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1 Leucocytes in adult Burrowing Parrots *Cyanoliseus patagonus* in the wild: variation between
2 contrasting breeding seasons, gender and individual condition

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14

15 **Abstract**

16 Wild birds exposed to stressors may modulate their investment into immunity. We studied the
17 leucocytes of breeding burrowing parrots (*Cyanoliseus patagonus*) in Patagonia during five
18 breeding seasons, during which global climate events such as a strong La Niña and a weak El
19 Niño occurred. We observed strong inter-annual variation in the ratio of heterophils to
20 lymphocytes (H/L), with higher H/L during the adverse conditions of La Niña compared to the
21 favourable conditions of El Niño for the studied region. Nevertheless, highest H/L were found
22 in the breeding season following a La Niña event, likely explained by a combination of long-
23 term detrimental effects of climatic conditions and other, e.g. biotic, stressors. Males had higher
24 H/L than females, and H/L ratios were negatively related to individual body condition.

25

26 **Keywords** body condition, blood cell counts, climatic conditions, El Niño Southern
27 Oscillation, ENSO, H/L ratio, Psittaciformes, sex differences

28

29 **Introduction**

30

31 Environmental stressors and their effects on individual growth, reproduction and pathogen
32 defence are important issues in ecological research and conservation biology. In this context, the
33 quantification of white blood cells (leucocytes) has become a useful tool in behavioural ecology.
34 In particular, the proportion of two types of leucocytes, heterophils and lymphocytes (*H/L*
35 ratio), has been used as an indicator of stress in birds (reviewed by Davis et al. 2008).

36 In response to exposure to various stressors, the relative number of heterophils
37 (phagocytosing cells of the innate immune system) in peripheral blood increases, while the
38 relative number of lymphocytes (part of the acquired immune system) decreases (Gross and
39 Siegel 1983; Maxwell 1993). These effects, mediated by glucocorticoids (mainly corticosterone
40 in birds), are seen in all five vertebrate taxa in response to either natural stressors or exogenous
41 administration of stress hormones (Siegel 1985; see also Davis et al. 2008). Therefore, high *H/L*
42 ratios in blood samples can reliably indicate high glucocorticoid levels and provide a reliable
43 assessment of stress in all vertebrate taxa (Davis et al. 2008). Obtaining *H/L* ratios from blood
44 smears has important advantages compared to hormone assays: (1) *H/L* ratios are not influenced
45 by handling times up to one hour (Davis 2005), (2) the method can easily be added to standard
46 sampling procedures in the field, as very small amounts of blood are needed, and (3) counting
47 leucocytes from blood smears is an inexpensive and repeatable method (see Davis et al. 2008).

48 Baseline information on leucocyte profiles from wild birds is scarce (but see e.g.
49 Merino et al. 1999; Vleck et al. 2000; Garamszegi et al. 2006; Owen and Moore 2006;
50 Quillfeldt et al. 2008). In Psittaciformes, haematology values, with a few exceptions (Joyner et
51 al. 1992; Karesh et al. 1997; Sheridan et al. 2004; Deem et al. 2005; Low et al. 2006; Tompkins
52 et al. 2006; Masello et al. 2009), correspond to captive birds (see Masello et al. 2009 and
53 references therein). Captivity, however, has been shown to influence avian leucocyte counts
54 significantly (Ewenson et al. 2001).

55 An important source of environmental stress affecting wild birds in South America is
56 the El Niño Southern Oscillation (ENSO). El Niño (warm) episodes are usually associated with
57 excess of rains in certain parts of the globe, while it is associated with deficit of rains in others

58 (e.g. Ropelewski and Halpert 1987). El Niño episodes of ENSO are often followed by La Niña
59 (cold) episodes, which roughly produce the opposite climate patterns of those observed during
60 El Niño. In southern South America, La Niña is associated mostly with droughts, affecting
61 primary productivity and crop production (see Masello and Quillfeldt 2004 and references
62 therein). Little information is available on the effects of ENSO on bird species in this region
63 (but see Masello and Quillfeldt 2004 and references therein). Also poor is our understanding of
64 the effects of ENSO on Psittaciformes (but see Masello and Quillfeldt 2004; Renton and
65 Salinas-Melgoza 2004). As Psittaciformes have become one of the most endangered orders of
66 birds (Snyder et al. 2000), a good understanding of the influences of climate events on the
67 condition of birds is needed for the identification of key risks and for the management and
68 conservation of the endangered populations of parrots.

69 Burrowing parrots *Cyanoliseus patagonus* are colonial Psittaciformes that, in Argentina,
70 mainly inhabit the phytogeographical province of ‘Monte’, a semi-desert scrubland,
71 characterized by bushy steppes and xerophytes forests. These parrots need sandstone, limestone
72 or earth cliffs to excavate nest burrows, where they breed once a year (September to January;
73 see Masello and Quillfeldt 2004 and references therein). Previous studies have shown that
74 ENSO dramatically impacts nestling survival and growth, and breeding success of burrowing
75 parrots (e.g. Masello and Quillfeldt 2004, 2008). Until now, information on haematological
76 parameters of this species was only available from nestlings in the wild (Masello et al. 2009) or
77 from captive adults (Polo et al. 1998).

78 In this study, ecological aspects of leucocyte parameters in adult burrowing parrots were
79 investigated during five breeding seasons. During our study, the region of North-eastern
80 Patagonia, Argentina, was affected by a severe drought associated with a La Niña event in
81 1998–1999, and by a weak El Niño in 2004–2005. Based on the stress-relatedness of leucocyte
82 parameters and the *H/L* ratio in particular, we predicted that:

- 83 1. *H/L* ratios would be elevated during La Niña compared to El Niño years
- 84 2. *H/L* ratios would be negatively correlated with individual body condition.

85 **Materials and Methods**

86

87 The study was carried out during five breeding seasons (1998 = breeding season from Oct. 1998
88 to Feb. 1999; 1999 = Nov. 1999 to Jan. 2000; 2001 = Nov. 2001 to Jan. 2002; 2003 = Nov.
89 2003 to Jan. 2004; 2004 = Nov. 2004 to Jan. 2005), at the largest colony of burrowing parrots
90 located in El C ndor, North-eastern Patagonia, Argentina (see Masello et al. 2009 and
91 references therein). In this region, precipitation is the main limiting factor of primary production
92 (e.g. Paruelo et al. 1998). The studied breeding seasons were characterized by very contrasting
93 weather conditions and hence, contrasting breeding success (Masello and Quillfeldt 2004). The
94 breeding season 1998 was subject to highly unfavourable environmental conditions (very low
95 precipitation) associated to La Ni a phase of ENSO that strongly reduced breeding success (see
96 Masello and Quillfeldt 2004, 2008), while during the breeding season 2004, the favourable
97 conditions of El Ni o (increased precipitation) prevailed in the study region (Masello and
98 Quillfeldt 2008).

99 According to accessibility, a sector of the colony was selected for monitoring and
100 sampling, as part of an ongoing study on the behavioural ecology of this species (see Masello et
101 al. 2009 and references therein). Nests were inspected every five days by climbing the cliff face.
102 Adult burrowing parrots were captured while attending their nestlings. Four morphometric
103 parameters of adults were recorded: (1) body mass (using a digital balance, to the nearest 1 g),
104 (2) tarsus length, (3) bill length (using callipers, to the nearest 0.1 mm) and (4) wing length
105 (distance from anterior surface of the radio-carpal joint to the tip of the longest primary,
106 flattened along a wing rule to the nearest 1 mm).

107 Blood samples of burrowing parrots were taken by puncture of the cutaneous ulnar vein,
108 using a hypodermic needle and heparinised capillary tubes, immediately after capture, i.e.
109 between 11 AM and 7 PM. Every individual was sampled once. As previously described (see
110 Masello et al. 2009 and references therein), molecular sex determination was carried out using
111 PCR amplification of a highly conserved W-linked gene. Blind duplicate and triplicate blood
112 samples of burrowing parrots were analysed in order to ensure the accuracy of the gender
113 determination. In all cases duplicates and triplicates confirmed the results. Blood sampling had

114 no detectable adverse effects on the birds. After measuring and sampling, the birds were
115 released in their burrows. No desertion occurred.

116 One drop of blood was smeared, air dried on a glass slide immediately after sampling,
117 and fixed with absolute methanol later in the laboratory. All smears were stained with Giemsa.
118 Following previous studies (see e.g. Merino et al. 1999; Masello et al. 2009), differential
119 leucocyte counts were carried out with a light microscope (1000 × magnification with oil
120 immersion) in parts of the smear where erythrocytes had separated in a monolayer. As in
121 Merino et al. (1999) the samples were crossed from down to up to minimize differences in the
122 thickness of the blood smear. Heterophils, eosinophils, basophils, lymphocytes and monocytes
123 were counted following Dein (1986) and Hawkey and Dennett (1989). A total of 100 leucocytes
124 were counted in each smear, thereby obtaining percentages of the different types of leucocytes
125 and the *H/L* ratio. Furthermore, the corresponding number of erythrocytes that were scanned in
126 order to detect 100 leucocytes was used to calculate the relative abundances of leucocyte types
127 per 10,000 erythrocytes. Counting leucocytes and corresponding erythrocytes took
128 approximately 15 min per smear. In total, blood smears of 138 male and female adult burrowing
129 parrots obtained in five breeding seasons were analysed (1998: 22 male and 24 females; 1999: 8
130 males and 10 females; 2001: 9 males and 7 females; 2003: 21 males and 21 females; 2004: 8
131 males and 8 females). Due to the absence or very low numbers of monocytes, basophils and
132 eosinophils in most of the blood smears, only *H/L* ratios, heterophils per 10,000 erythrocytes,
133 lymphocytes per 10,000 erythrocytes and the total relative abundance of leucocytes per 10,000
134 erythrocytes were considered in further statistical analyses. The *H/L* ratio has shown a low
135 measurement error under field conditions (e.g. Gross and Siegel 1983; Maxwell 1993; Merino et
136 al. 1999; see also review by Davis et al. 2008). Following Lessells and Boag (1987), we
137 calculated intra observer repeatability. Our results suggested a reasonable degree of repeatability
138 by the same observer ($r = 0.72$, ANOVA, $F = 6.18$, $d.f. = 23$, $P = 0.002$; the difference was below
139 0.08 for 10 out of 12 cases). All birds were sampled within 30 min of capture to ensure that the
140 *H/L* ratios did not changed due to routine handling (see Davis 2005).

141 Following previous studies (Masello et al. 2009 and references therein), and since body
142 mass is partly the result of structural body size and does not necessarily reflect the quantity of

143 body reserves, body mass was scaled to body size as a condition index. To determine body
144 condition, a multiple linear regression of body mass as dependent variable with wing, tarsus and
145 bill length as predictors, was carried out ($n = 91$, $R = 0.465$, $F = 7.999$, $P < 0.001$). This allowed
146 us to calculate an expected body mass for each combination of the three size factors as follows:
147 $\text{expected body mass} = 0.84(\text{wing length}) + 7.77(\text{tarsus length}) + 1.64(\text{bill length}) - 205.34$.
148 Then, body condition was calculated as the ratio between observed body mass and expected
149 body mass. Body condition was calculated for 66 males (of a total of 68 sampled individuals)
150 and 69 females (of a total of 70 individuals). This was due to the lack of precise bill
151 measurements for some individuals with broken bill tips.

152 Data were analysed using Sigma Stat 2.03 and SPSS 11.0. General Linear Models
153 (GLMs), based on Type III sums of squares, were fitted to the data. Separate models were fitted
154 for ‘ H/L ratio’, ‘heterophils/10.000 erythrocytes’, ‘lymphocytes/10.000 erythrocytes’ and ‘total
155 leucocytes/10.000 erythrocytes’ as response variables. Each model was initially calculated with
156 ‘year’ and ‘sex’ as categorical predictors and ‘body condition’, ‘sampling day’ (range = 16–72)
157 and ‘days after hatching of first chick’ (range = 1–66, an estimate of brood age) as continuous
158 predictor variables. Non-significant explanatory variables or interaction terms (all $P > 0.2$) were
159 deleted from initially saturated models in a stepwise manner and only reduced models are
160 shown. The covariates ‘sampling day’ and ‘days after hatching of first chick’ were non-
161 significant in all analyses. When linear models showed signs of non-normality of error terms or
162 heteroscedasticity, explanatory and response variables were transformed using natural logarithm
163 (\ln) transformations. As a measure of effect size partial Eta-squared values (η^2) were included,
164 i.e. the proportion of the effect + error variance that is attributable to the effect. The sums of the
165 η^2 values are not additive (e.g. http://web.uccs.edu/lbecker/SPSS/glm_effectsize.htm). All
166 presented means are estimated marginal means obtained from the fitted linear models (adjusted
167 for the covariate). Bonferroni correction was applied to multiple comparisons of estimated
168 marginal means. Throughout this study the significance level used is $P < 0.05$.

169 **Results**

170

171 Mean values of the differential leucocyte counts of 138 adult burrowing parrots obtained during
172 the five breeding periods studied are shown in Table 1. In both males and females, the highest
173 relative counts were obtained for heterophils, followed by lymphocytes. Relative numbers of
174 monocytes and eosinophils were extremely low, and no basophils were detected (Table 1).

175 Mean *H/L* ratios were significantly higher in 1998 and 1999 compared to 2003 and
176 2004, with intermediate values in 2001 (Fig. 1). This pattern of yearly variation was similar for
177 males and females. Male burrowing parrots had higher *H/L* ratios (Fig. 1, Table 1) and lower
178 lymphocyte counts per 10,000 erythrocytes (Fig. 2) compared to females throughout the study
179 period. For heterophil counts per 10,000 erythrocytes and the total abundance of leucocytes per
180 10,000 erythrocytes we found strong interaction patterns between variation among years and
181 gender differences (Table 2, Figs. 1-2). *H/L* ratios and heterophils per 10,000 erythrocytes
182 negatively covaried with body condition (Table 2, Fig. 3). This was the case in both sexes, as
183 apparent by the lack of a significant interaction term in the GLM.

184

185 **Discussion**

186

187 In the present study, we investigated leucocyte profiles of adult Burrowing Parrots in the wild.
188 As predicted, *H/L* ratios were higher during the breeding season affected by a La Niña event
189 (1998) compared to a breeding season affected by an El Niño event (2004). However, they were
190 highest in 1999, during the breeding season following La Niña event. Also as predicted, *H/L*
191 ratios were negatively correlated with individual body condition. In addition, we found
192 significant gender differences in leucocyte parameters and relatively high *H/L* values in
193 Burrowing Parrots compared to other groups, which likely represent an inherent variation in
194 immune physiology, rather than stress-related modulations.

195

196 Heterophilic profiles

197

198 In the majority of adult burrowing parrots, heterophil numbers exceeded those of lymphocytes.
199 This resulted in remarkably high mean *H/L* ratios for both, males and females (Table 1). Our
200 results differ from previously published data on captive burrowing parrots ($H/L < 1$; Polo et al.
201 1998). Experiments with zebra finches have shown that captivity can cause a significant and
202 rapid decrease in *H/L* ratios (Ewenson et al. 2001). This might explain the differences observed
203 between our present data on wild individuals and those previously described from captive ones.

204 Psittaciformes often have heterophilic profiles (e.g. Joyner et al. 1992; Sheridan et al.
205 2004; Deem et al. 2005; Low et al. 2006; Masello et al. 2009) as here reported, in contrast to the
206 lymphocytic profiles usually reported in Passeriformes (see review by Davis et al. 2008).
207 Whether this general pattern reflects a differential investment in innate and acquired immunity
208 (as e.g. discussed in Masello et al. 2009) remains to be tested by direct manipulation of both the
209 innate and acquired immune system (see Davis et al. 2008). Heterophilic profiles could as well
210 reflect current challenges of the innate immune system, e.g. by bacterial, viral or fungi
211 infections (see Hawkey and Dennett 1989; Davis et al. 2008). In any case, strong differences
212 between phylogenetic groups emphasize the importance of baseline information on sources of
213 variation in haematological parameters, such as presented in this study.

214

215 Differences of leucocyte profiles between breeding seasons

216

217 North-eastern Patagonia was affected by a strong drought associated to a long La Niña event
218 (1998–1999) during the breeding season 1998 (Oct. 1998–Feb. 1999). During that breeding
219 season only 5% (1.5 mm in December) of the long-term mean rainfall was recorded. This in
220 turn caused an important food shortage, and reduced the breeding success at the studied
221 Burrowing Parrot colony (Masello and Quillfeldt 2004). In contrast, during the breeding season
222 2004 (Nov. 2004–Jan. 2005), conditions associated to an El Niño event enhanced local
223 precipitations (76 mm in December, or 304% of the long-term average), which were the highest

224 so far recorded during our research in NE Patagonia. As a consequence, in the breeding season
225 2004, burrowing parrots had the highest reported breeding success (Masello and Quillfeldt
226 2008). In accordance with this, adult birds showed increased *H/L* ratios in the breeding season
227 1998 compared to the one in 2004 (Fig. 1a). Thus, the droughts associated with the La Niña
228 event not only affected nestling survival in burrowing parrots (see Masello and Quillfeldt 2004),
229 but also imposed physiological stress on the adults, reflected by increased *H/L* ratios. However,
230 the highest *H/L* values, and lowest body condition, were recorded during the breeding season
231 1999 (Nov. 1999–Jan. 2000), a period of average rainfall in the region, as well as intermediate
232 reproductive success in this species (Masello and Quillfeldt 2004, 2008).

233 In a previous analysis, burrowing parrots responded to unfavourable conditions by
234 maintaining their own body condition (adaptive mass regulation; Masello and Quillfeldt 2003).
235 During the breeding season of 1998, adults at the study site had higher body mass and body
236 condition, but decreased provisioning, than during years of average conditions (Masello and
237 Quillfeldt 2003). This higher investment in own survival (and thus in future offspring) might
238 explain why the highest *H/L* ratios were not observed in 1998. Another possible explanation for
239 higher body condition (and lower *H/L* ratios) in an unfavourable year could be that only those
240 birds in the best condition were breeding, while birds in worse state forego reproduction in
241 harsh years. However, this is very unlikely as there was no significant difference in the
242 proportion of active nests between the breeding season affected by La Niña (1998) and the
243 breeding season during average precipitation conditions (1999; Masello and Quillfeldt 2004).

244 What, however, might have caused the peak in *H/L* ratios in 1999, a breeding season of
245 average precipitation conditions and high breeding success? Two explanations might play a
246 role. Breeding success and nestling growth mainly depends on food availability during nestling
247 provisioning, and might therefore quickly and directly respond to changes in the resources.
248 Physiological stress of adults, in contrast, might also outlast the actual adverse period, as the
249 birds have to compensate for energetic losses. In our case, in spite of the local favourable
250 conditions during the breeding season 1999, birds might not have been fully recovered from the
251 previous breeding season under La Niña associated conditions, and might therefore have been
252 more sensitive to the general demands of breeding. This is supported by the observed lowest

253 body condition of birds in the breeding season 1999 (Fig. 1b). Secondly, leucocytes respond to
254 disease condition, and adult birds might have suffered high infection rates causing increased
255 stress and decreased body condition in 1999, without affecting breeding success and nestling
256 growth. While burrowing parrots seem to lack blood and intestinal parasites (Masello et al.
257 2006), no data on fungal, bacterial or viral infections are currently available.

258

259 Leucocytes, gender and body condition

260

261 The negative relationship between H/L and body condition suggests a stress-relatedness of this
262 parameter. This correlation appears to originate mostly from a relationship between condition
263 and heterophils, rather than lymphocytes (see Table 2).

264 While within gender H/L ratios are negatively related to body condition (Fig. 3), the
265 gender differences in H/L are, in contrast, paralleling the gender differences in condition (see
266 Fig. 2): males had higher H/L ratios and were in better body condition. Thus, the gender
267 differences in H/L seem to be not related to physiological stress, but probably represent some
268 inherent difference between the sexes in haematological parameters and immunity. Heterophil
269 abundances showed a much more complex pattern of variation among years and gender than
270 lymphocytes (Fig. 1), suggesting that other factors are influencing the abundance of these cells,
271 such as current immune challenges.

272

273 Suggestions for further research

274

275 Future studies could contribute to a more precise picture of the ecology of stress in burrowing
276 parrots by collecting information outside the breeding periods, regarding the unknown wintering
277 areas and migratory routes. Data on individual disease condition would be useful for a proper
278 interpretation of relationships with environmental conditions. Information on non-breeding
279 birds is necessary, since birds might selectively forego breeding periods depending on climate
280 and own condition, possibly creating a bias in the sample of breeding birds. Analysing data of
281 recaptured birds could help to evaluate the effects and importance of individual variation.

282 **Zusammenfassung**

283

284 **Leukozyten bei im Freiland brütenden Felsensittichen *Cyanoliseus patagonus*:**

285 **Abhängigkeiten von Brutzeiten, Geschlecht und individueller Kondition**

286

287 Unter dem Einfluss von Stressoren können wildlebende Vögel die Investition in ihr
288 Immunsystem verändern. Wir haben Leukozyten brütender Felsensittiche (*Cyanoliseus*
289 *patagonus*) in Patagonien in fünf Brutperioden untersucht. Während der Studie traten globale
290 Klimaereignisse mit Einfluss auf das Brutgebiet auf: ein starkes La Niña- und ein schwaches El
291 Niño-Ereignis. Zwischen den Brutperioden wurden große Unterschiede im Verhältnis der
292 Heterophilen zu Lymphozyten (H/L) beobachtet, wobei höhere H/L Quotienten unter den
293 widrigen Umweltbedingungen von La Niña im Vergleich zu den im Studiengebiet günstigen
294 Bedingungen von El Niño auftraten. Die höchsten H/L Werte wurden jedoch in einer auf La
295 Niña folgenden Brutperiode gefunden, möglicherweise verursacht durch längerfristige
296 Auswirkungen der vorangehenden Klimasituation in Kombination mit anderen, z.B. biotischen
297 Stressoren. Männchen zeigten höhere H/L Werte als Weibchen und H/L korrelierte negativ mit
298 individueller Körperkondition.

299

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310

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382 **Fig. 1** Variation in (a) H/L ratios and (b) \ln body condition among breeding seasons plotted
383 separately for male and female burrowing parrots. For H/L , homogeneous subsets are indicated,
384 as obtained from multiple comparisons of estimated marginal means

385

386 **Fig. 2** Variation in (a) heterophils per 10,000 erythrocytes and (b) lymphocytes per 10,000
387 erythrocytes among breeding seasons, plotted separately for males and females (estimated
388 marginal means obtained from GLMs were used and backtransformed to original scale)

389

390 **Fig. 3** Relationship among $\ln H/L$ ratios and \ln body condition of (a) male and (b) female
391 burrowing parrots during five breeding seasons

Table 1

Mean values of leucocyte counts of adult Burrowing Parrots in NE Patagonia, Argentina

	Males		Females	
	<i>n</i> = 68		<i>n</i> = 70	
	Mean ± SE	Range	Mean ± SE	Range
Heterophils (%)	76.6 ± 1.7	32.0 – 96.0	71.6 ± 1.5	43.0 – 94.0
Lymphocytes (%)	21.5 ± 1.6	4.0 – 68.0	25.8 ± 1.5	6.0 – 57.0
Eosinophils (%)	1.4 ± 0.2	0.0 – 7.0	2.3 ± 0.3	0.0 – 13.0
Monocytes (%)	0.4 ± 0.1	0.0 – 4.0	0.3 ± 0.1	0.0 – 4.0
<i>H/L</i> ratio	6.1 ± 0.7	0.5 – 24.0	4.3 ± 0.4	0.8 – 15.7
Heterophils / 10,000 erythrocytes	21.5 ± 1.6	4.1 – 65.8	21.1 ± 1.5	4.6 – 72.7
Lymphocytes / 10,000 erythrocytes	5.7 ± 0.7	1.0 – 38.4	7.3 ± 0.6	0.6 – 28.5
Eosinophils / 10,000 erythrocytes	0.4 ± 0.1	0.0 – 2.6	0.6 ± 0.1	0.0 – 4.6
Monocytes / 10,000 erythrocytes	0.1 ± 0.0	0.0 – 0.9	0.1 ± 0.0	0.0 – 0.9
Total leucocytes / 10,000 erythrocytes	27.6 ± 1.8	7.8 – 77.4	29.1 ± 1.9	5.8 – 92.1

Table 2

Sources of variation in leucocyte counts of Burrowing Parrots in NE Patagonia, Argentina

	Type III Sum of Squares	df	F	Sig.	η^2	t
<i>A) In H/L ratio</i>						
Year	14.615	4	7.353	0.000	0.184	
Gender	5.917	1	11.908	0.001	0.084	
Body condition	6.293	1	12.664	0.001	0.089	-3.559
Error	64.600	130				
<i>B) Heterophils / 10,000 erythrocytes</i>						
Year	1.283	4	1.058	0.380	0.032	
Gender	0.031	1	0.102	0.750	0.001	
Year x Gender	3.251	4	2.681	0.035	0.078	
Body condition	2.595	1	8.558	0.004	0.064	-2.925
Error	38.208	126				
<i>C) Lymphocytes / 10,000 erythrocytes</i>						
Year	11.053	4	5.240	0.001	0.138	
Gender	3.012	1	5.712	0.018	0.042	
Error	69.087	131				
<i>D) Total leucocytes / 10,000 erythrocytes</i>						
Year	0.660	4	0.629	0.642	0.020	
Gender	0.325	1	1.239	0.268	0.010	
Year x Gender	2.763	4	2.636	0.037	0.077	
Body condition	0.969	1	3.700	0.057	0.029	-1.923
Error	33.019	126				
<i>E) Body Condition</i>						
Year	0.087	4	4.273	0.003	0.115	
Gender	0.044	1	8.620	0.004	0.062	
Error	0.667	131				

Results correspond to General Linear Models (GLMs) with *H/L* ratios, Heterophils/10,000 erythrocytes, Lymphocytes/10,000 erythrocytes and Total leucocytes/10,000 erythrocytes as dependent variables, year and gender as fixed factors and body condition as covariate. Additionally, effects of year and gender on body condition were tested in a separate GLM. For the GLMs with body condition as covariate, *t*-values are included to indicate the direction of the relationship. Data from five years were included: 1998, 22 males, 24 females; 1999, 8 males, 10 females; 2001, 9 males, 7 females; 2003, 21 males, 21 females; 2004, 8 males, 8 females.

Figure 1

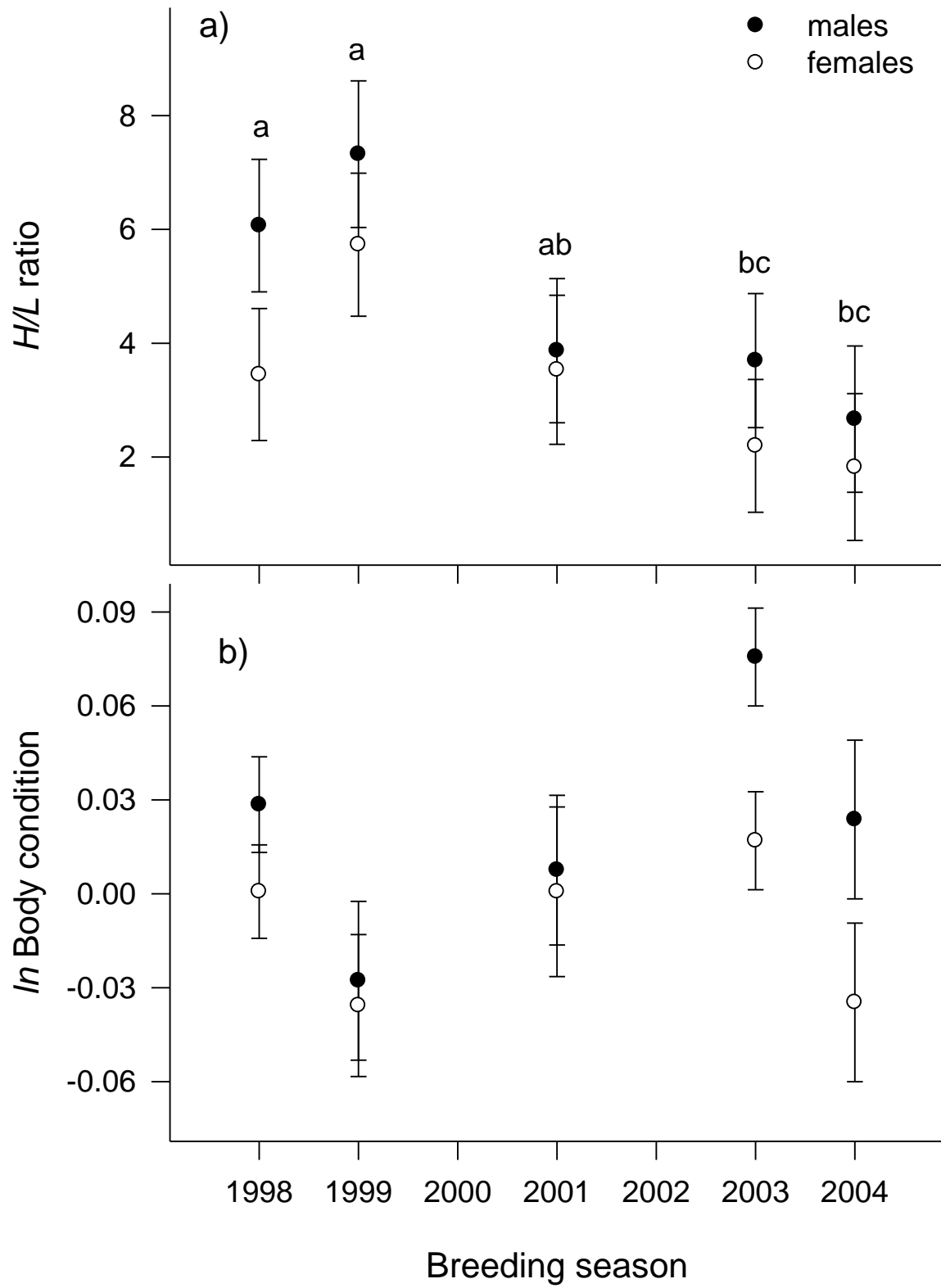


Figure 2

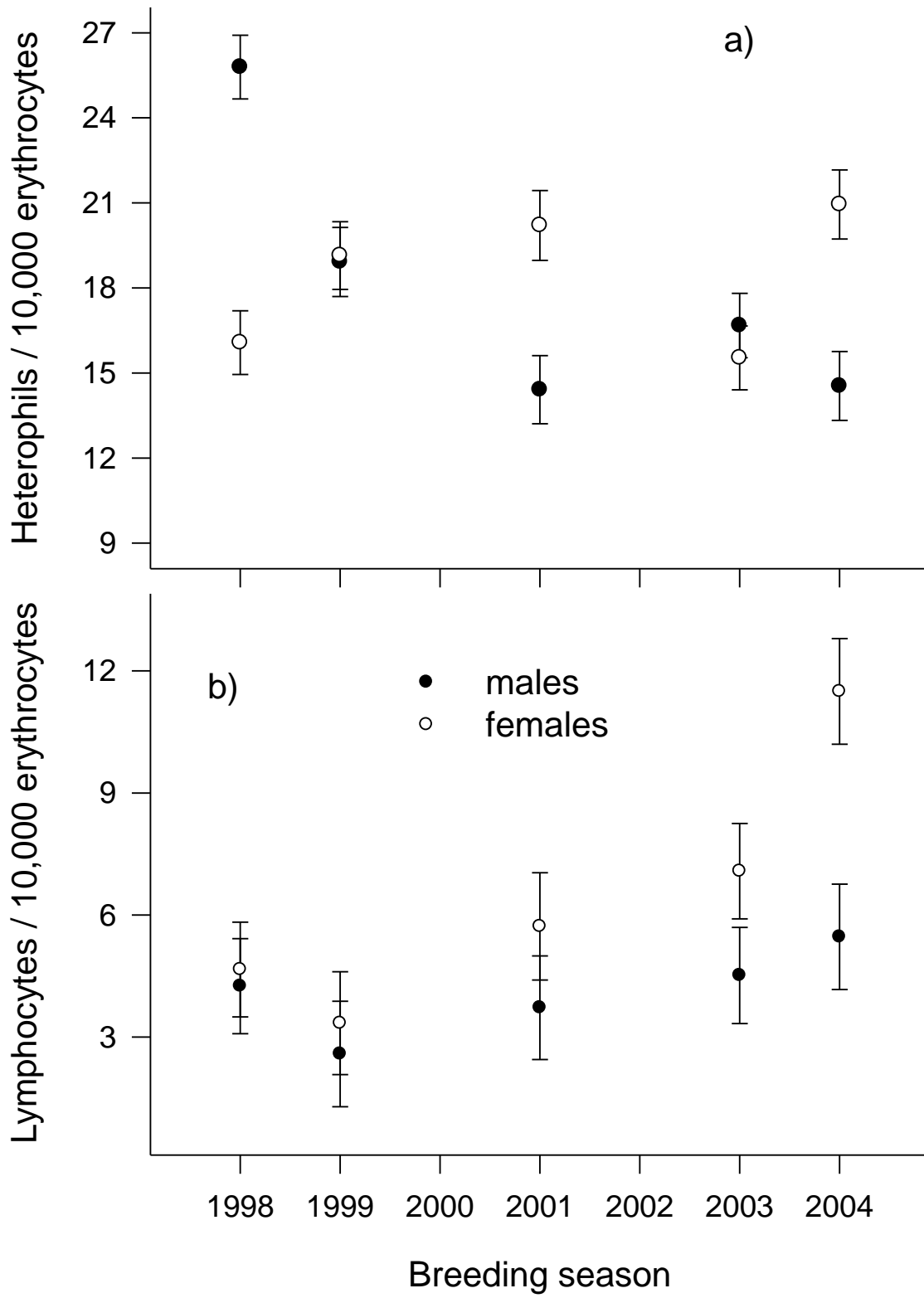


Figure 3

