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Behaviour and stress response during capture and handling of the red-billed chough *Pyrrhocorax pyrrhocorax* (Aves: Corvidae)

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We studied the effect of capture and handling on free-living red-billed choughs *Pyrrhocorax pyrrhocorax*. We analysed the association between bird behavioural response and individual health, breeding status, sex, and age. Active responses (aggression towards the ringer, screaming) were more common in the breeding period, and in individuals with a high heterophils to lymphocytes ratio, indicating poor physiological condition and/or high chronic stress. Adults were more aggressive than juveniles and yearlings, and females were more aggressive than males. Sex, age, and condition differences were also recorded in the spectrotemporal output of distress calls. Birds with a screaming/active response appeared to be more stress-susceptible than passive and silent individuals, and this response was stronger during the energy demanding period of reproduction. The results obtained suggest that the response of the red-billed chough during capture might primarily reflect stress-susceptibility, although a number of potential alternative explanations are discussed. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **96**, 846–855.

ADDITIONAL KEYWORDS: aggressive behaviour – distress calls – heterophil/lymphocyte ratio – physiological stress.

INTRODUCTION

Vertebrates have evolved specific physiological and behavioural responses to cope with sudden and unpredictable events, such as the attack of predators, psychosocial stressors, or unpredictable weather events (Wingfield, 2003). Physiological responses include increased heart and respiratory rates, higher body temperatures and the simultaneous release of corticosterone through activation of the hypothalamic-pituitary-adrenal axis, and release of epinephrine by the adrenal medulla or chromaffin tissue (Axelrod & Reisine, 1984). Concurrent with adrenal response may be a cellular response in which heterophil counts increase and lymphocyte numbers decrease (Gross & Siegel, 1983). Behavioural responses to stressful events span from enhanced restfulness to aggression and the utterance of fear screams (Högstedt, 1983; Koolhaas *et al.*, 1999).

Recent studies have shown that individuals of the same species and population may differ in their behavioural responses to stress or fear stimuli, even within the same age and sex and under standardized conditions (Carere *et al.*, 2005). These differences may depend upon heritable behavioural patterns maintained by natural selection, and are defined

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'personalities' by ethologists and psychologists or 'coping styles' by stress physiologists. The sudden attack of a predator or a dominant conspecific can trigger alternative strategies, normally termed 'fight-or-flight', or 'proactive-reactive' profiles, which differ in the magnitude of aggression and show characteristic hormonal responses (Koolhaas *et al.*, 1999). In birds, a prey phenotypic response to nearby predators may be simulated by the catching and handling protocol of ringing activities (Laiolo *et al.*, 2004). A standardized version of this technique is routinely used in avian stress studies and is referred to as the 'capture stress-protocol' (Romero & Romero, 2002).

In the present study, we investigated how free-living red-billed choughs respond behaviourally and physiologically to the unpredictable and stressful event of capture. We focused on natural occurring variation in both individual aggressiveness and distress calls during handling. We explored the relationship between variation in bird behavioural response and some individual specific features, such as physiological state, age, and sex. The concurrent analysis of all these potential determinants can help to shed light on the behavioural strategies when facing a threat, and permit testing of whether aggressive and vocal behaviour may result from the selection of predators or rather is an expression of stress-susceptibility. In the former case, we expect a positive correlation with individual health condition because prey may reliably signal their escaping abilities to the predator (Laiolo *et al.*, 2004), whereas, in the latter case, we expect the opposite (i.e. the most physiologically stressed birds respond actively when exposed to a sudden threat).

MATERIAL AND METHODS

The study was carried in Los Monegros (north-eastern Spain) from July 2005 to March 2006. At night, we captured 163 red-billed choughs by chasing after them with large butterfly nets in breeding or roosting farmhouses. Ringing permission was obtained from the 'Gobierno de Aragon' (LCE/mp 24/2005/1254; LCE/mp 24/2006/1054). Ten minutes after capture, birds were banded, weighed, and tarsus and wing length were measured. Sex and age were established on the basis of plumage and biometry (Blanco, Tella & Torre, 1996). We extracted 1 mL of blood from the brachial vein of 116 individuals. A drop was immediately used to make two blood smears, whereas the remaining blood was stored in 1.5-mL Eppendorff tubes using ethylenediaminetetraacetic acid as anticoagulant and transported in a chilled container to the laboratory on the day of collection. No anaesthetization was performed during handling.

Red-billed choughs can utter distress calls and attempt fleeing during catching and handling. We recorded these distress calls from individuals maintained in the same standard posture, with their feet held and wings set free. Recordings were made in open habitat at a distance ≤ 2 m with a Sony TC-D8 DAT recorder and a Sennheiser ME67 microphone. We also characterized individual behaviour during handling, by recording whether birds attempted to flee and attack the ringer with bill or claws; attempted flights and aggressions were concurrently produced by individual birds. Birds were defined as 'passive' when they remained quiet and as 'active' when they were restless and attempted to flee and attack.

One of us (E.B.) measured and extracted blood (7 min), and another one (P.L.) made audio recordings of the distress calls (3 min). Individual behavioural response (active or passive, calling, or silent) were consistent between different capture sessions in the study years. When considering the overall number of times birds were captured (the study population had been extensively marked from 1991 onward), no relationship was found between bird vocal behaviour and the number of times that it was captured (logistic regression: Wald $\chi^2 = 1.52$, $P = 0.22$, $N = 55$ recaptures). Birds with a long history of captures (more than three times) tended to be more active than birds captured fewer times (90% versus 40% of birds; Wald $\chi^2 = 13.9$, $P < 0.001$), although their proportion in the study sample was low (12%). We did not detect an effect of handling order on bird acoustic behaviour (we captured more than one bird at a time; Mann–Withney *U*-tests comparing the handling order of screaming versus silent and active versus passive birds: all $U > 43$, and $P > 0.11$, sexes and age classes tested separately). Only in the case of male juveniles captured after breeding did we find a significant difference in handling order between active and passive birds ($U = 12$, $P < 0.005$, $N = 24$ birds). In spite of this, some of these birds were recaptured and they maintained the same behavioural response irrespective of handling order ($N = 7$ birds).

BIRD PHYSIOLOGICAL STATE AND BODY CONDITION

Within 12 h of blood collection, one of us (J.A.L.) performed white and red cell count using an improved Neubauer haemocytometer and Natt and Herrick's solution in white blood cell dilution pipettes (Campbell, 1995). Blood smears prepared during captures were air dried, fixed in absolute methanol, stained, and examined with a light microscope. The proportions of heterophils, lymphocytes, eosinophils, monocytes, and basophils were determined by identifying 400 leukocytes under 1000 power in fields selected in an evenly distributed monolayer. From the leukocyte profile and total counts, we calculated the *H*/*L* ratio as the number of heterophils (*H*) divided by the number of lymphocytes (*L*). The *H*/*L* ratio is often used as an indicator of chronic or physiological stress, as blood circulating lymphocytes decrease, whereas circulating heterophils increase in response to infectious diseases, parasitemia, food or water deprivation, and injury (Hörak, Ots & Murumägi, 1998). Marked heterofilia and lymphopenia can also follow corticosteroid release in response to an immediate stress event (Gross & Siegel, 1983).

Blood was centrifuged in capillary tubes at 13 000 ***g*** for 10 min, to separate plasma from cells and both fractions were frozen separately until analysis. Haematocrit levels were calculated by dividing the red blood cell volume by the total blood volume. Low haematocrit levels are indicative of malnutrition, low oxygen consumption, and bacterial and parasite infections (Campbell & Dein, 1984). Haematocrit levels can also increase through dehydration as a result of the reduction in the volume of plasma (Dawson & Bortolotti, 1997). Plasma total solids were measured using a handheld temperature-regulated clinical refractometer (Eclipse Optical hand-held refractometer). Plasma total solids measure the amount of proteins in blood plasma due to diet quality, and can decrease in response to stress (Sturkie, 1976). The oxyhaemoglobin level was measured using a portable Hemocue (Hemocue 201+, HemoCue AB). Low levels of oxihaemoglobin can reveal anaemia, and may result from red blood cell loss provoked by chronic stress (Clinchy *et al.*, 2004). Stressful conditions also increase polychromasia (proportion of larger, immature red blood cells), as assessed on blood smears. The abundance of polychromatic cells may increase as a result of stress (McKilligan, 1996).Young leucocytes can be viewed as indicators of chronic disease (Campbell, 1995).

BIRD DISTRESS CALLING

One hundred and twenty individuals uttered distress calls, seven gave contact calls, and 36 did not vocalize at all. Here, we only focused on the distress call: a long, harsh, wide-band and loud alarm vocalization given when a predator is very close (Fig. 1; Laiolo, Palestrini & Rolando, 2000). From 2071 recorded calls, we measured the following spectrotemporal features *sensu* Laiolo *et al.* (2007) (Fig. 1): (1) call duration; (2) call rate (number of calls uttered per second); (3) mean peak frequency (the frequency band with the greatest energy, measured on the average spectrum); (4) mean fundamental frequency (the lowest frequency peak, measured on the average spectrum); (5) call harshness (the frequency range in which the signaler concentrate 50% of the call energy); and (6) harsh fraction (the duration of the harsh portion of the call, expressed as relative percentage of overall call duration). All sound analyses were carried out using Avisoft SASLab Pro, version 3.91, performing a Fast Fourier Transform (sampling frequency 22 050 Hz, FFT length 512, time resolution 8.9 ms, frequency resolution 43 Hz, window function: Bartlett; high-pass filter: cut-off frequency 900 Hz).

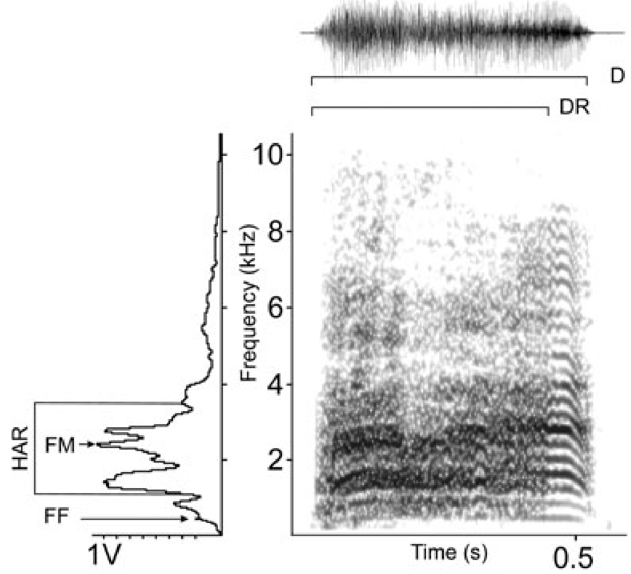

Figure 1. Waveform (top), sonogram (centre), and mean power spectrum (left) of a red-billed chough distress call. D, call duration; FM, mean peak frequency; FF, mean fundamental frequency; HAR, call harshness; DR, harsh fraction.

STATISTICAL ANALYSIS

For the present study, we entered in the analysis sound features and physiological data collected during one capture event per bird only because calls were recorded and blood extracted only once per individual. During a capture event, birds uttered distress calls repeatedly (an average of 14 calls per bird). We measured all vocalizations and estimated the mean value of the spectrotemporal variables for each bird. We used a principal components analysis (PCA) to summarize acoustic parameters into independent factors (PCA scores; for details of methodology, see Laiolo *et al.*, 2004). The scores of the first four principal components had eigenvalues > 1 and explained 82.7% of the variability of acoustic data. The first principal component was negatively correlated to call rate ($r = -0.86$; PC1 = Inverse Call Rate Factor), the second component was negatively correlated to the mean peak frequency ($r = -0.80$; PC2 = Inverse Peak Frequency Factor), the third was positively correlated to the mean fundamental frequency ($r = 0.94$; PC3 = Fundamental Frequency Factor), whereas the percent length of the harsh component provided the greatest loading to the fourth component ($r = -0.97$; PC4 = Inverse Harsh Fraction Factor).

We performed generalized linear models to analyse the determinants of distress calling (0: silent; 1: calling), bird behavioural profile (0: passive; 1: active) and call spectrotemporal variables (four continuous PCA scores). We used a binomial distribution with a logit link function in the two former cases, and a normal distribution with an identity link function in the latter. Generalized linear models were performed with the procedure GENMOD in SAS, version 8.2 (SAS Institute). As independent predictors, we entered sex, age (yearling, adult, and juvenile), an indicator of body size (wing length), the index of body condition (size corrected-body mass), period (breeding or nonbreeding stage), *H*/*L* ratio, haemoglobin levels, and proportion of young leukocytes. Plasma total solids, haematocrit and polychromasia levels had a significant correlation with the *H*/*L* ratio (negative in the former two cases and positive in the latter; $r = -0.28$, -0.30 and $+0.23$ respectively, all $P < 0.05$) and were therefore excluded from the models. These correlations suggest a connection between physiological and immunological state, and corroborate the *H*/*L* ratio role with respect to revealing physiological stress in general, given the association with nutritional conditions (total solids and haematocrit) and anaemia level (polychromatic, regenerating red blood cells).

We used the bias-corrected version of Akaike's Information Criterion ($\mathrm{AIC_c}$) to identify the best approximating statistical models. Models were ranked on the basis of the differences between the $\mathrm{AIC_c}$ of a given model and the $\mathrm{AIC_c}$ of the highest ranked model ($\Delta\mathrm{AIC_c}$), and those models that were separated by less than 2 $\mathrm{AIC_c}$ points were considered as equally probable (Buckland, Burnham & Augustin, 1997). To minimize the potential for overfitting and significance-by-chance effects, models included those single variables and interactions that ranked lowest $\mathrm{AIC_c}$. Continuous variables that were not normally distributed were log-transformed before carrying out statistical tests.

RESULTS

BEHAVIOURAL RESPONSE

Distress calling was more common in active than in passive birds: 94% for active red-billed choughs versus 50% for passive birds ($\chi^2 = 37.7$, $P < 0.001$).

The best statistical model for call utterance highlighted the importance of body measurements, *H*/*L* ratio, and period (AICc = 115.23–116.6) for calling behaviour (Table 1). Calling choughs had a higher *H*/*L* ratio and a poorer body condition (lower body weight in relation to body size) (Fig. 2). Distress calls were also more commonly uttered in breeding than outside the breeding season (Fig. 3).

Sex, age, and haematological parameters, such as young leukocytes and haemoglobin levels, were not associated with the probability of calling. Conversely, bird behaviour during handling did vary with the age and sex of the caller: adults were more active than yearlings and juveniles, females were more active than males. Bird behavioural profile also varied in relation to the *H*/*L* ratio, with the greatest stress (highest *H*/*L* ratio) being recorded in active individuals (Fig. 2). Active and (concurrently) calling birds showed the greatest *H*/*L* ratios in the sample ($F_{3,108} = 4.0$, $P = 0.01$). Apart from giving more distress calls, birds were also more active in the breeding stage, either when considering all age classes or when including adults only (Fig. 3). Three models including different combinations of sex, age, *H*/*L* ratio, and

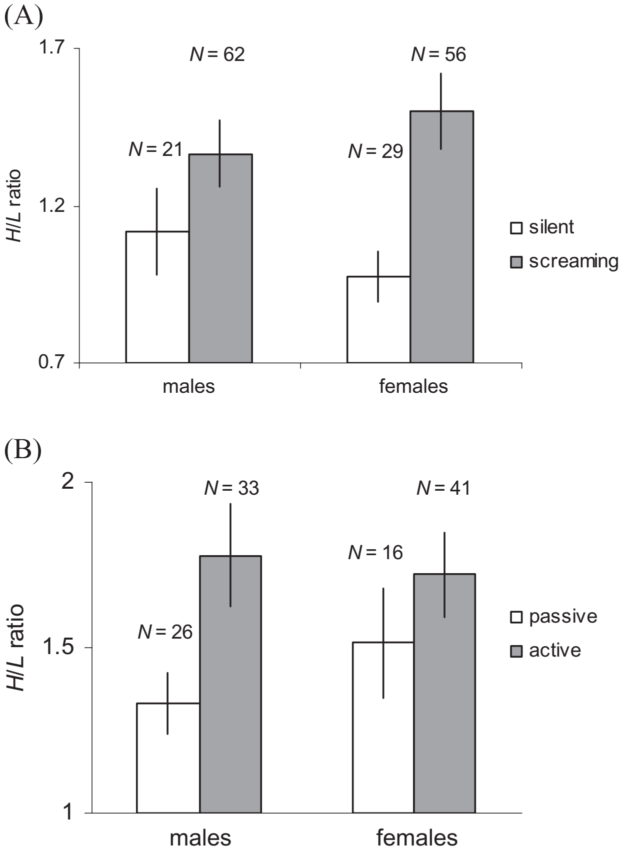

Figure 2. Differences in heterophil/lymphocyte (*H*/*L*) ratios between (A) individuals giving distress calls (screaming) and individuals remaining silent and (B) active and passive individuals. Male and female data (mean ± SE) are presented separately. Sample sizes are also shown.

Table 1. Ranking of the models used to explain variation in screaming and active responses of captured red-billed choughs

	Hypothesized model	AIC$_c$	ΔAIC$_c$	*P*-values of each model predictor	Overall explained deviance (%)
Screaming	Period + *H*/*L* ratio	115.3	0.00	(0.0002) + (0.026)	40.23
	Period + *H*/*L* ratio + body weight + tarsus length	115.8	0.45	(0.0004) + (0.076) + (0.07) + (0.050)	40.16
	Period + *H*/*L* ratio + body weight + wing length	116.6	0.83	(0.0004) + (0.076) + (0.07) + (0.050)	39.94
	H/*L* ratio	125.7	12.26	(0.011)	30.60
	Young leucocytes	131.9	18.53	(0.70)	26.95
	Haemoglobin	133.1	19.72	(0.95)	26.26
	H/*L* ratio + body weight + tarsus length	128.5	13.9	(0.04) + (0.28) + (0.22)	31.46
	Period	166.9	53.48	(0.0007)	6.61
	Body weight + tarsus length + wing length	128.0	12.7	(0.87) + (0.06) + (0.10)	6.81
	Age	172.9	59.5	(0.069)	3.11
	Wing length	175.7	62.24	(0.21)	1.49
	Sex	178.3	64.84	(0.90)	0.01
Aggression	Period + *H*/*L* ratio	117.4	0	(0.0001) + (0.0249)	44.43
	Period + sex + *H*/*L* ratio	118.3	0.96	(0.0001) + (0.095) + (0.033)	45.05
	Period + age + *H*/*L* ratio	119.1	1.62	(0.0001) + (0.047) + (0.0065)	44.72
	Period + age + sex + *H*/*L* ratio	120.2	2.83	(0.0001) + (0.047) + (0.096) + (0.0095)	45.24
	H/*L* ratio	144.4	27.03	(0.0016)	29.55
	Young leucocytes	152.3	34.94	(0.14)	25.51
	Haemoglobin	155.7	38.38	(0.46)	23.76
	Period	165.7	48.31	(0.0001)	18.67
	Age	192.1	74.7	(0.006)	5.21
	Sex	196.8	79.44	(0.019)	2.79
	Body weight + tarsus length + wing length	198.62	81.26	(0.57) + (0.27) + (0.92)	4.04
	Wing length	200.3	82.94	(0.58)	1.01

The best models are those separated by less than 2 points on the bias-corrected version of Akaike's Information Criterion (AIC$_c$) (ΔAIC$_c$ < 2) from the most probable model. *P*-values of each predictor in the model and the overall explained deviance are also shown.
H/*L*, heterophil/lymphocyte ratio.

period were equally likely to explain variation in bird behavioural profiles (Table 1).

ACOUSTIC RESPONSE

The *H*/*L* ratio appears to affect also the acoustic response of birds, although its weight was lower than in the case of the behavioural responses; see *P*-values in Table 2. Stressed birds with a high *H*/*L* ratio tended to give calls at a faster rate and with a higher pitch than birds presenting a low *H*/*L* ratio (Fig. 4). Call rate also increased significantly with body size and age; adults gave a mean ± SD of 0.91 ± 0.38 ($N = 80$) calls per second, whereas juveniles and yearlings only 0.74 ± 0.36 ($N = 28$) and 0.78 ± 0.33 ($N = 14$) calls per second, respectively.

Bird age and sex were associated with two frequency PCA scores (PC2 and PC3; Table 2). Young birds and females tended to give calls with higher fundamental and peak frequencies than adults and males, and body size (indicated by wing length) was significantly related to call peak frequency, with larger birds uttering deeper sounds. Two haematological parameters correlated with the Inverse Harsh Fraction Factor (PC4), and proportionately longer harsh calls were uttered by birds with greater levels of haemoglobin and young leucocytes (Table 2).

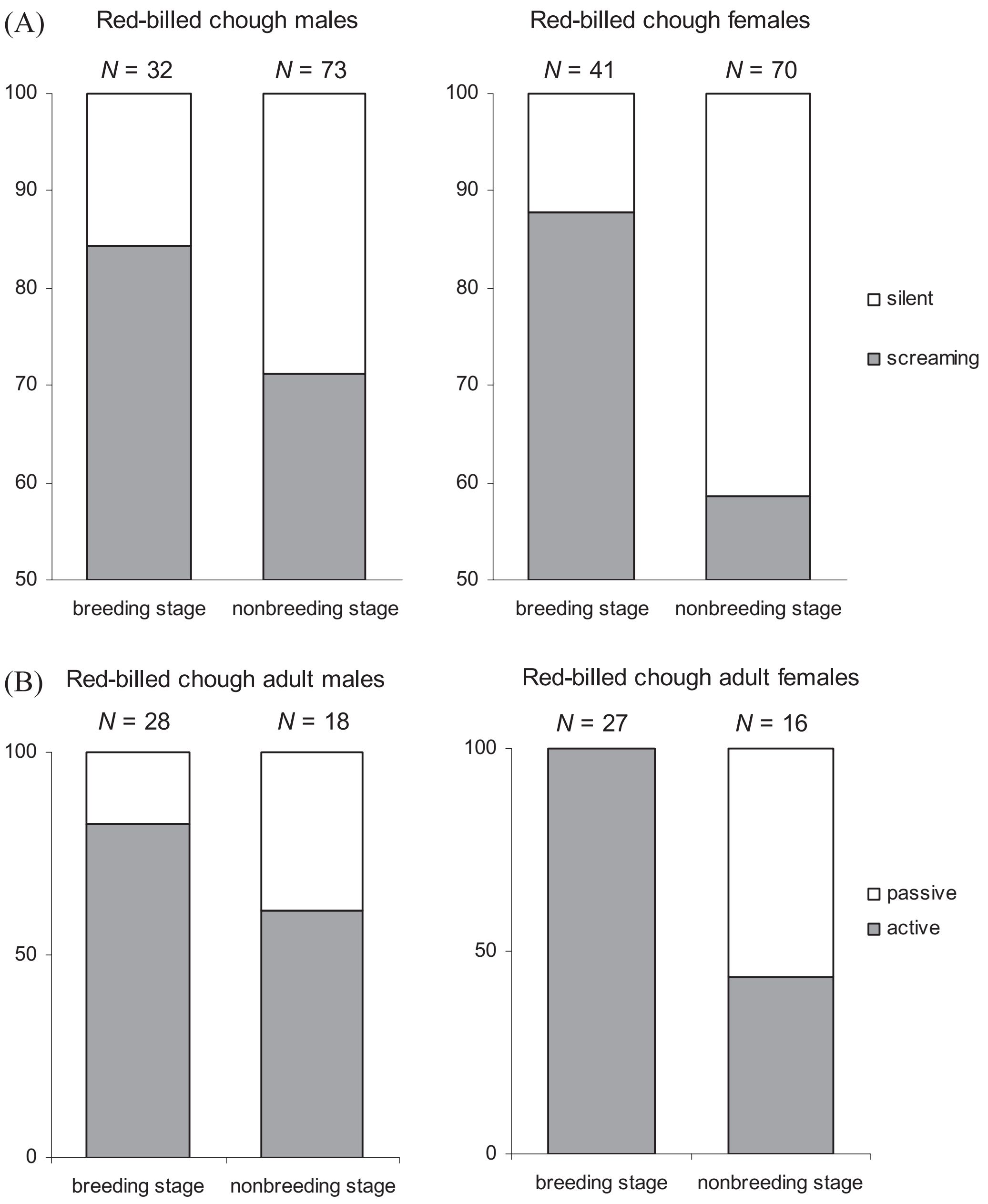

Figure 3. A, relative proportions of screaming and silent individuals in the sample of captured red-billed choughs. B, relative proportions of active and passive individuals in the sample of red-billed choughs (only adult are shown). Sample sizes are also shown.

DISCUSSION

The magnitude of individual aggressiveness and screaming in response to the stress of capture varied among red-billed chough individuals, and differences were mostly associated with the sex and age of birds, with their condition, and with the time of the year that they were trapped. The *H*/*L* ratio, which reflects the level of physiological stress, was one of the most important predictors of red-billed chough aggressive and acoustic behaviour together with the time of the year. Physiologically stressed birds were more active during handling and gave distress vocalizations. In a study on the Chinstrap penguin (*Pygoscelis antarctica*), Martín *et al.* (2006) showed that weaker chicks were more frightened when facing a threat, and that birds in worse body and immunological conditions had a stronger response to approaching predators, fleeing sooner and running farther away. The association between bird behaviour and physiological

Table 2. Ranking of the models used to explain variation in the four principal components (PC) analysis scores of distress call spectrotemporal parameters

	Hypothesized model	AIC$_{\mathrm{c}}$	ΔAIC$_{\mathrm{c}}$	*P*-values of each model predictor	Overall explained deviance (%)
PC1 (inverse call rate factor)	Period + *H/L* ratio + age + wing length	240.93	0.00	(0.0097) + (0.67) + (0.047) + (0.085)	39.22
	H/L ratio + age + wing length	241.76	0.83	(0.59) + (0.14) + (0.0041)	36.81
	H/L ratio	249.29	8.36	(0.69)	27.11
	Young leucocytes	249.44	8.51	(0.90)	27.00
	Haemoglobin	256.44	15.51	(0.88)	24.16
	Wing length + age	328.30	87.37	(0.0016) + (0.063)	12.71
	Wing length	331.28	90.35	(0.009)	8.80
	Body weight + tarsus length + wing length	332.82	92.07	(0.35) + (0.77) + (0.001)	9.57
	Age	333.67	92.74	(0.019)	9.64
	Period + age + wing length	334.55	93.62	(0.0084) + (0.020) + (0.045)	14.41
	Period	339.59	98.66	(0.012)	5.06
	Sex	343.35	102.42	(0.12)	2.03
PC2 (inverse peak frequency factor)	*H/L* ratio + sex + age	244.08	0.00	(0.32) + (0.012) + (0.38)	35.03
	H/L ratio + wing length	245.50	1.41	(0.33) + (0.035)	32.09
	H/L ratio + wing length + age + sex	246.47	2.39	(0.32) + (0.08) + (0.38) + (0.08)	35.04
	H/L ratio	247.73	3.65	(0.34)	28.40
	Young leucocytes	247.81	3.72	(0.36)	28.36
	Haemoglobin + young leucocytes	249.83	5.74	(0.35) + (0.36)	28.54
	Haemoglobin	252.55	8.46	(0.67)	27.46
	Body weight + tarsus length + wing length	338.10	94.02	(0.38) + (0.42) + (0.19)	5.88
	Wing length	338.54	94.46	(0.17)	2.97
	Sex	341.64	97.56	(0.042)	3.40
	Period	342.78	98.70	(0.086)	2.48
	Age	345.09	101.00	(0.70)	0.60
PC3 (fundamental frequency factor)	Period + *H/L* ratio + wing length + age	222.95	0.00	(0.010) + (0.22) + (0.060) + (0.03)	50.82
	H/L ratio + sex + age	226.88	3.93	(0.29) + (0.033) + (0.068)	46.85
	H/L ratio + age	229.02	6.07	(0.30) + (0.068)	43.99
	H/L ratio + wing length + age	229.54	6.59	(0.29) + (0.03) + (0.28)	45.25
	H/L ratio	232.20	9.25	(0.31)	40.28
	Young leucocytes	233.17	10.22	(0.78)	39.59
	Haemoglobin	239.76	16.81	(0.61)	37.27
	Body weight + tarsus length + wing length	320.32	93.44	(0.001) + (0.55) + (0.0002)	18.74
	Body size	328.17	105.22	(0.003)	11.00
	Sex	335.32	112.37	(0.0012)	8.20
	Age	336.58	113.63	(0.012)	7.26
	Period	341.19	118.24	(0.034)	3.60
PC4 (inverse harsh fraction factor)	Young leucocytes + haemoglobin	248.78	0.00	(0.039) + (0.040)	29.36
	Young leucocytes + *H/L* ratio × *H/L* ratio + *H/L* ratio + haemoglobin	249.73	0.96	(0.084) + (0.064) + (0.059) + (0.11)	32.38
	Young leucocytes	250.83	2.05	(0.043)	25.71
	H/L ratio × *H/L* ratio + *H/L* ratio	251.16	2.39	(0.018) + (0.015)	27.35

Table 2. *Continued*

Hypothesized model	AIC_c	ΔAIC_c	*P*-values of each model predictor	Overall explained deviance (%)
Young leucocytes + *H*/*L* ratio	252.83	4.05	(0.54) + (0.047)	25.91
Haemoglobin	254.38	5.61	(0.029)	25.85
H/*L* ratio	254.55	5.78	(0.55)	22.38
Body weight + tarsus length + wing length	333.40	84.63	(0.35) + (0.77) + (0.001)	9.12
Wing length	338.58	89.80	(0.11)	2.87
Period	342.71	93.93	(0.085)	2.46
Age	342.93	94.16	(0.26)	2.29
Sex	344.05	95.28	(0.21)	1.36

The best models are those separated by less than 2 points on the bias-corrected version of Akaike's Information Criterion (AIC_c) ($\Delta AIC_c < 2$) from the most probable model. *P*-values of each predictor in the model and the overall explained deviance are also shown.
H/*L*, heterophil/lymphocyte ratio.

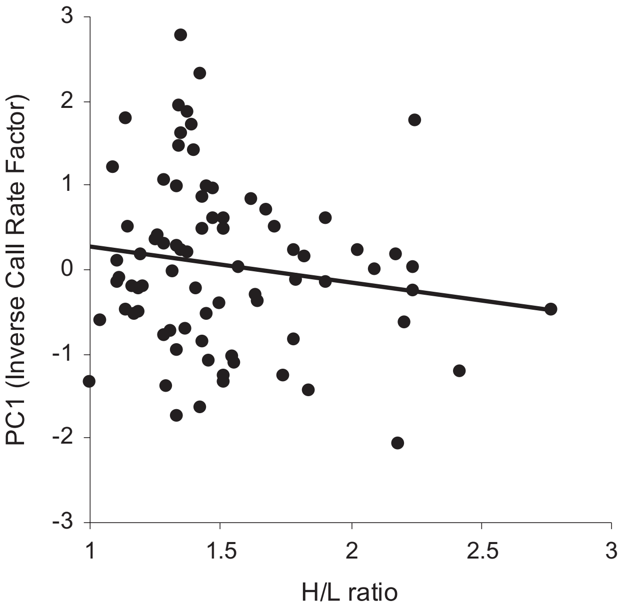

Figure 4. Relationships between the first principal components analysis score of acoustic data (inversely correlated to call rate) and heterophil/lymphocyte (*H*/*L*) ratio. The five most extreme values of *H*/*L* ratio (range 3.76–5.25) were excluded as outliers.

stress could be explained if we take into account that vertebrates respond to acute stressors with the immediate secretion of adrenocorticosteroid hormones into the blood, which prepare the organisms to cope behaviourally and physiologically with the event. In both wild birds and poultry, these increases in plasma levels of corticosterone can elicit a concomitant augment of *H*/*L* ratios (Maxwell, 1993; Newman *et al.*, 2005).

The association between active behaviour and physiological stress was also supported by the resulting behavioural shifts between breeding and non-breeding stages, between sexes, and between age classes. Several vertebrates modulate their stress susceptibility seasonally in relation to individual physical condition and breeding state (Nelson *et al.*, 2002). In the breeding season, the intense competition for mates, nesting or foraging sites lead to increased stress responsiveness (Romero, 2002). Hence, the active response of captured red-billed chough in the breeding period, and in the adult stage, might be associated with such increase in susceptibility. In the present study, females were more active than males during the breeding period. It has been shown that sex is an important predictor of vulnerability to stress, although responses are often contrasting among species (Carere & van Oers, 2004). In the case of breeding red-billed choughs, adults were captured just before the critical egg-laying phase, when females were facing higher energetic costs than males, and this might have enhanced sex-differences. In support of this hypothesis, we found that adult females have greater *H*/*L* ratios during than after breeding (two-tailed *t*-test: $t_{30} = 2.09$; $P = 0.044$). Outside this energy-demanding period, aggressiveness of adult females dropped to values even lower than those of males (Fig. 3). In spite of the above relationship between breeding and the *H*/*L* ratio, the time of the year was indeed a significant predictor of bird behaviour irrespective of the *H*/*L* ratio. The observed aggressiveness during breeding might be associated with nest defence, and the necessity in protecting eggs and nestlings from predators (Montgomerie & Weatherhead, 1988). In the study area, brooding females were only occasionally preyed (1% of breeding females only), but nest predation was high (50% of nests; Banda, 2007).

Besides affecting the occurrence of active behaviour and stress vocalizations, sexes and age classes also differed in the acoustic features of distress calling. Female, yearling and juvenile red-billed choughs gave calls with greater fundamental and peak frequencies than males and adults in general. This is in line with studies showing that the smaller individuals (young and females in the red-billed chough) give higher pitched calls than the larger ones because the frequency of vocalizations normally depends upon the size of vocal apparatus, which in turn correlates with body size (Laiolo *et al.*, 2000; Laiolo & Rolando, 2002).

In their study on lesser short-toed lark *Calandrella rufescens*, Laiolo *et al.* (2004) found a correlation among bird health or body condition and the spectrotemporal characteristics of distress calls, and hypothesized that birds may reveal their health quality to potential predators through the acoustic features of their distress calls. Lesser short-toed larks give distress calls when flying away; this behaviour may convey information on bird escaping qualities, aiming to discourage the predator from further chasing (Laiolo *et al.*, 2004). Conversely, the calls of the red-billed chough are uttered during handling, which would correspond to the situation where the prey is already grasped by the predator. Thus, it appears unlikely that the species is communicating (its stress) to the predator. We cannot exclude an alternative hypothesis proposing that the signal receiver may be a conspecific (Bergstrom & Lachmann, 2001). The red-billed chough is a social species, and the screaming of birds that feel in poor condition may serve to warn and attract flock and roost mates that could mob the predator and thus help the caller to escape. The fact that passive birds also utter screams suggests that calling is not just a consequence of the anxious attitude of birds, and the observation of captured red-billed choughs attracting several conspecifics provides further evidence to support this alternative hypothesis. In their study on another corvid, the Siberian Jay *Perisoreus infaustus*, Griesser & Ekman (2005) and Griesser (2008) found that different alarm calls were able to elicit situation specific responses from other group members, and that these calls were most commonly directed to kin group members.

In conclusion, the present study reveals the complex nature of distress calling and its compound association with aggressive behaviour. Observed behavioural patterns can respond to individual susceptibility to stress, and can depend upon a species life history traits. The response may also vary in relation to the bird's experience of the stressor event, which could affect its immediate stress copying behaviour. In the light of our findings, we suggest that studies evaluating the levels of both stress-induced and baseline glucocorticoids in species with different behavioural strategies during capture should be carried out to better clarify the nature of these strategies, namely as pursuit-deterrent behaviours or unavoidable responses to fear and captive situation (Högstedt, 1983; Hasson, 1991).

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