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Abstract

Southern Rockhopper Penguins (*Eudyptes chrysocome chrysocome*) have a strongly synchronised breeding cycle with a fixed pattern of nest attendance for males and females. We studied leucocyte profiles and the development of granulocyte/lymphocyte (G/L) ratios as an indicator of stress. Variation in G/L ratios were related to sex and breeding stage, but not individual body condition. G/L ratios were similar for males and females during the first part of the incubation period ("shared incubation", when males and females both attend the nest), but in the second part of the incubation ("single incubation", only one adult attends the nest), females had significantly higher G/L ratios and lower body condition than males. The lowest G/L ratios were registered during the crèche of the chicks at the end of the breeding season. Our results show that G/L ratios in breeding southern rockhopper penguins on the population-scale reflect the temporally and sexually different timing of fasting and refeeding related to the breeding cycle. However, this measurement was not subtle enough to reveal an effect of body condition on G/L ratios on an individual scale.

Keywords: Southern rockhopper penguin, breeding biology, Falkland Islands, immunology, G/L ratios, body condition, stress

Zusammenfassung

Spiegeln Leukozyten-Profile zeitliche und sexuelle Variation in der Körperkondition während des Brutzyklus des südlichen Felsenpinguins wider?

Südliche Felsenpinguine (*Eudyptes chrysocome chrysocome*) haben einen stark synchronisierten Brutzyklus. Während der Inkubation und Kükenaufzucht wechseln sich Männchen und Weibchen am Nest in einem festgelegten Muster ab. Wir untersuchten Leukozyten-Profile und die Entwicklung des Granulocyten/Lymphozyten- (G/L) Verhältnisses als Indikator von Stress. Das G/L Verhältnis war abhängig von Geschlecht und Brutphase (Inkubation, Kükenaufzucht), aber nicht individueller

Körperkondition. Männchen und Weibchen hatten ähnliche G/L Verhältnissein der ersten Phase der Inkubation ("gemeinsame Inkubation", wenn Männchen und Weibchen gemeinsam das Nest bewachen und die Eier bebrüten).Weibchen hatten signifikant höhere G/L Verhältnisse und geringere Körperkondition als Männchen in der zweiten Phase der Inkubation ("einzelne Inkubation", nur ein Elternteil bleibt am Nest). Wir fanden die niedrigsten G/L Verhältnisse während der Crèche (Küken bleiben tagsüber allein und formen kleine Gruppen, sog. Crèches) am Ende des Brutzyklus. Unsere Ergebnisse zeigen, dass das G/L Verhältnis das zeitliche Muster von Fasten und Gewichtszunahme während des Brutzyklus für Männchen und Weibchen des südlichen Felsenpinguins auf Populationsebene gut widerspiegelt. Auf individueller Ebene war diese Methode jedoch nicht subtil genug um einen Effekt von Körperkondition auf das G/L Verhältnis nachzuweisen.

Introduction

During the breeding period, parental birds must cope with high energetic demands, resulting in a trade-off between reproduction and self-maintenance. This trade-off can also influence investment into the immune system (e.g. Sheldon & Verhulst 1996; Lee 2006). As the defence mechanism against pathogens and parasites, the immune system is necessary for the health and survival of animals, but it can be suppressed when the resources are needed for other body maintenance functions (Davis et al. 2008; Lee 2006).

Leucocytes, or white blood cells, are the main element of the immune system. Depending on their function, they are attributed either to the innate or to the acquired arm of the immune system (Roitt et al. 1993). The innate immunity works predominantly by means of phagocytosis and provides initial protection against a variety of different pathogens (Maxwell & Robertson 1998; Roitt et al. 1993). It is based on monocytes and three different types of granulocytes (heterophils, eosinophils and basophils), which react to different pathogens. Heterophils, the most frequent type of granulocytes, increase during inflammatory processes, chronic stress, and infection (Maxwell & Robertson 1998; Coles 1997). The acquired immunity is more pathogen-specific and works by means of cell-mediated and humoral responses of lymphocytes (Roitt et al. 1993). Under nutritional stress, animals have been found to suppress their cell-mediated immunity, leading to a decrease of lymphocyte concentrations (Gross & Siegel 1983; Hõrak et al. 1998a).

Since heterophils (H) usually increase with stress while lymphocytes (L) decrease in the same conditions, the ratio of heterophils to lymphocytes (H/L ratio) is often a useful indicator of stress (Gross & Siegel 1983; Ots & Hõrak 1996; Davis et al. 2008). Elevated H/L ratios have been found to be related to a range of stressors and energetically demanding phases, such as long-distance migration (Owen & Moore 2006), injuries (Vleck et al. 2000), and parasitic infection (Lobato et al. 2005). H/L ratios have also been found to differ between

80 males and females (Hõrak et al. 1998b) and between chicks and adults (Quillfeldt et al. 2008
81 and references therein) in some bird species. As heterophils are the most frequent type of
82 granulocytes (G) compared to eosinophils and basophils (e.g. Davis et al. 2008), the ratio of
83 granulocytes to lymphocytes (G/L ratio) can also be used instead of the H/L ratio (e.g. Hoi-
84 Leitner et al. 2001). For example, a high G/L ratio was also reported to reflect starvation in
85 serin (*Serinus serinus*) chicks (Hoi-Leitner et al. 2001).

86 As infections and diseases lead to an increase of leucocytes, a condition called
87 leucocytosis (Fudge 1989), the ratio of leucocytes to erythrocytes (red blood cells) might
88 provide additional information on the immune status. In fact, leucocyte numbers per 10,000
89 erythrocytes have been used as a measure of health status in reproducing great tits (*Parus*
90 *major*; Ots et al. 1998) and Magellanic penguins (*Spheniscus magellanicus*; Moreno et al.
91 2002).

92 Moreover, the interpretation of H/L ratios sometimes necessitates the estimation of
93 leucocyte numbers per erythrocytes. For example, Masello et al. (2009) observed a positive
94 correlation between body condition and the H/L ratio in wild burrowing parrot (*Cyanoliseus*
95 *patagonus*) nestlings: nestlings in better body condition invested more energy into their innate
96 immunity, which led to an increase in the number of heterophils. In contrast, the number of
97 lymphocytes was independent of body condition.

98 Thus, leucocyte profiles can help to understand how animals distribute limited
99 resources between the different arms of the immune system. Yet, data of free-living birds,
100 including penguins, are still scarce. Even though there are reference haematological values for
101 some penguin species in the wild (Hawkey et al. 1989; Karesh et al. 1999; Nicol et al. 1988),
102 immunological findings are rarely related to ecology or breeding stages (Merino & Barbosa
103 1997; Moreno et al. 1998, 2002; Sergent et al. 2004; Vleck et al. 2000). However, for
104 penguins, while both parents participate in incubation, brooding and feeding (Williams 1995),

105 they also show different investment during the breeding stages. Thus, it is likely that sexual
106 differences in haematological values represent different parental effort.

107 Penguins invest heavily in each breeding attempt, from their arrival at the colony until
108 the departure of the fledgling. Breeding adults undergo fasting and a serious loss of body
109 weight during incubation (Williams 1995). Indeed, correlations between body condition and
110 haematological values were found in wild little penguins (*Eudyptula minor*) (Sergent et al.
111 2004). Vleck et al. (2000) reported differences in H/L ratios between different breeding stages
112 in Adélie penguins (*Pygoscelis adeliae*). Additionally, reproductive output has been linked
113 with health status (evaluated by counting leucocyte numbers per 10,000 erythrocytes) in
114 Magellanic penguins (Moreno et al. 2002).

115 The breeding cycles of crested penguins (genus *Eudyptes*) include long periods of both
116 parents at the nest, during which they fast alternatively for several weeks (Warham 1972). In
117 southern rockhopper penguins (*Eudyptes chrsysocome chrysocome*), males arrive at the
118 colony about 3-4 weeks before laying, and about 10 days before females (Fig. 1; Strange
119 1982). Both parents stay at the colony until laying and during the first incubation ("shared
120 incubation") stage. About 10 days after laying, males leave the colony for a 7-10 day feeding
121 trip while the females stay at the nests alone ("female single incubation" stage) (Strange
122 1982). After the return of the males, females leave for a feeding trip ("male single incubation"
123 stage) and return by the time the young hatch (Strange 1982). The chicks are initially brooded
124 continuously by the males while the females feed them every 1-2 days until they are about
125 three weeks old. Then chicks are no longer guarded permanently and form crèche groups
126 ("crèche" stage). They are fed by both parents in regular intervals of usually less than two
127 days until they fledge at about 10 weeks old (Williams 1982; Raya Rey et al. 2007).

128 As rockhopper penguins show a strongly synchronised breeding cycle with long
129 alternating fasting periods, they provide a good model to test whether parental care is equally
130 demanding for males and females, and whether there are differences between the breeding

stages. We studied leucocyte profiles and granulocyte/lymphocyte (G/L) ratios in adult southern rockhopper penguins on New Island, Falkland Islands, to investigate if G/L ratios reflect the sexually different temporal patterns of fasting and refeeding during breeding. Thereby, we interpret the G/L ratio as an indicator of persistent stressors during the breeding season (Vleck et al. 2000), especially in reference to energetic demands.

Specifically, we expected to find (1) a negative correlation between individual body condition and G/L ratios for both sexes, (2) little to no sex differences in immunological parameters and body condition during crèche but (3) lower body condition and higher G/L ratios in females compared to males during single incubation due to the possibility of foraging for males but not females prior to this incubation period. We had no clear expectations of G/L ratios for the shared incubation phase, as males had fasted longer, but females had produced eggs.

Material and Methods

Study area and study species

Field work was conducted in the "Settlement Colony" on New Island, Falkland/Malvinas Islands (51°43'S, 61°17'W) between October 2006 and February 2007 as part of an ongoing study on southern rockhopper penguins (Poisbleau et al. 2008; 2009a; 2009b). The colony holds about 5,000 breeding pairs of southern rockhopper penguins. We captured males and females during three stages of their breeding cycle: shared incubation, single incubation, and crèche. Birds were captured from their nests by hand. To minimize disturbance, the bird's head was covered during the measurements. Blood samples were collected immediately (end of the sampling within 3 minutes after capture) from the brachial vein with a 1 ml heparinised syringe and a 25 gauge needle. Flipper length (extended from axilla) was measured with a ruler to the nearest millimeter. Bill length (exposed culmen) and bill depth (at junction of gonys and inter-ramal region) were measured to the nearest 0.1 mm using callipers (following Poisbleau et al. in press). Penguins were weighed with an electronic balance to the nearest 20g. Sex was determined using morphological measurements (Poisbleau et al. in press). Following Ruiz et al. (2002), one drop of blood was smeared on a glass slide and air-dried. Samples were later fixed with methanol (100%) and stained with Giemsa prior to counting.

Leucocyte counts

Blood smears were scanned with a light microscope (1,000x, oil immersion) for a monolayer of blood cells. Differential leucocyte counts were accomplished along the short-axis of the slide to prevent differences in thickness of blood cells, following previous authors (e.g. Merino et al. 1999, Masello et al. 2009). A minimum of 100 leucocytes were counted per slide and distinguished as granulocytes, lymphocytes, and monocytes following the criteria of Hawkey & Dennett (1989). We could not clearly distinguish between eosinophils and

heterophils, therefore all heterophils, eosinophils, and basophils were classified as granulocytes. Relative numbers of each leucocyte type were calculated as the percentage of all leucocytes ("relative leucocyte numbers"). Leucocyte numbers per 10,000 erythrocytes were calculated by counting the number of all erythrocytes in one microscopic visual field and multiplying it with the number of the microscopic visual fields that were scanned until reaching 100 leucocytes, following Lobato et al. (2005), who found a high repeatability of this method.

Statistics and data evaluation

Statistical tests were performed with SPSS 11.0. Data were tested for normality (Kolmogorov-Smirnov tests) and homogeneity of variances (Levene's test). Data of G/L ratios, granulocytes [%], granulocytes per 10,000 erythrocytes, and lymphocytes per 10,000 erythrocytes were ln transformed. Monocytes per 10,000 erythrocytes were ln transformed after adding 1, as they contained zero values. Means are given with standard errors throughout.

Body condition was calculated separately for males and females as they differ in size (e.g. Warham 1972, Strange 1982). We used a multiple linear regression of body mass as the dependent variable; flipper length, bill length and bill depth as predictors ($N = 42$, $R = 0.505$, $F = 4.334$ and $P = 0.010$ for males and $N = 44$, $R = 0.488$, $F = 4.175$ and $P = 0.012$ for females). The resulting regression equations were taken to calculate the expected body mass for males = 48.19*(flipper length) + 28.24*(bill length) – 120.71*(bill depth) – 4370.25, and for females = 9.89*(flipper) + 18.69*(bill length) + 115.06*(bill depth) – 1795.12. Body condition was then calculated as the residual between the observed and expected body mass.

The data set contained samples taken during shared incubation (14 females, 14 males), single incubation (15 females, 14 males) and crèche (15 females, 14 males). Each individual was represented in the dataset only once to ensure independent data. We used General Linear

196 Models (GLMs) to test for differences in leucocyte parameters between the three breeding
197 stages. Initially, we included both sexes into one GLM and tested for the influence of sex
198 (fixed factor), breeding stage (fixed factor) and body condition (covariate) as well as the
199 interactions on leucocyte parameters (as dependent variable). This resulted in a significant
200 interaction between sex and breeding stage. As main effects cannot be interpreted when
201 interactions are significant (see e.g. McDonald 2008), we had to separately analyse the effects
202 for males and females. Therefore, we tested the influence of "breeding stage" (as a fixed
203 factor) and "body condition" (as a covariate) on differential leucocyte counts and G/L ratio
204 (as dependent variables) separately for males and females. GLMs were based on Type III Sum
205 of Squares and we give partial Eta-squared values (η^2) as a measurement of the effect size.
206 For significant relationships, we also present t-values to indicate the direction of covariate
207 relationship. Additionally, we conducted t-tests to test directly for differences in differential
208 leucocyte counts and body condition between males and females, separately for the different
209 breeding stages. However, multiple testing of essentially the same H0 and/or the same data set
210 required some error-level correction. We did this using Fisher's omnibus test. This procedure
211 combines a number of P-values into a single chi-square-distributed variable with its degrees
212 of freedom equaling twice the number of P-values (Haccou and Meelis 1994, Quinn and
213 Keough 2003). Thus, we conducted Fisher's omnibus tests for each breeding stage separately,
214 including all leucocyte parameters.
215
216

Results

Development of body condition over the breeding cycle

Body condition differed between breeding stages in both sexes (Fig. 2; ANOVA for differences between breeding stages: $F_{2,42}$ = 25.2 and P < 0.001 for males, and $F_{2,44}$= 32.56 and P < 0.001 for females).

Body condition of females decreased from shared incubation towards single incubation and was highest during crèche. In comparison, body condition of males increased from shared incubation to single incubation and peaked during crèche (Fig. 2). Males were therefore in a lower body condition during shared incubation, but in a higher body condition during single incubation compared to females (t = -5.1 and 7.6, df = 26 and 27, P < 0.001 and < 0.001, respectively). During crèche, body condition was similar between the sexes (t = -0.6, df = 27, P = 0.584). For comparison, we present the changes in body mass for males and females in table 1.

Leucocyte profiles and body condition

Rockhopper penguins had slightly more lymphocytes than granulocytes, resulting in mean G/L ratios of 0.86 ± 0.40 (range 0.34 - 2.23, Table 2). Monocytes were less frequent.

Relative granulocytes and lymphocytes, as well as G/L ratios varied depending on breeding stage for both sexes (Tables 3 and 4). Even though body condition was found to be high in those stages when the G/L ratio was low (Figs. 2 and 3), individual body condition was not identified as a significant influence on leucocyte parameters except for monocytes in females (Tables 3 and 4). Indeed, monocyte counts (both relative and per 10,000 erythrocytes) were independent of breeding stage and body condition in males, but were negatively affected by body condition in females. In females, there was an interaction between body condition and breeding stage for lymphocytes per 10,000 erythrocytes, and granulocytes per 10,000 erythrocytes were related with breeding stage (Table 4). Granulocytes per 10,000 erythrocytes

were elevated in females during single incubation compared to females during crèche (ANOVA for differences within breeding stages: $F_{1,44} = 3.86$, P = 0.029).

Relative numbers of granulocytes and lymphocytes and G/L ratios did not differ between males and females during the shared incubation stage (Fig. 3; Fisher's omnibus test P = 0.673). With ongoing incubation period, females had increased granulocyte and decreased lymphocyte levels, while males showed the opposite trend (Fisher's omnibus test P = 0.012; Fig. 3). The resulting G/L ratios were higher in females than males in the single incubation stage (Fig. 3, $t = -2.2$, $df = 25.2$, $P = 0.037$; $N_{females} = 15$, $N_{males} = 14$). Females later decreased granulocytes and G/L ratios from the single incubation to the crèche stage, while the levels remained similar for males. During crèche, males had elevated G/L ratios compared to females (Fig. 3, $t = 2.3$, $df = 27$, $P = 0.029$; Fisher's omnibus test for crèche P = 0.005). This was mainly explained by lowered lymphocytes compared to females (Fig. 4; $t = -2.8$, $df = 27$, $P = 0.009$ for lymphocytes, and $t = 1.936$, $df = 27$, $P = 0.063$ for granulocytes; $N_{females} = 15$, $N_{males} = 14$; tests with ln-transformed data).

Relative numbers of monocytes did not change among breeding stages, but were generally higher in males than females, though not significantly (Fig. 3).

Discussion

In the present study, body condition differed significantly between sexes and breeding stages, and we observed associated changes in leucocyte parameters. In contrast, data of individuals reflected hardly any influence of body condition on leucocyte parameters. Instead, breeding stage alone explained most of the variation in G/L ratios, relative counts of lymphocytes and granulocytes. In the rockhopper penguins, G/L ratios were not sufficiently sensitive or did not change quickly enough to reveal an effect of body condition on G/L ratios on an individual scale.

Absolute leucocyte counts per 10,000 erythrocytes

Total leucocytes per 10,000 erythrocytes were independent of any tested factor and remained stable throughout the breeding season. This indicates that influences of increased energetic demand during the breeding season in rockhopper penguins are mainly traded off between the different leucocyte types, while for absolute leucocyte counts only granulocytes/10,000 erythrocytes were affected by breeding stage in females.

This is in a contrast to sex differences not only in relative leucocyte numbers, but also lymphocytes per 10,000 erythrocytes and total leucocytes/10,000 erythrocytes found in breeding Magellanic penguins (Moreno et al. 2002). These results could indicate that breeding stages have a more serious impact on the health status, including leucocytosis, in Magellanic penguins compared to rockhopper penguins.

G/L ratio and relative leucocyte counts

As predicted, the results of relative leucocyte numbers and G/L ratios revealed significantly higher G/L ratios and therefore higher stress in females compared to males during single incubation. Moreover, G/L ratios were higher during shared incubation compared to crèche for both sexes. Contrasting these results with the time scale of the breeding cycle and the

287 body condition, G/L ratios well reflect the burden related to the different activities of males
288 and females during the distinct breeding stages.
289
290 During shared incubation, both males and females stay at the nest permanently and therefore
291 do not forage. This explains high G/L ratios in both sexes due to fasting and low body
292 condition, but maybe also inter- and intraspecific competition and aggression within the
293 colony. As males arrive in the colony about 10 days earlier than females and defend their nest
294 territory more aggressively than females (Strange 1982), one could expect higher stress levels
295 in males due to longer fasting, higher activity, and a significantly lower body condition than
296 females. Despite this, G/L ratios of males and females during shared incubation were similar.
297 Potentially, females are energetically strained by producing and laying two eggs and therefore
298 have elevated G/L ratios, similar to those of males despite a better body condition. The
299 differences might also reflect sex differences in hormonal regulation of parental effort versus
300 self-maintenance independently of body condition, for example differences among males and
301 females in prolactin levels (e.g. Riou et al. in press).
302
303 During single incubation, G/L ratios of females increased and were higher than during shared
304 incubation. By this time, females had already been fasting for about four weeks, resulting in a
305 low body condition and elevated G/L ratios. When males were sampled in their following
306 single incubation stage, they had recently returned from a foraging trip and hence were in a
307 good nutritional stage. This could explain low G/L ratios and the significant differences in
308 G/L ratios and body condition compared to the females during single incubation.
309
310 Although G/L ratios were significantly higher in males than in females during crèche, the G/L
311 ratios in both sexes were very low compared to the previous breeding stages. As the crèche
312 period offers the possibility of regular foraging for both genders, the nutritional status and

313 therefore body condition of adults improved compared to the previous breeding stages. This
314 elevation in body condition could also explain the low G/L ratios. The sexual difference in the
315 G/L ratio during crèche might be related to the previous guard stage. During the
316 approximately 3 weeks of guarding, males attend the nest and fast, while females forage daily
317 to feed the chicks. On closer examination, high G/L ratios in males during crèche were caused
318 by significantly lowered lymphocytes, not granulocytes (Fig. 3). As lymphocytes have a
319 lifespan of up to 200 days (Reece 2004), low lymphocyte numbers during crèche could still
320 reflect immunosuppression and therefore an extra strain for males during guard.
321
322 *G/L ratio and body condition*
323 Although the G/L ratio appeared to reflect changes in body condition throughout the breeding
324 stages in both sexes, we could not verify a significant correlation between the G/L ratio and
325 individual body condition. This is in contrast to findings in northern goshawks (*Accipiter*
326 *gentilis*; Hanauska-Brown et al. 2003) and burrowing parrots (Plischke et al. 2010), but in
327 agreement with results from Quillfeldt et al. (2008) in thin-billed prions (*Pachyptila belcheri*).
328
329 *Leucocyte profiles as a measure of the parental demand*
330 Breeding season has been shown to affect immunological parameters in several bird species,
331 sometimes with different effects for each sex. However, there is no consistent trend of the
332 G/L or H/L ratio with ongoing breeding season within the studied species. While the H/L ratio
333 increases over the breeding season in thin-billed prions (Quillfeldt et al. 2008) and female
334 great tits (Hõrak et al. 1998b), Vleck et al. (2000) found the opposite for Adélie penguins. As
335 elevated H/L ratios imply high stress levels, they are likely to connote higher parental
336 demands, as suggested by Quillfeldt et al. (2008). For both Adélie and rockhopper penguins,
337 incubation is more demanding for parental birds than chick-rearing (Davis 1982). In addition
338 to the aspect of mass loss due to fasting, Vleck et al. (2000) named several more potential

reasons for higher demands and stress levels in adult breeding penguins during incubation compared to later breeding stages: During incubation, the nest site and eggs have to be defended against conspecifics and skuas (*Catharacta sp.*), respectively. In comparison, during crèche, chicks are large enough to be left alone, thus releasing parental penguins from staying in the colony permanently. This also leads to a lower density of penguins in the colony and thus less aggression (Vleck et al. 2000). Even the need to provide the chick with enough food during chick-rearing, allows for additional effort in foraging, it does not seem to compensate for these benefits. On the contrary, during foraging trips, adults can also maintain their own nutritional and water homeostasis (Vleck et al. 2000).

In contrast to penguins, courtship for parental thin-billed prions is less demanding than foraging for the chick and themselves during chick-rearing (Quillfeldt et al. 2008), which explains increasing H/L ratios with ongoing breeding season. The same argument applies to great tits, where females are more heavily affected than males (Hõrak et al. 1998b, Ots et al. 1998). The results from the present study are consistent with this overall picture, where the G/L ratio during the breeding season changes in respect to parental demands and body condition, and may differ between the sexes for the same reason, but as a rule does not increase or decrease with ongoing breeding season. Particularly the findings of Vleck et al. (2000), who reported elevated H/L ratios during incubation, are consistent with our results and reflect the similar breeding ecology of Adélie penguins and southern rockhopper penguins.

In summary, we were able to show that relative leucocyte numbers and the G/L ratio reflect the pattern of fasting and refeeding during the breeding cycle in southern rockhopper penguins. High G/L ratios coincided with periods of fasting and low body condition. Even though these relationships are correlational and lack causality, these results indicate that the G/L ratio provides a useful measurement of long-termed stress related to the breeding cycle.

365

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Table 1. Body mass (in g) in 86 free-living adult southern rockhopper penguins throughout the breeding season. N = 14 for each breeding stage in males, 14 for females during shared incubation, and 15 for females during single incubation and crèche, respectively. Sampling dates for the different breeding stages are provided in column 1.

Breeding Stage	**Males**		**Females**	
	Mean ± SE	Range	Mean ± SE	Range
Shared incubation 6.11.-17.11.2006	2603 ± 33	2340 - 2760	2706 ± 58	2280 - 2980
Single incubation Females: 17-25.11.2006 Males: 30.11.-5.12.2006	3318 ± 38	3040 - 3500	2384 ± 56	2080 - 2960
Crèche 3.1.-4.2.2007	3084 ± 118	2180 - 3940	2988 ± 48	2700 - 2988

483 **Table 2.** G/L ratios and differential leucocyte counts of 86 free living adult southern
484 rockhopper penguins (42 males and 44 females).

485

	Mean ± SE	Range
G/L ratio	0.86 ± 0.43	0.34 – 2.23
Granulocytes [%]	42.48 ± 1.04	24.00 – 67.65
Lymphocytes [%]	53.59 ± 1.04	30.39 – 71.00
Monocytes [%]	3.93 ± 0.28	0.00 – 13.00
Granulocytes/10,000 erythrocytes	33.26 ± 1.31	11.39 – 70.94
Lymphocytes/10,000 erythrocytes	42.87 ± 1.92	13.40 – 108.39
Monocytes/10,000 erythrocytes	3.03 ± 0.24	0.00 – 9.74
Total leucocytes/10,000 erythrocytes	79.15 ± 2.82	35.56 – 171.33

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Table 3. Results of GLMs with independent data, showing the effects of breeding stage and body condition on leucocyte profiles in adult male southern rockhopper penguins. Leucocyte parameters were used as dependent, breeding stage as fixed factor, and body condition as covariate. η^2 was included to indicate the effect size. N = 42, df = 2.

Parameter	Breeding stage (BS)	Body condition (BC)	Interaction (BC*BS)
G/L ratio*	F=5.242, **P=0.010**, η^2=0.226	F=2.772, P=0.105, η^2=0.071	F=1.993, P=0.151, η^2=0.100
Granulocytes [%]*	F=4.353, **P=0.020**, η^2=0.195	$F_{2,42}$=2.077, P=0.158, η^2=0.182	F=1.785, P=0.182, η^2=0.090
Lymphocytes [%]	F=5.809, **P=0.007**, η^2=0.244	F=2.163, P=0.130, η^2=0.081	F=2.163, P=0.130, η^2=0.107
Monocytes [%]	F=0.327, P=0.723, η^2=0.018	F=0.181, P=0.673, η^2=0.005	F=0.191, P=0.827, η^2=0.011
Granulocytes/ 10000 erythrocytes*	F=0.641, P=0.533, η^2=0.034	F=1.122, P=0.296, η^2=0.030	F=0.275, P=0.761, η^2=0.015
Lymphocytes/ 10000 erythrocytes*	F=2.111, P=0.136, η^2=0.105	F=0.213, P=0.647, η^2=0.006	F=2.247, P=0.120, η^2=0.111
Monocytes/ 10000 erythrocytes*	F=0.344, P=0.711, η^2=0.019	F=0.104, P=0.749, η^2=0.003	F=0.044, P=0.957, η^2=0.002
Total Leucocytes/ 10000 erythrocytes	F=0.711, P=0.498, η^2=0.038	F<0.001, P=0.983, η^2<0.001	F=1.813, P=0.178, η^2=0.092

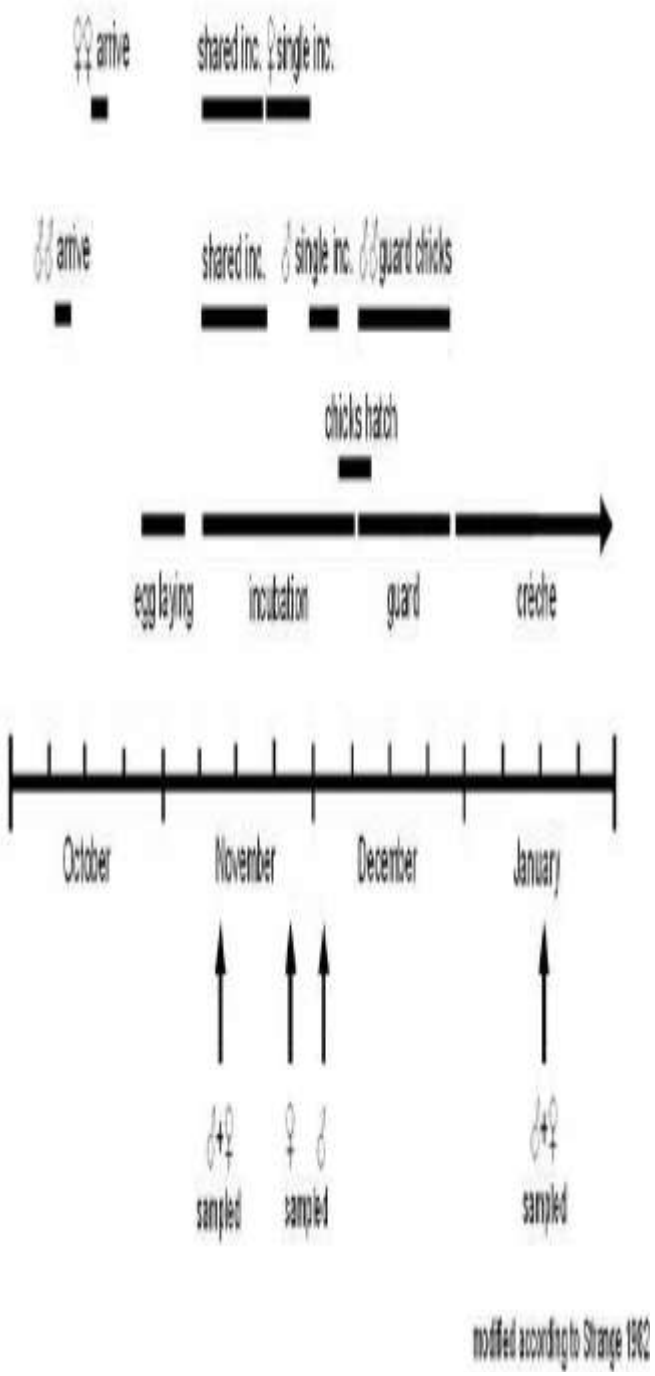
* transformed data. G/L ratio, Granulocytes [%], Granulocytes/10,000 erythrocytes and Lymphocytes/10,000 erythrocytes were transformed using the natural logarithm, Monocytes/10,000 erythrocytes were transformed ln(1+monocytes/10,000 erythrocytes).

Table 4. Results of GLMs with independent data showing the effects of breeding stage and body condition on leucocyte profiles in adult female southern rockhopper penguins. Leucocyte parameters were used as dependent, breeding stage as fixed factor, and body condition as covariate. η^2 was included to indicate the effect size. If P-values revealed significance, we additionally give the t-value to indicate the direction of the relationship. N = 44, df = 2.

Parameter	Breeding stage (BS)	Body condition (BC)	interaction (BC*BS)
G/L ratio*	F=3.588, **P=0.037**, η^2=0.159	F=2.470, P=0.124, η^2=0.061	F=2577, P=0.089, η^2=0.119
Granulocytes [%]*	$F_{2,44}$=3.298, **P=0.048**, η^2=0.148	F=2.453, P=0.126, η^2=0.061	F=2.672, P=0.082, η^2=0.123
Lymphocytes [%]	F=3.424, **P=0.043**, η^2=0.153	F=1.645, P=0.207, η^2=0.041	F=2.537, P=0.092, η^2=0.118
Monocytes [%]	F=0.954, P=0.394, η^2=0.048	F=6.332, **P=0.016**, t=-1.172, η^2=0.143	F=0.112, P=0.895, η^2=0.006
Granulocytes/ 10000 erythrocytes*	F=3.528, **P=0.039**, η^2=0.157	F=0.806, P=0.375, η^2=0.021	F=1.035, P=0.365, η^2=0.052
Lymphocytes/ 10000 erythrocytes*	F=1.428, P=0.252, η^2=0.070	F=0.430, P=0.516, η^2=0.011	F=4.694, **P=0.015**, η^2=0.198
Monocytes/ 10000 erythrocytes*	F=1.273, P=0.292, η^2=0.063	F=5.424, **P=0.025**, t=-0.591, η^2=0.125	F=0.461, P=0.634, η^2=0.024
Total Leucocytes/ 10000 erythrocytes	F=1.238, P=0.301, η^2=0.061	F=0.058, P=0.812, η^2=0.002	F=2.781, P=0.075, η^2=0.128

* transformed data. G/L ratio, Granulocytes [%], Granulocytes/10,000 erythrocytes and Lymphocytes/10,000 erythrocytes were transformed using the natural logarithm, Monocytes/10,000 erythrocytes were

509 **Fig. 1.** Overview of the attendance patterns of males and females in the colony during the
510 breeding season. Arrows indicate when samples were collected. Note that the samples during
511 crèche were taken over a period of 4 weeks.



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513 **Fig. 2.** Body condition (means ± standard errors) of adult male and female rockhopper
514 penguins during shared incubation, single incubation and crèche stages. N= 14, 15, 15 for

females during shared incubation, single incubation and crèche, respectively, and 14 for males for all three breeding stages. Body condition was calculated separately for males and females, using residuals of a linear regression. Results from t-tests for differences between males and females within each breeding stage are included in the graph. * for $P < 0.05$, ** for $P < 0.01$, n.s. for not significant values.

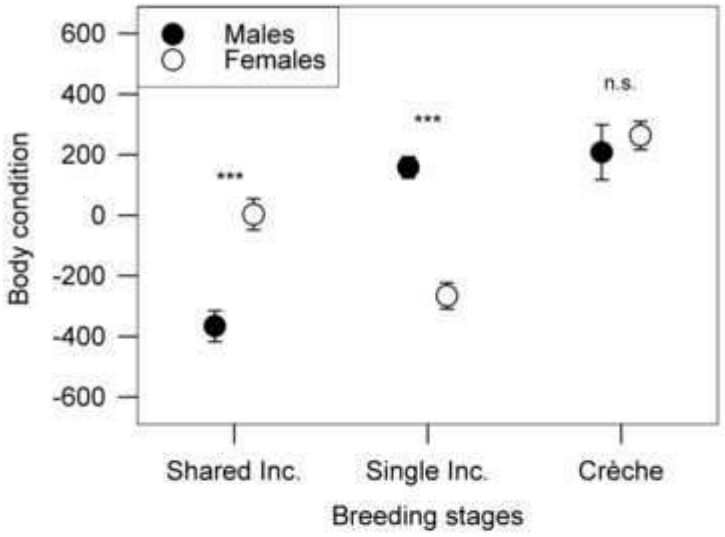

Fig. 3. G/L ratios and leucocyte types (means ± standard errors) of adult southern rockhopper penguins during shared incubation, single incubation and crèche stages. Values for males are shown with filled circles, values of females with open circles. Sample sizes as in Fig. 2. Results from t-tests for differences between males and females within each breeding stage are included in the graph. * for $P < 0.05$, ** for $P < 0.01$, n.s. for not significant values.