterophil counts ($H_{3, 107}$ = 46.6, Multiple Comparisons, $P < 0.007$), and consequently the H/L ratios ($H_{3, 107}$ = 50.7, $P < 0.007$, Multiple Comparisons, $P < 0.007$) found in these species (tables 1 and 2). Furthermore, several kinds of stressors such as site temperature, severe heat, extreme exercise, food or water deprivation, contamination and/or exposure to novel social situations, can also cause an increase in H/L ratios (Davis *et al*., 2008 and references therein).

Magellanic penguins showed higher numbers of eosinophils ($H_{3, 107}$ = 54.5, Multiple Comparisons, $P < 0.007$) and monocytes ($H_{3, 107}$ = 35.5, Multiple Comparisons, $P < 0.007$) (table 1), which could be related to infestations of gastrointestinal parasites (Campbell, 1995) and Magellanic penguins exhibit more diverse and greater helminth parasite loads (table 2). Chinstrap penguins showed the highest lymphocyte counts ($H_{3, 107}$ = 42.7, $P < 0.007$) whereas gentoo penguins showed the lowest (Multiple Comparisons, $P < 0.007$, table 1). Raised lymphocyte counts could be indicative of a good

TABLE 2

Comparison of gastrointestinal parasite parameters between Antarctic pygoscelid penguins at Isla 25 de Mayo/King George Island and the non-Antarctic Magellanic penguins at Península Valdés. Data are from Díaz *et al*., 2010 and Díaz *et al*., 2013.
[Comparación de parámetros representativos de la carga de parásitos gastrointestinales entre pingüinos pigoscélidos antárticos en la isla 25 de Mayo/King George y el pingüino magallánico (no antártico) en Península Valdés. Según datos de Díaz et al.*, 2010 y Díaz* et al.*, 2013.]*

	Antarctic pygoscelid penguin			**Non-Antarctic penguin**
	Gentoo (*P. papua*) (N = 37)	Adélie (*P. adeliae*) (N = 9)	Chinstrap (*P. antarctica*) (N = 3)*	Magellanic (*S. magellanicus*) (N = 30)
Prevalence (%)	62	55	100	100
Mean abundance	22.6	9.7	64	1064
Mean intensity	36.3	17.6	64	1064
Species richness	4	2	3	5

* Data of parasites in chinstrap do not differ from data obtained in Deception Island with a higher sample size (N = 64) (Vidal *et al*., 2012).

state of health or, conversely, could indicate an immune response to a current infection (Davis *et al*., 2008). In both cases, more analyses of body condition should be addressed to establish the state of health.

Adélie penguins displayed the highest basophil counts among the penguin species in this study, which is difficult to explain (table 1). However, it has been reported that some stressors such as severe heat may cause variation in phagocyte counts (Davis *et al*., 2008) and that some birds respond to a stress situation, for example an abrupt increase in temperature, both with heteropenia (a fall in heterophils in blood) and significant basophilia (an increase in basophils in blood) (Maxwell and Robertson, 1995). In the present study, an extraordinary weather event consisting of extreme temperature fluctuations took place at the site during the Adélie sampling (Bases Argentinas: http://www.dna.gov.ar), leading to clutch losses because the snow melted and flooded the eggs (pers. obs.). This may explain why this population displayed unexpected high levels of basophils and also eosinophils ($H_{3, 107} = 46.5$ and $H_{3, 107} = 54.5$ respectively, Multiple Comparisons, $P < 0.007$, table 1). It is important to note that the Adélie population at Stranger Point has been declining dramatically over the past 15 years, probably due to the effect of climate warming in this region (Carlini *et al*., 2009), to which this species seems to be very sensitive.

Other factors, such as differences related to nesting behaviour or coloniality, which can affect immune investment (Møller and Erritzøe, 1996; Møller *et al*., 2001), may also explain the differences found. Whereas Magellanic penguins nest in burrows, the pygoscelid penguins breed in densely packed and open nests, which could facilitate parasite/pathogen transmission, resulting in a high immune investment (Møller *et al*., 2001). Infective agents that cause diseases (e.g. *Salmonella* sp., *Chlamydia* sp., Influenza, Paramyxovirus, Infectious Bursal Disease, Feather loss disorder) have been reported in all four penguin species studied (Barbosa and Palacios, 2009; Kane *et al*., 2010), although at present there is no information about interspecific differences in prevalence. Therefore, in this case, such factors do not seem to explain our results since we found differences in immune investment in the four species, but surely the intensity of infection may be variable and more important than presence/absence.

Finally, leucocyte counts can also vary in response to different factors intrinsic to the individuals, such as body condition or age. Although we have no data on body condition, it is unlikely that consistent interspecific differences in this variable exist and affect our results, as variation at this level is more relevant at the intraspecific level. In fact this is the case for humoral immunity as interspecific differences among Antarctic penguin species in immunoglobulin levels were not related to body mass (Barbosa *et al*., 2007). Regarding age, all individuals sampled were adult breeders and it is thus very unlikely that a bias towards younger birds in a species existed.

We realise that leucocyte counts inform only on the relative proportions of leucocyte types that are currently circulating in blood (Davis *et al*., 2008). They do not indicate if leucocytes are stored elsewhere in the body, or (without doing another smear after a stress paradigm) how many would be released or redistributed in response to a stressor or infectious agent (Davis *et al*., 2008). Leucocyte counts as described here can be used as a reference parameter for the four penguin species. We provide new data for leucocyte counts for pygoscelid Antarctic penguins and, for the first time, leucocyte counts for Magellanic penguins. These baseline data are important for future comparisons in order to assess likely changes produced by environmental changes in the region.

ACKNOWLEDGEMENTS.—We appreciate the hospitality and logistic support of the Argentinian Antarctic Base "Carlini" and Estancia San Lorenzo in Península Valdés, Argentina. We thank the Spanish Polar Ship "Las Palmas" which provided us with transport to Antarctica. We thank the logistic support provided by the Instituto Antártico Argentino. Permissions to work were given by Dirección Nacional del Antártico, the Spanish Polar Committee, and Government of Chubut. We are grateful to J. Benzal, B. Fussaro, L. Longarzo, M. Santos, M. Libertelli, M. Gasco, W. Cóppola, C. Villanueva and A. Varisco for their invaluable help during fieldwork. We thank M. G. Palacios for language revision of the manuscript. We are also grateful to the reviewers for their valuable suggestions. This study was funded by the Spanish Ministry of Science and Innovation and European Regional Developments Fund by project CGL2007-60369. V.L.D. had a CONICET Postdoctoral fellowship and V.V.B. was supported by a doctoral grant from the Spanish Council of Scientific Research at the time of this study. This study is a contribution to the International Polar Year Birdhealth project, and to the following projects: PINGUCLIM II and "Evaluación del turismo en una colonia reproductiva de pingüinos de Magallanes (*Spheniscus magellanicus*) en Península Valdés, Chubut".

BIBLIOGRAPHY

BARBOSA, A., MERINO, S., BENZAL J., MARTÍNEZ, J. and GARCIA-FRAILE, S. 2007. Geographic variation in immunoglobulin levels in pygocelid penguins. *Polar Biology*, 30: 219-225.

BARBOSA, A. and PALACIOS, M. J. 2009. Health of Antarctic birds: a review of their parasites, pathogens and diseases. *Polar Biology*, 32: 1095-1115.

BERTELLOTTI, M. 2014. *Pingüino de Magallanes: Embajador de la Patagonia*. Vázquez Manzini. Buenos Aires.

BOERSMA, D. 2008. Penguins as marine sentinels. *BioScience,* 58: 597-607.

CAMPBELL, T. W. 1995. *Avian Hematology and Cytology*. Iowa State University Press. Ames, Iowa.

CARLINI, R., CORIA, N. R., SANTOS, M. M., NEGRETE, J., JUARES, M. A. and DANERI, G. A. 2009. Responses of *Pygoscelis adeliae* and *P. papua* populations to environmental changes at Isla 25 de Mayo (King George Island). *Polar Biology*, 32: 1427-1433.

DAVIS, A. K., MANEY, D. L. and MAERZ, J. C. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22: 760-777.

DEHNHARD, N., POISBLEAU, M., DEMONGIN, L. and QUILLFELDT, P. 2011. Do leucocyte profiles reflect temporal and sexual variation in body condition over the breeding cycle in southern rockhopper penguins? *Journal of Ornithology*, 152: 759-768.

DÍAZ, J. I., CREMONTE, F. and NAVONE, G. 2010. Helminths of the Magellanic penguin, *Spheniscus magellanicus* (Sphenisciformes), during the breeding season in Patagonian coast, Chubut, Argentina. *Comparative Parasitology*, 77: 172-177.

DÍAZ, J. I., FUSARO, B., LONGARZO, L., CORIA, N. R., VIDAL, V., JEREZ, S., ORTIZ, J. and BARBOSA, A. 2013. Gastrointestinal helminths of gentoo penguins (*Pygoscelis papua*) from Stranger Point, 25 de Mayo/King George Island, Antarctica. *Parasitology Research*, 112: 1877-1881.

FARGALLO, J. A., LAAKSONEN, T., POYRI, V. and KORPIMÄKI, E. 2002. Inter-sexual differences in the immune response of Eurasian kestrel nestlings under food shortage. *Ecology Letters*, 5: 95-101.

HARVELL, C. D., MITCHELL, C. E., WARD, J. R., ALTIZER, S., DOBSON, A. P., OSTFELD, R. S. and SAMUEL, M. D. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science*, 296: 2158-2163.

HAWKEY, C., SAMOUR, H. J., HENDERSON, G. M. and HART, M. G. 1985. Haematological findings in captive gentoo penguins (*Pygoscelis papua*) with bumblefoot. *Avian Pathology*, 14: 251-256.

HORROCKS, N. P. C., HEGEMANN, A., MATSON, K. D., HINE, K., JAQUIER, S., SHOBRAK, M., WILLIAMS, J. B., TINBERGEN, J. M. and TIELEMAN, B. I. 2012. Immune indexes of larks from desert and temperate regions show weak associations with life history but stronger links to envi-

ronmental variation in microbial abundance. *Physiological and Biochemical Zoology*, 85: 504-515.

KANE, O. J, SMITH, J. R., BOERSMA, D. P., PARSONS, N. J., STRAUSS, V., GARCIA-BORBOROGLU, P. and VILLANUEVA, C. 2010. Feather-loss Disorder in African and Magellanic Penguins. *Waterbirds*, 33: 415-421.

LESSELLS, C. M. and BOAG, P. T. 1987. Unrepeatable repeatabilities: a common mistakes. *The Auk*, 104: 116-121.

MAXWELL, M. H. and ROBERTSON, G. W. 1995. The avian basophilic leukocyte: a review. *World's Poultry Science Journal*, 51: 307-325.

MAXWELL, M. H. and ROBERTSON, G. W. 1998. The avian heterophil leukocyte: a review. *World's Poultry Science Journal*, 54: 155-178.

MØLLER, A. P. and ERRITZØE, J. 1996. Parasite virulence and host immune defense: host immune response is related to nest re-use in birds. *Evolution*, 50: 2066-2072.

MØLLER, A. P. and ERRITZØE, J. 2003. Climate, body condition and spleen size in birds. *Oecologia*, 442: 621-626.

MØLLER, A. P., MERINO, S., BROWN, C. R. and ROBERTSON, R. J. 2001. Immune defense and host sociality: a comparative study of swallows and martins. *American Naturalist*, 158: 136-145.

NORRIS, K. and EVANS, M. R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology*, 11: 19-26.

PALACIOS, M. G., CUNNICK, J., WINKLER, D. and VLECK, C. M. 2007. Immunosenescence in some but not all immune components in a free-living vertebrate, the tree swallow. *Proceedings of the Royal Society of London B*, 274: 951-957.

RABASSA, J. 2010. El cambio climático global en la Patagonia desde el viaje de Charles Darwin hasta nuestros días. *Revista de la Asociación Geológica Argentina*, 67: 139-156.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, 43: 223-225.

ROITT, I., BROSTOFF, J. and MALE, D. 2001. *Immunology*. Mosby. London.

TURNER, J., COLWELL, S., MARSHALL, G., LACHLAN-COPE, T., CARLETON, A., JONES P., LAGUN, V., REID P. and IAGOVKINA, S. 2005. Antarctic climate change during the last 50 years. *International Journal of Climatology*, 25: 279-294.

VIDAL, V., ORTIZ, J., DÍAZ, J. I., RUIZ DE YBÁÑEZ, M. R., AMAT, M., PALACIOS, M. J., BENZAL, J., VALERA, F., DE LA CRUZ, C., MOTAS, M. and BARBOSA, A. 2012. Gastrointestinal parasites in chinstrap penguins from Deception Island, South Shetlands, Antarctica. *Parasitology Research*, 111: 723-727.

VLECK, C. M., VERTALINO, N., VLECK, D. and BUCHER, T. 2000. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adélie penguins. *Condor*, 102: 392-400.

ZINSMEISTER, V. A. P. and VAN DER HEYDEN, M. J. N. 1987. Differential leucocyte counts from Pygoscelid penguins of Antarctica. *Journal of Wildlife Diseases*, 23: 521-523.

ZUK, M. and STOEHR, A. M. 2002. Immune defense and host life history. *American Naturalist*, 160: 9-22.

Received: 15 July 2013
Accepted: 10 March 2014

Editor: Carola Sanpera