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Individual variation in corticosterone and personality traits in the blue tit *Cyanistes caeruleus*

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1 **Summary**

2

3 Variation in personality traits is predicted to reflect physiology, but the extent to which variations in
4 stress hormones derive from differences in personality and/or state-dependent factors remains
5 unclear. To investigate this, wild blue tits *Cyanistes caeruleus* were briefly brought into captivity and
6 scored for personality and corticosterone (Cort) concentrations. More active females had lower
7 baseline Cort than less active individuals. Exploratory tendency and neophobia did not co-vary with
8 baseline Cort. Stress-induced Cort concentrations were correlated negatively with exploratory
9 tendency and haematocrit, but positively with mass gain in captivity. Baseline and stress-induced
10 Cort concentrations in wintering blue tits were associated with state-dependent variables sex, age
11 and personality traits. Key to interpreting the physiology of personality traits seems to be their
12 interactions with other traits that mediate ability to utilise resources, and thus influence an
13 individual's perception of its current and future energy balance.

14

1 Introduction

2

3 When exposed to the same environmental or social stimuli, under standardised conditions, the
4 behavioural (Verbeek et al., 1996; Gosling, 2001) and physiological responses (Cockrem, 2007) of
5 individuals of the same species often differ. Behavioural differences that are stable within individuals
6 measured repeatedly across a range of situations or contexts are often referred to as 'personality
7 traits' (Gosling, 2001). While the heritability of personality traits (Drent et al., 2003; Oers et al., 2004;
8 Oers et al., 2005; Martins et al., 2007) suggests that they have a genetic basis, they can also be
9 affected by early environmental conditions (e.g. (Arnold et al., 2007; Stamps & Groothuis, 2010).
10 Similarly, both genes and early environmental conditions have been shown to affect physiological
11 systems such as the stress axis of adults (Monaghan, 2008; Koolhaas et al., 2010). The consistent
12 nature of these differences between individuals suggests they may contribute towards variability in
13 fitness-related traits among individuals (Buchanan, 2000; Both et al., 2005; Blas et al., 2007; Bonier
14 et al., 2009).

15 In birds, the physiological stress response is modulated by both basal levels and the acute
16 release of the adrenal steroid corticosterone (Cort), amongst other hormones. Basal concentrations
17 of Cort have been reported to vary in response to environmental variables such as weather
18 (Astheimer et al., 1995; Rubenstein, 2007) food availability (Jenni-Eiermann et al., 2008), as well as
19 state-dependent variables such as an individual's body condition (Raja-aho et al., 2010) or health
20 status (Martin, 2009). These changes in basal Cort can trigger a series of physiological and
21 behavioural changes that should restore homeostasis. For example the increase in Cort seen
22 following food restriction simulates increased motivation to feed (Lohmus et al., 2003; Long &
23 Holberton, 2004; but see Piersma et al., 2000). Chronic elevations of basal Cort, due to long term or
24 repeated exposure to a stressor, have been reported to have negative effects, suppressing insulin
25 production hence rendering the animal prone to fatigue and disease (reviewed in Buchanan, 2000,
26 see also (Goutte et al., 2010; Hau & Goymann, 2015). However, high basal Cort might not necessarily

1 signal challenging conditions: basal Cort also varies consistently across individuals under the same
2 conditions, with some individuals showing relatively high and others showing low levels of Cort. The
3 extent to which such differences in basal Cort reflect individual differences in personality and fitness
4 are still the focus of discussion (Bonier et al., 2009; Angelier et al., 2010; Dingemanse et al., 2010;
5 Koolhaas et al., 2010; Hau & Goymann, 2015).

6 Rapid on-set, acute stress responses evoked in response to an 'emergency' often involve an
7 immediate increase in blood glucose, to facilitate rapid behavioural reactions such as fight or flight
8 (Cockrem & Silverin, 2002a) and are generally considered to be adaptive (Buchanan, 2000). In Parids,
9 like many small passerines (Wingfield & Kitayskya, 2002), the acute stress response is generally
10 characterised by a rise in Cort that usually peaks between ten and approximately 40 minutes after
11 the stressor before declining (Cockrem & Silverin, 2002b; Henderson et al., 2014)). The level and
12 timing of the peak in Cort varies both within and between species (Bokony et al., 2009; Cockrem et
13 al., 2009; Henderson et al., 2014). Extensive blood sampling is not possible in small birds, so
14 researchers generally present data on 'stress-induced' (rather than strictly peak) Cort
15 concentrations, measured after an animal has been held for a set time under standardised restraint
16 conditions (Wingfield, 1994). As physiological responses to stress are often accompanied by changes
17 in behaviour, there is a prediction, with some supporting data, that personality types might show
18 associated consistent differences in both basal and acute Cort levels (Cockrem, 2007; Hau &
19 Goymann, 2015). These effects might be directly related, for example more neophobic juvenile rats
20 have a higher glucocorticoid response as adults (Cavigelli & McClintock, 2003), or indirect, for
21 example where personality affects the ability to acquire or control food, which might have an impact
22 on both stress-induced and baseline Cort (Koolhaas et al., 2010). Similarly, age, sex and dominance
23 status can affect resource holding potential and thus energetic balance (Gosler, 1996); Fat reserves
24 reflect a trade-off between starvation (high fat reserves are beneficial) and predation (high fat
25 reserves slow down escape flight, so are costly) resulting in great tits *Parus major* living under high
26 perceived predation risk carrying lower fat reserves than under low perceived predation risk.

1 Moreover, dominant birds (adult males) carried the least fat, presumably because they were able to
2 displace subordinate birds (juvenile females) at feeders, so had a low risk of starvation despite their
3 low fat reserves (Gentle & Gosler, 2000). Thus, age, sex and dominance status might also influence
4 both baseline and stress-induced Cort (Rubenstein, 2007). So although there is evidence of
5 correlations between an individual's personality and its glucocorticoid profile (Carere et al., 2003;
6 Cavigelli & McClintock, 2003; Cockrem, 2007; Martins et al., 2007; Cavigelli et al., 2009; Koolhaas et
7 al., 2010), causality is difficult to assign.

8 Assessments of 'personality traits' in animals result in individuals being traditionally defined
9 as falling somewhere on a linear continuum: neophobic-neophilic or bold-shy (Wilson et al., 1994);
10 aggressive-passive; (Huntingford, 1976); active-inactive (Sih et al., 1992) and fast-slow explorers
11 (Verbeek et al., 1994). Such personality traits are often highly correlated within individuals, for
12 example activity and exploration (Dingemanse et al., 2007; Martin & Reale, 2008), or boldness and
13 aggression (Bell, 2005). A commonly described trait correlation is the "proactive-reactive" syndrome
14 (Carere, 2003; Koolhaas et al., 2007). Such correlations imply proximate links between traits, via
15 genetic linkage or shared physiology (Verbeek et al., 1994). In a review, Cockrem (2007) concluded
16 that across avian species, birds with 'proactive' (bold, fast exploring, aggressive etc.) personalities
17 have relatively active behavioural responses and low stress-induced Cort concentrations, whilst birds
18 with reactive personalities have relatively passive behaviour and large stress-induced Cort
19 concentrations. However, correlations can also occur between traits that are not mechanistically
20 connected but rather subject to the same selection pressures or jointly determined by other factors
21 (Bell & Sih, 2007; Dingemanse et al., 2007; Ruiz-Gomez et al., 2008; Carere et al., 2010).

22 Furthermore, not all species show correlations between such suites of behaviours (e.g. (Bell & Sih,
23 2007; Herborn et al., 2010; Herborn et al., 2011) which would suggest that the regulation of these
24 traits may differ between species. Thus, while neophobia and exploratory tendency are correlated in
25 many but not all species (Herborn et al., 2010) they could differ in their associations with baseline
26 and stress-induced Cort concentrations within individuals and across populations. This is in line with

1 Cockrem et al.'s (2009) assertion that there is no optimum Cort response for all conditions that
2 maximises fitness.

3 Whether or not an individual's hormonal stress response is a function of environmental and
4 state-dependent variables or associated with personality is important for understanding the
5 mechanisms underlying fitness variation. In this study, we investigated the environmental, state-
6 dependent and behavioural factors that might be associated with inter-individual differences in
7 baseline and stress-induced Cort in wild blue tits *Cyanistes caeruleus*. In contrast to studies of the
8 closely related great tit (Verbeek et al., 1996), we have previously shown that neophobia and
9 exploratory tendency are uncorrelated in blue tits (Herborn et al., 2010). This would suggest that in
10 the blue tit these traits can potentially differ in their relationships with basal and/or stress-induced
11 Cort. Moreover, we have found that personality types differed in antioxidant defences, and it was
12 the combination of an individual's personality traits that proved important (Arnold et al., 2015). This
13 suggests that the physiology underlying personality traits is complex. Here, we specifically addressed
14 whether an individual's baseline or stress-induced plasma Cort concentrations were correlated with:
15 1) weather variables on the day of capture, as these would affect opportunities to forage and thus
16 metabolic state; 2) personality traits (neophobia and two measures of behaviour in a novel
17 environment: exploratory tendency and activity); 3) state-dependent variables (condition,
18 haematocrit, change in mass in captivity and motivation to feed) and permanent inter-individual
19 differences (or variables that would not change within a season i.e sex, age and size), as these may
20 also affect foraging opportunities in the wild and therefore hormonal status.

21

22 **Material and methods**

23

24 The study was conducted over two winters between 2007 and 2009 on a population of blue tits
25 living in oak dominated woodland on the east bank of Loch Lomond, UK (56°08'N 4°37'W) (Arnold et
26 al., 2007). In October 2007, eight feeding stations were established at approximately 500m intervals

1 (Herborn et al., 2010). These feeding stations were removed at the end of February 2008 and
2 reinstalled in the same positions between October 2008 and February 2009. Between November and
3 February, birds were captured as they approached the feeding stations, using mist-nets. Mist-netting
4 was conducted between dawn and noon, three times at each feeding station in the winter of 2007-8
5 and twice in 2008-9. Birds arrived in captivity generally between 10:00 and 12:00, within 15 minutes
6 journey time from their capture site. A total of 125 blue tits were caught, with 69 birds (54 male: 15
7 female) for which we had a Cort sample and a full set of behavioural data being included in this
8 study. For the other 56 individuals, in most cases we did not have a large enough blood sample to
9 run the Cort analyses as well as other planned blood assays, in a few cases the bird did not perform
10 properly in one of the behavioural trials so had to be excluded and/or a morphometric measurement
11 was missing. This sex ratio bias reflected that of the population of birds caught at the feeders
12 (Herborn et al., 2010). Birds were then taken into captivity for 2 days in 2007/8 and 3 days in 2008/9.
13 In 2007/8 roughly two hours after blood sampling, birds were then released at the site of capture. In
14 2008/9, birds were released on day 3 following the second exploration trial.

15

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Environmental variables on the day of capture

17 Variables that may have affected foraging immediately prior to capture and hence metabolic state
18 were recorded. These included day length, rainfall (mm) and minimum and maximum temperature
19 for the day of capture. Weather data were collated from records for Glasgow Bishopton sent to us
20 by the Met office.

21

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Personality traits

23 After removal from the mist-net, birds were held in cloth bags and transported, up to 3km, to the
24 Scottish Centre for Ecology and the Natural Environment. After arrival birds were scored for their
25 morphometrics and housed indoors, at a temperature of $17^{\circ}\text{C}\pm 1^{\circ}\text{C}$ on a 12:12 hour light:dark cycle
26 (to enable us to conduct all of the tests and minimise the time the birds would be held in captivity

1 whilst standardising conditions across birds) . Trials ran from mid-November until the end of
2 January, during which time the natural daylength was at maximum 8hr30 and minimum 7hr. Each
3 bird was housed individually in a 150cm x 50cm x 50cm high cage, with six cages per room arranged
4 such that birds were visually but not acoustically occluded from one another. Birds were fed peanut
5 granules, Haiths' Prosecto™ insectivorous mix and water *ad libitum*, supplemented with around ten
6 *Tenebrio molitor* and two *Galleria mellonella* larvae per day. All birds were observed eating within 10
7 minutes of arrival in captivity. After arrival birds were left undisturbed for a minimum of 2 hours and
8 a maximum of 3 hours before the start of personality trait assessments. As we wanted to return the
9 birds to the wild as soon as possible, we did not allow the birds a long acclimation period in captivity.
10 We know that the personality traits measured in captivity predicted neophobia and exploratory
11 tendency measured for the same individuals in the wild weeks or even months later (Herborn et al.
12 2010). So we have some evidence that the short acclimation period was not having significant
13 negative impacts on our behavioural assays. Three behavioural traits, exploratory tendency, activity
14 and neophobia were assayed as fully described in Herborn et al (2010):

15 **Exploratory tendency** was assessed within what would become the home cage of the focal
16 bird. Each cage was split into two halves with an opaque cage divider (Figure S1 in Electronic
17 Supplementary material (ESM)). On arrival into captivity, the bird was introduced to one side of the
18 cage, selected at random, and allowed to acclimate for two hours thereby creating a “familiar” and,
19 behind the divider, a “novel” environment. To assay exploratory tendency and not neophobia, the
20 arrangement of plastic plants and perches was the same in each cage half, so that the novel
21 environment was novel only in that it was unexplored. At the start of the trial, the food bowl and any
22 spilt food were removed and 30 mins after that the water bowl was also removed, this was to
23 ensure that the birds did not spent the whole trial eating or drinking, thus making it difficult to
24 determine their motivation for moving. After a further 30 mins, the researcher removed the opaque
25 cage divider and observed the focal bird for 10 mins. Unlike other exploration trials (e.g. (Verbeek et
26 al., 1994), individuals had the option of remaining within the familiar environment. This allowed us

1 to distinguish activity due to exploration which is related to information gathering about a novel
2 environment, from activity due to escape behaviours which is not necessarily linked to information
3 gathering (Mettke-Hofmann et al., 2009). The number of movements (hop or flight) in each side of
4 the cage was recorded, with the endpoint of each movement defining the side of the cage: novel or
5 familiar. Exploratory tendency was calculated as the number of movements in the novel
6 environment minus the number of movements in the familiar environment. In 2008/9, the
7 exploratory tendency trial was repeated between 08:00 and 011:00 on day 3 of captivity before
8 releasing the birds. This was to allow us to calculate the repeatability of this behavioural trait. To
9 create a new novel environment, the paper lining the cage was changed from white to brown or vice
10 versa. Also, the arrangement and size of perches and artificial plant material were similar between
11 these cage types, but different leaf shapes were used in the brown versus white-lined cages.

12 **Activity** was derived from the same exploration trial, as the number of movements in both
13 the familiar and novel side of the cage. We described behaviour in the exploration trial in these two
14 ways to determine whether patterns relating to exploration were due to individual variation in true
15 exploratory tendency, as reflected by space use within the trial, or simply activity levels, as
16 commonly reported in other exploration studies (Verbeek et al., 1994; Martins et al., 2007).

17 **Neophobia** was assessed over two trials conducted between 13:00 and 17:00 on day 1 and
18 08:00 and 11:00 on day 2. Each trial had two phases: a novel object phase and a disturbance control
19 phase. Food and water were removed for 30 mins prior to each phase. Water was removed so that
20 during the experiment only one resource, food, was available. In the novel object phase, the
21 observer then returned the food bowl with one of two novel objects (different novel objects were
22 used in the two trials) placed inside. The objects were a bright pink plastic frog and half of a purple
23 rubber ball, of similar size (approximately 4cm diameter and 2cm height) (Figure S2 in ESM). The
24 latency to land on familiar food bowl was recorded. The object was then removed and the water
25 returned. The disturbance control phase was completed so as to allow interpretation of the results
26 relative to any possible differences in an individual's motivation to feed, or their response to

1 disturbance by the observer returning the food bowl to the cage (van Oers et al., 2005). Thus in a
2 random order either one hour before or after the novel object phase, the latency to feed was
3 measured by food bowl removal and replacement as before but without a novel object. Neophobia
4 was defined as the latency in the novel object phase minus the latency in the disturbance phase.
5 Mean latency to approach the food bowl was significantly greater when a novel object was present
6 (Wilcoxon Signed ranks $Z = -3.34$, $N = 68$, $p = 0.001$), than when absent. Thus the presence of the
7 novel object modified behaviour so could be said to induce neophobia.

8 Repeatability of neophobia, exploratory tendency or activity scores between day 1 and day 2
9 was calculated using the mean squares from an analysis of variance, following Lessells and Boag
10 (1987). Neophobia across days 1 and 2 (ANOVA; $r = 0.29$, $F_{63,68} = 1.811$, $p = 0.009$), as well as
11 exploratory tendency ($r = 0.27$, $F_{36,68} = 1.71$, $p = 0.04$) and activity ($r = 0.42$, $F_{36,68} = 2.56$, $p = 0.001$)
12 across days 1 and 3 were repeatable. In the following results, only data from the first of each trial
13 per individual were analysed as they were therefore considered representative of the individual's
14 personality traits but ensured no carry-over effects through learning or habituation to the
15 experiments. This also meant that we had a larger sample size for exploratory tendency and activity
16 than if we had taken a mean of both trials.

17

18 *State-dependent and permanent inter-individual differences*

19 Next, we determined whether state-dependent (i.e. changeable within a season) variables, and
20 permanent inter-individual differences (fixed at least within one season) predicted an individual's
21 Cort concentration. An individual's state was assessed as its body condition (residual of mass at
22 blood sampling against tarsus length), haematocrit and change in mass in captivity (mass at blood
23 sampling after 24 hours of captivity – mass at capture).

24 The duration of the first and second feeding bouts in each disturbance trial (see below) were
25 taken as a measure of hunger and motivation to feed. Neither of these measures differed
26 significantly between the two years, juveniles and adults or between the sexes (Mann-Whitney U-

1 test $p > 0.11$ in all cases), so the data were pooled. The duration of the two feeding bouts were
2 correlated, i.e. consistent, within individuals (Kendall's tau $b = 0.23$, $N = 56$, $p = 0.027$), so from the
3 first disturbance trial we analysed just the duration of the second feeding bout as an index of hunger
4 and motivation to feed in case there was any disturbance associated with returning the bowl to the
5 cage in the first feeding bout.

6 Permanent variables (unchanged within a field season) were also assessed and recorded for
7 each bird. Wing length was used as a measure of overall body size (not measured in one bird). Age
8 (juvenile/adult) was determined from plumage traits (Svensson, 1992). Sex was determined from a
9 blood sample using primers P2 and P8 (Arnold et al., 2007).

10

11 *Blood sampling*

12 Approximately 24 hours after capture, birds were blood sampled. Birds were sampled after
13 undergoing behavioural assays as there is some evidence that blood sampling and/or associated
14 handling can change the physiology and behaviour of birds (e.g. (Le Maho et al., 1992; Cockrem et
15 al., 2009; Dickens et al., 2009). Moreover, in captivity all the birds had received *ad libitum* food,
16 constant temperature and light/dark cycles and minimal social interactions, thus many of the
17 environmental factors known to affect Cort concentrations had been standardised across individuals
18 (Romero et al., 2000; Pravosudov et al., 2001). During blood sampling, two birds per room were
19 removed from their cages with the order randomly assigned. For baseline Cort, birds were blood
20 sampled within 3 mins of the researcher entering the aviary room, which is generally considered to
21 be representative of baseline Cort concentrations (Romero & Reed, 2005). For the stress-induced
22 Cort, the bird was put into a cloth bag upon capture, removed after 14 mins and blood sampled 15
23 mins after initial disturbance. Caged birds were then left undisturbed for one hour before re-entry to
24 each room to collect samples from different birds. The last birds to be sampled had therefore been
25 disturbed a maximum of twice that day, but disturbance frequency did not significantly influence
26 either baseline (ANOVA $F_{2,35} = 0.065$, $p = 0.94$) or stress-induced Cort (ANOVA $F_{2,29} = 0.092$, $p =$

1 0.91). To collect the blood, a sterile 25 gauge needle was used to prick the brachial vein. Blood
2 samples were collected in 75 µl heparinized capillaries and centrifuged for 5 minutes at 14,000 g. A
3 haematocrit score (a measure of anaemia) was taken for each tube before the red cells and plasma
4 were split, red cells mixed with ethanol and samples stored at -70 °C until assayed. Less than 10% of
5 the circulating blood volume was collected, as defined by the UK Home Office. Collecting this volume
6 of is not considered to have negative impacts on the welfare of a bird. As we could only take enough
7 blood from each bird for a single Cort assay, due to ethical considerations and the limits defined by
8 the UK Home Office, we only have either baseline or stress-induced Cort concentration from each
9 individual.

10 *Corticosterone assays*

11 Corticosterone concentrations were measured after extraction of 5-20µl aliquots of plasma in
12 diethyl ether, using a double antibody radioimmunoassay (Wingfield et al., 1992). Primary antibody
13 Esoterix B183, Secondary antibody Sigma goat anti-rabbit and [3H]-corticosterone label (GE
14 Healthcare, UK). See Henderson et al. (2013) for full methods. Extraction efficiency was $93 \pm 8\%$
15 (mean \pm SD). Recoveries were measured for samples independently and individual corticosterone
16 concentrations adjusted accordingly. The assay detection limit was 0.03 ng/ml. Samples were run
17 within two assays. Inter- and intra-assay coefficients of variation were 6.68% and $4.35 \pm 0.001\%$
18 respectively.

19 *Ethical Note*

20 All work was carried out in accordance with ASAB/ABS's guidelines for the treatment of animals in
21 research. Work was under license of the UK Home Office and subject to ethical review by
22 WALTHAM® Centre for Pet Nutrition and the University of Glasgow. Captive studies were completed
23 and feeders removed 2 months before the first record of nest building in the area. Birds had on
24 average gained ($2.97\% \pm 7.3\%$) body mass whilst in captivity. 108 out of the total 125 birds released
25 were later recorded using the feeders or were re-trapped in the area.
26

1

2 *Statistical Methods*

3 Analyses were carried out using SPSS (Ver 20). Prior to analysis, data were checked for normality,
4 homogeneity of variance and co-linearity. Where these assumptions were violated, data were
5 transformed or non-parametric tests were carried out. The distribution of stress-induced Cort
6 concentrations, for example, showed a slight skew and a log transformation improved the situation.
7 In addition, Cook's distances were calculated for all co-variates and subsequently neophobia data
8 from three individuals and exploration data from one individual were excluded as extreme outliers
9 that would have distorted the outcome of the analyses. Exploratory tendency (Shapiro–Wilks test:
10 $W_{120} = 0.94$, $p < 0.0001$), activity during the exploration trial ($W_{120} = 0.95$, $p < 0.0001$) and neophobia
11 (Shapiro–Wilks test: $W_{78} = 0.89$, $p < 0.0001$) were leptokurtic and it was not possible to normalise
12 their distributions. Exploration scores (Mann-Whitney U-test Age: $z = -0.38$, $N = 64$, $p = 0.71$; sex: $z =$
13 -0.23 , $N = 60$, $p = 0.82$), activity levels (Age: $z = -0.39$, $N = 64$, $p = 0.70$; sex: $z = -0.94$, $N = 60$, $p = 0.35$)
14 and neophobia scores (Mann-Whitney U-test Age: $z = -1.25$, $N = 63$, $p = 0.21$; sex $z = -0.88$, $N = 59$, p
15 $= 0.38$) did not differ between adults and juveniles or males and females, therefore data were
16 pooled to analyse other sources of between-individual variation. With the Bonferroni correction
17 threshold p-value of 0.004, all other morphometric (mass at capture, mass at blood sampling, wing
18 length) and environmental variables (rainfall, minimum temperature and maximum temperature on
19 day of capture) were non-significant in relation to neophobia, exploratory tendency and activity.
20 Therefore analyses of these traits were calculated using actual scores, rather than, for example,
21 residuals or transformed data.

22 General linear models (GLMs) were run with Cort plasma concentration (basal or peak) as
23 the dependent variable, sex and age (adult or juvenile) as factors, and change in mass in captivity
24 (mass on day 2 – mass on day1), wing length, haematocrit and personality traits as co-variates. The
25 interactions between sex or age and personality traits or change in mass in captivity were also
26 included in the full model. All tests were two-tailed. Means \pm S.E. are presented.

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Results

Neither basal nor stress induced Cort concentrations differed significantly between years (Mann-Whitney U-test $z = -0.36$, $N = 69$, $p = 0.72$) so data were pooled. Baseline Cort concentrations varied among individuals, from 0.33 – 13.21 ng/ml. Stress-induced plasma Cort concentrations were significantly higher (mean = 10.55 ng/ml \pm 0.94, range from 1.32 – 23.83 ng/ml) than baseline concentrations (mean = 4.17 ng/ml \pm 0.55; GLM $F_{1, 66} = 30.81$, $p < 0.0001$), although we did not have both measures from the same individual.

Environmental variables on the day of capture

Neither baseline nor stress-induced Cort concentrations were significantly related with daily rainfall (Baseline: Kendall's tau = 0.13, $N = 38$, $p = 0.27$; Stress-induced: Kendall's tau = 0.07, $N = 28$, $p = 0.63$), minimum daily temperature (Baseline: Kendall's tau = 0.02, $N = 38$, $p = 0.87$; Stress-induced: Kendall's tau = -0.04, $N = 28$, $p = 0.75$) or maximum daily temperature on the day of capture (Baseline: Kendall's tau = 0.00, $N = 38$, $p = 1.0$; Stress-induced: Kendall's tau = 0.06, $N = 28$, $p = 0.63$).

Personality traits

As mentioned above, we only used data from the first of each trial per individual. In these first trials, neophobia scores varied from -117s to 597s with a mean of 85.59s \pm 19.18 (six birds scored zero and six birds failed to complete the trial), with positive scores indicating highly neophobic birds and negative scores meaning that the birds were not neophobic. Exploratory tendency ranged from -280 to 226 with a mean of 9.69s \pm 11.79 (three birds scored zero and two birds failed to complete the trial) and total activity level from 0 – 467 with a mean of 233.42s \pm 15.90 (two birds scored zero, i.e. did not move, and two failed to complete the trial). High scores indicated highly exploratory and active birds respectively.

1 Neophobia scores were not correlated with either exploratory tendency (Kendall's tau = -
2 0.04, N = 62, p = 0.69) or activity levels during the exploration trial (Kendall's tau = 0.01, N = 62, p =
3 0.89). Activity levels and exploratory tendency were uncorrelated (Kendall's tau = 0.051, N = 67, p =
4 0.54). Thus, exploratory tendency, activity levels during the exploration trial and neophobia are
5 distinct personality traits (i.e. were repeatable across time), not simply three measures of the same
6 trait or by-products of differences in activity levels. Also, we did not find evidence of a behavioural
7 syndrome.

8 Baseline Cort concentrations were significantly related to the interaction between sex and
9 activity ($F_{1,36} = 5.46$, $p = 0.026$; sex $F_{1,36} = 8.82$, $p = 0.006$, activity level $F_{1,36} = 10.09$, $p = 0.003$; Fig.
10 1a). Specifically, more active birds had lower baseline Cort than inactive birds. The slope of this
11 relationship was much steeper for females than males. Using a backwards stepwise process, non-
12 significant interactions then age, exploratory tendency (Fig. 1b) and neophobia (Fig. 1c) were
13 removed from the model. The sample size for females in this analysis was only six, so we repeated
14 the analysis just for males. For males there was no significant correlation between activity levels and
15 baseline Cort (Pearson correlation = -0.244, N = 31, $p = 0.185$).

16 Stress-induced Cort was uncorrelated with activity ($F_{1,28} = 0.02$, $p > 0.8$; Fig. 1d). There was a
17 non-significant tendency for more neophobic birds to have lower stress-induced Cort concentrations
18 than less neophobic birds ($F_{1,24} = 3.40$, $p = 0.079$; Fig. 1e). Stress-induced Cort was significantly
19 associated with exploratory tendency ($F_{1,24} = 5.04$, $p = 0.035$; Fig. 1f), with more exploratory
20 individuals having lower stress-induced Cort concentrations than slow explorers.

21
22

State-dependent and permanent inter-individual differences

23 Mass at capture did not differ between age classes (adult mean = 11.24 ± 0.11 g, juvenile mean =
24 11.15 ± 0.11 g; GLM $F_{1,64} = 0.38$, $p = 0.54$) neither did condition at capture (GLM $F_{1,64} = 0.66$, $p = 0.43$).
25 At capture, males weighed more (male mean = 11.33 ± 0.09 g, female mean = 10.89 ± 0.09 g; GLM
26 $F_{1,67} = 6.12$, $p = 0.016$) and were in better condition (GLM $F_{1,66} = 6.46$, $p = 0.013$) than females. Birds

1 that were in poor condition upon capture put on significantly more mass in captivity than those in
2 better condition (Kendall's tau = -0.32, N = 68, $p < 0.0001$).

3 Following GLM modelling to investigate the relationship(s) between baseline Cort
4 concentrations and state-dependent traits, all variables dropped out of the model ($p > 0.2$) except
5 the interaction between age and change in mass (GLM $F_{1,35} = 5.92$, $p = 0.021$; age $F_{1,35} = 2.76$, $p =$
6 0.11 ; change in mass $F_{1,35} = 0.36$, $p = 0.55$; Fig. 2). Baseline Cort was lower in juveniles that increased
7 in mass whilst in captivity than compared with those that lost mass, with the opposite relationship in
8 adults.

9 When the above model was re-run with stress-induced Cort as the dependent variable;
10 change in mass ($F_{1,28} = 5.26$, $p = 0.030$; Fig. 3a) and haematocrit level ($F_{1,28} = 5.40$, $p = 0.044$; Fig. 3b)
11 remained in the GLM. Across all individuals, birds that put on more mass in captivity, typically those
12 that were in poor condition at capture, had higher stress-induced Cort concentrations than those
13 that lost mass in captivity. Also, birds that had lower haematocrit levels (i.e. were more anaemic)
14 had higher stress-induced Cort concentrations than those with higher haematocrit scores.

15 Change in mass and haematocrit contributed additively to the model and were not
16 correlated with one another (Kendall's tau b = - 0.13, N= 65, $p = 0.14$). Age, sex, condition at blood
17 sampling and feeding bout duration (an index of hunger and motivation to feed on day 1) dropped
18 out of the model.

19

20 **Discussion**

21

22 In this study, we investigated extrinsic and intrinsic correlates of baseline and stress-induced plasma
23 Cort concentrations in wild blue tits held under standard conditions. Both baseline and stress-
24 induced Cort concentrations varied widely despite all animals being maintained at a standard
25 temperature with *ad libitum* food availability for the 24 hours prior to blood sampling. Weather on
26 the day of capture, which might indicate the condition of birds on entry to the study, was not related

1 to Cort. Notably, neophobia and exploratory tendency, although significantly repeatable within
2 individuals, at least over a 2-3 day interval, did not predict and were not related to baseline Cort
3 concentrations. There was a hint that birds that were generally more active in the exploration trial
4 had lower baseline Cort than less active individuals, but this was driven by data from a small number
5 of females. Unfortunately, given the small number of females sampled, we cannot interpret this sex-
6 difference further. With stress-induced Cort, more exploratory individuals had lower Cort
7 concentrations than less exploratory birds. In contrast, there was a tendency for more neophobic
8 birds to exhibit lower stress-induced Cort than less neophobic birds. There were also relationships
9 between Cort and individual state. Variation in baseline Cort concentrations was determined by a
10 significant interaction between age and change in mass in captivity. Juveniles that had gained mass
11 in captivity had a lower baseline Cort concentration than those that declined in mass. Conversely
12 adults that put on more mass in captivity had higher baseline Cort than those that lost mass. With
13 stress-induced Cort though, all birds that put on mass in captivity had higher stress-induced Cort
14 concentrations than those that lost mass, although condition at blood sampling did not contribute
15 significantly to the model. Birds with a low haematocrit level also had higher stress-induced Cort
16 concentrations than birds with higher haematocrit levels.

17 Our mixed evidence for associations between behavioural responses to novel and
18 potentially stressful situations and physiological responses to stress are intriguing but not without
19 precedent (Carere et al., 2003; Martins et al., 2007; Scheid & Noe, 2009; Schoech et al., 2009; Scheid
20 & Noe, 2010). One interpretation of our results is that our behavioural assays did not generate
21 neophobia in blue tits. This was unlikely because most birds took significantly longer to approach the
22 food bowl in the presence of the novel object than in the disturbance phase, although a few
23 individuals were clearly not neophobic, approaching the food faster in the presence than absence of
24 the novel object. This is consistent with the notion that the object created a motivational conflict
25 between desire to feed and to avoid the object which is perceived as a risk (Mettke-Hofmann et al.,
26 2002). However, whether the novel objects induced 'stress' is harder to determine. The classic flight

1 or fight response to acute stress, is typically (Buchanan, 2000) but not always (Muller et al., 2006)
2 associated with an elevation in Cort in wild animals. The few studies on birds comparing Cort before
3 and after presentation of a novel object, or more broadly Cort concentrations in high neophobic
4 versus low neophobic individuals, find different responses depending on the experimental design.
5 Generally, those placing a novel object with food identify a Cort response but those presenting the
6 novel object in a neutral location do not (Daisley et al., 2005; Martins et al., 2007; Tobler & Sandell,
7 2007; but see Apfelbeck & Raess, 2008). This absence of a Cort response (although of course other
8 stress hormones might have been elevated) in neutral studies comes even when accompanied by
9 typical “stress” behaviours – e.g. aversions, tonic responses, distress call (Daisley et al., 2005).
10 Furthermore, within the same species, latency to approach a novel object in a neutral location is not
11 necessarily correlated with latency to approach a novel object with food, suggesting they stimulate
12 different behaviours (Mettke-Hofmann et al., 2002). However, stereotypical stress behaviours are
13 not necessarily evidence of physiological stress (Muller et al., 2006). Neophobia, i.e. the avoidance of
14 novelty, might be a behavioural strategy to avoid a potentially stressful situation. Therefore, we
15 cannot assume that neophobia is an index of responsiveness to stressors in general. Neophobia is
16 simply a behavioural response to something unfamiliar which may or may not be dangerous or
17 stressful. Indeed, contrary to predictions, we found a tendency for more neophobic birds to show a
18 lower hormonal stress response than low neophobia birds, but this needs to be interpreted with
19 caution. Moreover, we also have the issue that we might have captured a biased sample of the least
20 neophobic individuals in the population. Certainly we caught fewer females than males which might
21 suggest that we were lacking data on individuals who were subordinate at feeders, but also
22 potentially those that avoided novel food sources in the wild. The ecological significance of
23 neophobia in the wild is still to be fully assessed.

24 Exploration has been previously shown to be linked with stress-induced Cort in some but
25 not all species studied (reviewed in Cockrem, 2007), although interpretation of the patterns can be
26 complex. For example, in captive populations of passerines subject to selection on either

1 behavioural or hormonal responses to stressors, Cort and behaviour are sometimes related: In two
2 lines of great tits (*Parus major*) selected to be either fast or slow explorers, Carere et al. (2003)
3 found that slow exploring birds had higher stress-induced Cort metabolites in their faeces and less
4 impairment in activity than fast birds when stressed (Carere et al., 2003). In zebra finches
5 (*Taeniopygia guttata*), over five generations researchers selected for high or low stress-induced
6 Cort. In the low Cort line, stress-induced Cort concentration was positively correlated with
7 exploratory behaviour but no relationship was present in the high Cort or control lines (Martins et
8 al., 2007). Thus, the results were ambiguous, but provide some evidence of heritable coping styles
9 suggesting selective maintenance of variation in Cort secretion patterns within populations. In
10 contrast to these studies in which the measurement of exploration and activity were confounded
11 (Carere et al., 2003; Martins et al., 2007) we found no relationship between exploration and baseline
12 Cort. On the contrary, experimentally increasing circulating Cort can increase activity levels in birds
13 (Breuner et al., 1998). Thus we suggest that exploratory tendency *per se* is not a behavioural
14 indicator of chronic stress, or that individuals that are chronically stressed are less likely to gather
15 information about a novel environment. Comparison of our behavioural results with those of others
16 (Marchetti & Drent, 2000; van Overveld & Matthysen) suggests that interpretation of “exploratory
17 tendency”, and particularly its importance in explaining differences in variation in information
18 acquisition and use, is dependent on the method by which exploration is scored. The differing
19 patterns of covariation between circulating Cort concentrations and personality traits suggests that
20 the physiological mechanisms and potentially the costs of different personality traits will vary. Thus,
21 we would emphasize the need to check the assumptions that exploration, activity and neophobia /
22 risk responsiveness are always correlated or that they represent three measures of the same trait
23 (Reale et al., 2007).

24 One issue to be explored is the impact of bringing birds into captivity on baseline Cort
25 measurements and both behavioural and hormonal stress responsiveness. In order to standardise
26 the food, temperature and day length experienced by the birds and to ensure that time spent in the

1 mist net did not influence the baseline Cort measure (Romero & Reed, 2005), we decided to blood
2 sample birds after 24 hours in captivity. However, it might be considered that this reflects a
3 disturbed state. Recent work by Dickens et al (2009) on chukars (*Alectoris chukar*) showed that on
4 the first day of captivity (which involved transport of 100km rather < 3km as in our study) birds lost
5 weight, haematocrit decreased and both baseline and stress-induced Cort concentrations were
6 raised. These effects took 3 to 9 days to fully attenuate. In contrast, we found that after 24 and 48
7 hours of captivity, the majority of blue tits had gained rather than lost body mass. Moreover, the
8 plasma concentrations of Cort we measured in captivity were similar to those measured in the field
9 at the capture site (basal Cort in the field in 2008 only: 3.2 ng/ml \pm 1.8, n = 36; in captivity: 4.2 ng/ml
10 \pm 3.1, n = 16, t-test P = 0.22; (Henderson et al., 2013)). Thus, the elevated stress markers seen in
11 chukars (Dickens et al., 2009) might have been associated with the long distance transport, during
12 which animals often do not feed or drink, rather than captivity *per se*, as has been observed in
13 livestock (Fazio & Ferlazzo, 2003). More research is needed to determine whether individuals with
14 different personality traits vary in how they cope with captivity. It should be noted that we have
15 previously shown that neophobia and exploratory tendency measured in the wild have been shown
16 to be consistent over several weeks to months and was predicted by measurements taken in
17 captivity using analogous methods and the same individuals (Herborn et al., 2010). Thus, whilst only
18 reflecting a snapshot in time, we feel that our results have some ecological relevance at least for our
19 study population.

20 Our data suggest that both baseline and stress-induced Cort concentrations in wintering
21 blue tits are associated with state-dependent and permanent variables, as well as personality traits.
22 This is in line with evidence that Cort is involved in maintaining a homeostatic energy balance,
23 allowing individuals to respond to food shortages and environmental perturbations (Wingfield &
24 Kitayskya, 2002; Bonier et al., 2009; Hau & Goymann, 2015). For adults only, in the case of baseline
25 Cort, and birds (independent of age), in the case of elevated Cort, birds that put on more mass in
26 captivity had higher plasma Cort concentrations. Moreover birds that were light on entry into

1 captivity gained more mass during captivity than relatively heavy individuals. What we do not know,
2 and could only find out with manipulative experiments, is whether the poor quality birds gained
3 mass because of their high Cort concentrations (which can stimulate hyperphagia (Landys et al.,
4 2004)) or the rapid increase in mass resulted in raised baseline and stress-induced Cort. There was
5 no sex difference in the relationship between basal Cort and change in mass, although on entry into
6 captivity males were in better condition than females. In contrast to adults, for juveniles the
7 relationship between baseline Cort and mass gain was negative. However, juveniles and adults did
8 not, on average, differ significantly in mass upon capture or their mass change in captivity. Thus,
9 juveniles may have a different stress physiology and/or feedback mechanisms from adults (Bonier et
10 al., 2009). Experience in coping with environmental perturbations and/or relative dominance status
11 have also been shown to influence how birds respond to changes in food supply (Gosler, 1996;
12 Grieco et al., 2002), and thus potentially Cort levels.

13 For stress-induced Cort, high concentrations were associated with low haematocrit
14 (indicating poor condition) and a greater mass increase. These data indicate that high Cort may
15 signal individuals in poor condition – which might be true for both basal and stress-induced Cort.
16 However, this needs further study, not least because one limitation of our study is that we did not
17 have basal and stress-induced Cort from the same individual for welfare reasons. Thus, we do not
18 know whether blue tits with high basal Cort also had high stress-induced Cort (which might actually
19 indicate a relatively small change in Cort in response to stress) or those with low basal Cort could
20 have high stress-induced Cort (i.e. had a relatively great physiological response to restraint).
21 Selection experiments have shown that basal and stress-induced Cort levels are not necessarily
22 linked (Martins et al., 2007), but this requires more investigation in relation to behavioural
23 responses.

24 To conclude, exploratory tendency, activity and neophobia in blue tits were uncorrelated
25 and showed differing patterns of co-variation with state-dependent, permanent and physiological
26 traits. As individuals vary along these three independent axes of behavioural variation, the

1 underlying physiological mechanisms, and indeed the costs (Herborn et al., 2011), may vary in a
2 complex fashion. More research both under captive and wild conditions is needed to determine
3 whether both baseline and stress-induced Cort, i.e. physiological responses to chronic and acute
4 stress, are linked with individual variation in behaviour directly or indirectly via homeostatic control
5 of energetic balance. Of particular interest is how personality traits and other factors that affect
6 ability to control resources interact, and thus influence an individual's current and future energy
7 balance.

8

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10

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16

17 **References**

- 18 ANGELIER, F., WINGFIELD, J. C., WEIMERSKIRCH, H. & CHASTEL, O. (2010). Hormonal correlates of individual quality in a
19 long-lived bird: a test of the 'corticosterone-fitness hypothesis'. — *Biology Letters* 6, 846-849.
- 20 APFELBECK, B. & RAESS, M. (2008). Behavioural and hormonal effects of social isolation and neophobia in a
21 gregarious bird species, the European starling (*Sturnus vulgaris*). — *Hormones and Behavior* 54, 435-
22 441.
- 23 ARNOLD, K. E., HERBORN, K. A., ADAM, A. & ALEXANDER, L. (2015). Individual variation in the oxidative costs of
24 personality traits. — *Functional Ecology* 29, 522-530.
- 25 ARNOLD, K. E., RAMSAY, S. L., GOULD, C. & ADAM, A. (2007). Parental prey selection affects risk taking behaviour
26 and spatial learning in avian offspring. — *Proc R Soc Lond B* 274, 2563-2569.
- 27 ASTHEIMER, L. B., BUTTEMER, W. A. & WINGFIELD, J. C. (1995). Seasonal and acute changes in adrenocortical
28 responsiveness in an arctic breeding bird. — *Horm Behav* 29, 442-457.
- 29 BELL, A. M. (2005). Behavioural differences between individuals and two populations of stickleback
30 (*Gasterosteus aculeatus*). — *Journal of Evolutionary Biology* 18, 464-473.
- 31 BELL, A. M. & SIH, A. (2007). Exposure to predation generates personality in threespined sticklebacks
32 (*Gasterosteus aculeatus*). — *Ecology Letters* 10, 828-834.
- 33 BIRO, P. A. & DINGEMANSE, N. J. (2009). Sampling bias resulting from animal personality. — *Trends in Ecology &*
34 *Evolution* 24, 66-67.

- 1 BLAS, J., BORTOLOTTI, G. R., TELLA, J. L., BAOS, R. & MARCHANT, T. A. (2007). Stress response during development
2 predicts fitness in a wild, long lived vertebrate. — *Proceedings of the National Academy of Sciences of*
3 *the United States of America* 104, 8880-8884.
- 4 BOKONY, V., LENDVAI, A. Z., LIKER, A., ANGELIER, F., WINGFIELD, J. C. & CHASTEL, O. (2009). Stress response and the
5 value of reproduction: Are birds prudent parents? — *American Naturalist* 173, 589-598.
- 6 BONIER, F., MARTIN, P. R., MOORE, I. T. & WINGFIELD, J. C. (2009). Do baseline glucocorticoids predict fitness? —
7 *Trends in Ecology & Evolution* 24, 634-642.
- 8 BOTH, C., DINGEMANSE, N. J., DRENT, P. J. & TINBERGEN, J. M. (2005). Pairs of extreme avian personalities have
9 highest reproductive success. — *Journal of Animal Ecology* 74, 667-674.
- 10 BREUNER, C. W., L, G. A. & C, W. J. (1998). Noninvasive corticosterone treatment rapidly increases activity in
11 Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). — *General and Comparative*
12 *Endocrinology* 111, 386-394.
- 13 BUCHANAN, K. L. (2000). Stress and the evolution of condition-dependent signals. — *Trends in Ecology and*
14 *Evolution* 15, 156-160.
- 15 CARERE, C. (2003). Personalities as epigenetic suites of traits. — Ph.D., Groningen.
- 16 CARERE, C., CARAMASCHI, D. & FAWCETT, T. W. (2010). Covariation between personalities and individual differences
17 in coping with stress: Converging evidence and hypotheses. — *Current Zoology* 56, 728-740.
- 18 CARERE, C., GROOTHUIS, T. G. G., MOSTL, E., DAAN, S. & KOOLHAAS, J. M. (2003). Fecal corticosteroids in a territorial
19 bird selected for different personalities: daily rhythm and the response to social stress. — *Hormones*
20 *& Behavior* 43, 540-548.
- 21 CAVIGELLI, S. A. & MCCLINTOCK, M. K. (2003). Fear of novelty in infant rats predicts adult corticosterone dynamics
22 and an early death. — *Proceedings of the National Academy of Sciences of the United States of*
23 *America* 100, 16131-16136.
- 24 CAVIGELLI, S. A., RAGAN, C. M., MICHAEL, K. C., KOVACSICS, C. E. & BRILISCKE, A. P. (2009). Stable behavioral inhibition
25 and glucocorticoid production as predictors of longevity. — *Physiology & Behavior* 98, 205-214.
- 26 COCKREM, J. F. (2007). Stress, corticosterone responses and avian personalities. — *Journal of Ornithology* 148,
27 S169-S178.
- 28 COCKREM, J. F., BARRETT, D. P., CANDY, E. J. & POTTER, M. A. (2009). Corticosterone responses in birds: Individual
29 variation and repeatability in Adelie penguins (*Pygoscelis adeliae*) and other species, and the use of
30 power analysis to determine sample sizes. — *General and Comparative Endocrinology* 163, 158-168.
- 31 COCKREM, J. F. & SILVERIN, B. (2002a). Sight of a predator can stimulate a corticosterone response in the great tit
32 (*Parus major*). — *General and Comparative Endocrinology* 125, 248-255.
- 33 —. (2002b). Variation within and between birds in corticosterone responses of great tits (*Parus major*). —
34 *General and Comparative Endocrinology* 125, 197-206.
- 35 DAISLEY, J. N., BROMUNDT, V., MOSTL, E. & KOTRSCHAL, K. (2005). Enhanced yolk testosterone influences behavioral
36 phenotype independent of sex in Japanese quail chicks *Coturnix japonica*. — *Hormones and Behavior*
37 47, 185-194.
- 38 DICKENS, M. J., EARLE, K. A. & ROMERO, L. M. (2009). Initial transference of wild birds to captivity alters stress
39 physiology. — *General and Comparative Endocrinology* 160, 76-83.
- 40 DINGEMANSE, N. J., EDELAAR, P. & KEMPENAEERS, B. (2010). Why is there variation in baseline glucocorticoid levels?
41 — *Trends in Ecology & Evolution* 25, 261-262.
- 42 DINGEMANSE, N. J., WRIGHT, J., KAZEM, A. J. N., THOMAS, D. K., HICKLING, R. & DAWNAY, N. (2007). Behavioural
43 syndromes differ predictably between 12 populations of three-spined stickleback. — *Journal of*
44 *Animal Ecology* 76, 1128-1138.
- 45 DRENT, P. J., OERS, K. V. & NOORDWIJK, A. J. V. (2003). Realized heritability of personalities in the great tit (*Parus*
46 *major*). — *Proceedings of the Royal Society B-Biological Sciences* 270, 45-51.
- 47 FAZIO, E. & FERLAZZO, A. (2003). Evaluation of stress during transport. — *Veterinary Research Communications*
48 27, 519-524.
- 49 GENTLE, L.K. and GOSLER, A.G., 2001. [The effects of altering perceived predation risk on the fat reserves of](#)
50 [wild Great Tits, *Parus major*, *Proceedings of the Royal Society of London Series B. Proceedings of the*](#)
51 [Royal Society of London Series B](#), 268, 487-491.
- 52 GOSLER, A. G. (1996). Environmental and social determinants of winter fat storage in the great tit *Parus major*.
53 — *Journal of Animal Ecology* 65, 1-17.
- 54 GOSLING, S. D. (2001). From mice to men: What can we learn about personality from animal research? —
55 *Psychological Bulletin* 127, 45-86.

- 1 GOUTTE, A., ANGELIER, F., WELCKER, J., MOE, B., CLEMENT-CHASTEL, C., GABRIELSEN, G. W., BECH, C. & CHASTEL, O. (2010).
2 Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes. — *Gen Comp*
3 *Endocrinol* 167, 246-51.
- 4 GRIECO, F., VAN NOORDWIJK, A. J. & VISSER, M. E. (2002). Evidence for the effect of learning on timing of
5 reproduction in blue tits. — *Science* 296, 136-138.
- 6 HAU, M. & GOYMANN, W. (2015). Endocrine mechanisms, behavioral phenotypes and plasticity: known
7 relationships and open questions. — *Frontiers in Zoology* 12, 1-15.
- 8 HENDERSON, L. J., EVANS, N. P., HEIDINGER, B. J., ADAMS, A. & ARNOLD, K. E. (2014). Maternal condition but not
9 corticosterone is linked to offspring sex ratio in a passerine bird. — *PloS one* 9, e110858.
- 10 HENDERSON, L. J., HEIDINGER, B. J., EVANS, N. P. & ARNOLD, K. E. (2013). Ultraviolet crown coloration in female blue
11 tits predicts reproductive success and baseline corticosterone. — *Behavioral Ecology* 24, 1299-1305.
- 12 HERBORN, K., COFFEY, J., LARCOMBE, S., ALEXANDER, L. & ARNOLD, K. E. (2011). Oxidative profile varies with
13 personality in European greenfinches. — *Journal of Experimental Biology* 214, 1732-9.
- 14 HERBORN, K. A., MACLEOD, R., MILES, W. T. S., SCHOFIELD, A. N. B., ALEXANDER, L. & ARNOLD, K. E. (2010). Personality in
15 captivity reflects personality in the wild. — *Animal Behaviour* 79, 835-843.
- 16 HUNTINGFORD, F. A. (1976). Relationship between anti-predator behavior and aggression among conspecifics in
17 3-spined Stickleback, *Gasterosteus aculeatus*. — *Animal Behaviour* 24, 245-260.
- 18 JENNI-EIERMANN, S., E, E. G., GRUEBLER, M., SCHWABL, H. & L, L. J. (2008). Glucocorticoid response to food
19 availability in breeding barn swallows (*Hirundo rustica*). — *Gen Comp Endocrinol* 155, 558-565.
- 20 KOOLHAAS, J. M., DE BOER, S. F., BUWALDA, B. & VAN REENEN, K. (2007). Individual variation in coping with stress: A
21 multidimensional approach of ultimate and proximate mechanisms. — *Brain Behavior and Evolution*
22 70, 218-226.
- 23 KOOLHAAS, J. M., DE BOER, S. F., COPPENS, C. M. & BUWALDA, B. (2010). Neuroendocrinology of coping styles:
24 towards understanding the biology of individual variation. — *Front Neuroendocrinol* 31, 307-21.
- 25 LANDYS, M. M., RAMENOFKY, M., GUGLIELMO, C. G. & WINGFIELD, J. C. (2004). The low-affinity glucocorticoid
26 receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow
27 *Zonotrichia leucophrys gambelii*. — *Journal of Experimental Biology* 207, 143-154.
- 28 LE MAHO, Y., KARMANN, H., BRIOT, D., HANDRICH, Y., ROBIN, J. P., MIOSKOWSKI, E., CHEREL, Y. & FARNI, J. (1992). Stress
29 in birds due to routine handling and a technique to avoid it. — *American Journal of Physiology* 263,
30 R775-R781.
- 31 LOHMUS, M., SANDBERG, R., HOLBERTON, R. L. & MOORE, F. R. (2003). Corticosterone levels in relation to migratory
32 readiness in red-eyed vireos (*Vireo olivaceus*). — *Behavioral Ecology and Sociobiology* 54, 233-239.
- 33 LONG, J. A. & HOLBERTON, R. L. (2004). Corticosterone secretion, energetic condition, and a test of the migration
34 modulation hypothesis in the hermit thrush (*Catharus guttatus*), a short-distance migrant. — *Auk* 121,
35 1094-1102.
- 36 MARCHETTI, C. & DRENT, P. J. (2000). Individual differences in the use of social information in foraging by captive
37 great tits. — *Animal Behaviour* 60, 131-140.
- 38 MARTIN, J. G. A. & REALE, D. (2008). Temperament, risk assessment and habituation to novelty in eastern
39 chipmunks, *Tamias striatus*. — *Animal Behaviour* 75, 309-318.
- 40 MARTIN, L. B. (2009). Stress and immunity in wild vertebrates: Timing is everything. — *General and Comparative*
41 *Endocrinology* 163, 70-76.
- 42 MARTINS, T. L. F., ROBERTS, M. L., GIBLIN, I., HUXHAM, R. & EVANS, M. R. (2007). Speed of exploration and risk-taking
43 behavior are linked to corticosterone titres in zebra finches. — *Hormones and Behavior* 52, 445-453.
- 44 METTKE-HOFMANN, C., LORENTZEN, S., SCHLICHT, E., SCHNEIDER, J. & WERNER, F. (2009). Spatial Neophilia and Spatial
45 Neophobia in Resident and Migratory Warblers (*Sylvia*). — *Ethology* 115, 482-492.
- 46 METTKE-HOFMANN, C., WINKLER, H. & LEISLER, B. (2002). The significance of ecological factors for exploration and
47 neophobia in parrots. — *Ethology* 108, 249-272.
- 48 MONAGHAN, P. (2008). Review. Early growth conditions, phenotypic development and environmental change. —
49 *Philos Trans R Soc Lond B Biol Sci* 363, 1635-45.
- 50 MULLER, C., JENNI-EIERMANN, S., BLONDEL, J., PERRET, P., CARO, S. P., LAMBRECHTS, M. & JENNI, L. (2006). Effect of
51 human presence and handling on circulating corticosterone levels in breeding blue tits (*Parus*
52 *caeruleus*). — *Gen Comp Endocrinol* 148, 163-71.
- 53 OERS, K. V., DRENT, P. J., GOEDE, P. D. & NOORDWIJK, A. J. V. (2004). Realized heritability and repeatability of risk-
54 taking behaviour in relation to avian personalities. — *Proc. Roy. Soc. B.* 271, 65-73.
- 55 OERS, K. V., JONG, G. D., NOORDWIJK, A. J. V., KEMPENAERS, B. & DRENT, P. J. (2005). Contribution of genetics to the
56 study of animal personalities: a review of case studies. — *Behaviour* 142, 1185-1206.

- 1 PIERSMA, T., RENEERKENS, J. & RAMENOFKY, M. (2000). Baseline corticosterone peaks in shorebirds with maximal
2 energy stores for migration: A general preparatory mechanism for rapid behavioral and metabolic
3 transitions? — *General and Comparative Endocrinology* 120, 118-126.
- 4 PRAVOSUDOV, V. V., KITAYSKY, A. S., WINGFIELD, J. C. & CLAYTON, N. S. (2001). Long-term unpredictable foraging
5 conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). — *General
6 and Comparative Endocrinology* 123, 324-331.
- 7 RAJA-AHO, S., SUORSA, P., VAINIO, M., NIKINMAA, M., LEHIKONEN, E. & EEVA, T. (2010). Body condition is associated
8 with adrenocortical response in the barn swallow (*Hirundo rustica* L.) during early stages of autumn
9 migration. — *Oecologia* 163, 323-332.
- 10 REALE, D., READER, S. M., SOL, D., MCDUGALL, P. T. & DINGEMANSE, N. J. (2007). Integrating animal temperament
11 within ecology and evolution. — *Biological Reviews* 82, 291-318.
- 12 ROMERO, L. M. & REED, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good
13 enough? — *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*
14 140, 73-79.
- 15 ROMERO, L. M., REED, J. M. & WINGFIELD, J. C. (2000). Effects of weather on corticosterone responses in wild free-
16 living passerine birds. — *General and Comparative Endocrinology* 118, 113-122.
- 17 RUBENSTEIN, D. R. (2007). Stress hormones and sociality: integrating social and environmental stressors. —
18 *Proceedings of the Royal Society B-Biological Sciences* 274, 967-975.
- 19 RUIZ-GOMEZ, M. D., KITTILSEN, S., HOGLUND, E., HUNTINGFORD, F. A., SORENSEN, C., POTTINGER, T. G., BAKKEN, M.,
20 WINBERG, S., KORZAN, W. J. & OVERLI, O. (2008). Behavioral plasticity in rainbow trout (*Oncorhynchus
21 mykiss*) with divergent coping styles: When doves become hawks. — *Hormones and Behavior* 54, 534-
22 538.
- 23 SCHEID, C. & NOE, R. (2009). The performance of rooks in a cooperative task depends on their temperament. —
24 *Anim Cogn* 13, 545-53.
- 25 SCHEID, C. & NOE, R. (2010). The performance of rooks in a cooperative task depends on their temperament. —
26 *Animal Cognition* 13, 545-553.
- 27 SCHOECH, S. J., RENSEL, M. A., BRIDGE, E. S., BOUGHTON, R. K. & WILCOXEN, T. E. (2009). Environment, glucocorticoids,
28 and the timing of reproduction. — *General and Comparative Endocrinology* 163, 201-207.
- 29 SIH, A., KATS, L. B. & MOORE, R. D. (1992). Effects of Predatory Sunfish on the Density, Drift, and Refuge Use of
30 Stream Salamander Larvae. — *Ecology* 73, 1418-1430.
- 31 STAMPS, J. & GROOTHUIS, T. G. G. (2010). The development of animal personality: relevance, concepts and
32 perspectives. — *Biological Reviews* 85, 301-325.
- 33 SVENSSON, L. (1992). Identification Guide to European Passerines. — BTO, Thetford, Norfolk.
- 34 TOBLER, M. & SANDELL, M. I. (2007). Yolk testosterone modulates persistence of neophobic responses in adult
35 zebra finches, *Taeniopygia guttata*. — *Hormones and Behavior* 52, 640-645.
- 36 VAN OERS, K., KLUNDER, M. & DRENT, P. J. (2005). Context dependence of personalities: risk-taking behavior in a
37 social and a nonsocial situation. — *Behavioral Ecology* 16, 716-723.
- 38 VAN OVERVELD, T. & MATTHYSEN, E. (2010). Personality predicts spatial responses to food manipulations in free-
39 ranging great tits (*Parus major*). — *Biology Letters* 6, 187-190.
- 40 VERBEEK, M. E. M., BOON, A. & DRENT, P. J. (1996). Exploration, aggressive behavior and dominance in pair-wise
41 confrontations of juvenile male great tits. — *Behaviour* 133, 945-963.
- 42 VERBEEK, M. E. M., DRENT, P. J. & WIEPKEMA, P. R. (1994). Consistent individual-differences in early exploratory-
43 behavior of male great tits. — *Animal Behaviour* 48, 1113-1121.
- 44 WILSON, D. S., CLARK, A. B., COLEMAN, K. & DEARSTYNE, T. (1994). Shyness and Boldness in Humans and Other
45 Animals. — *Trends in Ecology & Evolution* 9, 442-446.
- 46 WINGFIELD, J. C. (1994). Modulation of the adrenocortical response to stress in birds. — In: *Perspectives in
47 Comparative Endocrinology*, National Research Council of Canada, Ottawa (K. G. D. e. al., ed), p. 520-
48 528.
- 49 WINGFIELD, J. C. & KITAYSKYA, A. S. (2002). Endocrine responses to unpredictable environmental events: Stress or
50 anti-stress hormones? — *Integrative and Comparative Biology* 42, 600-609.
- 51 WINGFIELD, J. C., VELCK, C. M. & MOORE, M. C. (1992). Seasonal changes of the adrenocortical response to stress
52 in birds of the Sonoran desert. — *J Exp Zool* 264, 419-428.

1 **Figure legends**

2

3 Fig. 1: Relationships between personality traits and plasma Cort concentrations. Baseline Cort
4 concentrations were related to a) activity level in females (crosses, solid line, N = 6), but not in males
5 (circles, dashed line, N = 31); and unrelated to b) exploratory tendency (N = 37); and c) neophobia (N
6 = 35). Stress-induced Cort concentrations were d) unrelated to activity levels (N = 29); e) marginally
7 related to neophobia (N = 24); and f) significantly related to exploratory tendency (N = 29).

8

9 Fig. 2: Relationships between change in mass over two days of captivity (mass at blood sampling on
10 day 2 – mass at capture from wild) and baseline plasma Cort concentrations for juveniles (circles,
11 dashed line, N = 13) and adults (stars, solid line, N = 23).

12

13 Fig. 3: Relationships between stress-induced plasma Cort concentrations and a) change in mass over
14 two days of captivity (mass at blood sampling on day 2 – mass at capture from wild)(N = 30); b)
15 Mean haematocrit level (N = 30).

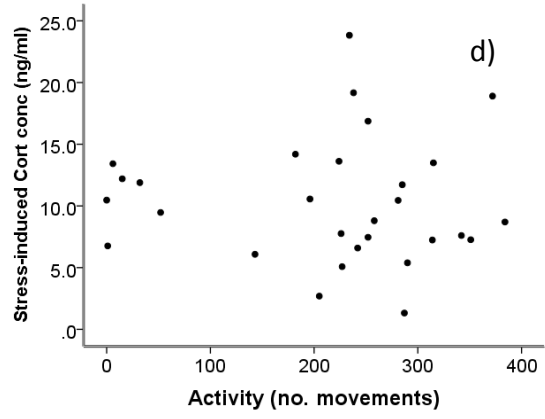
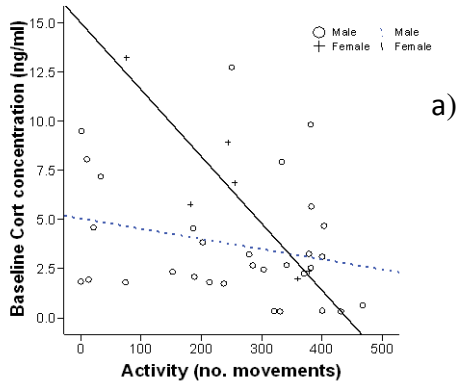
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1 Figure 1

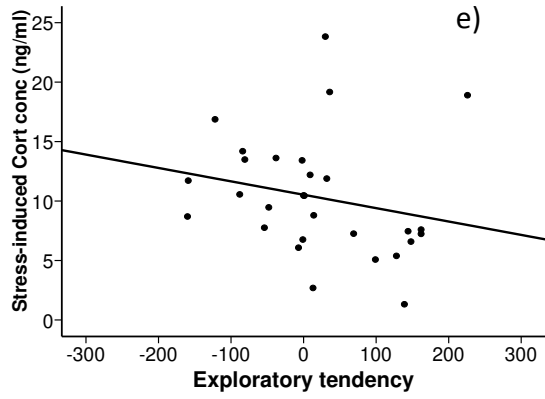
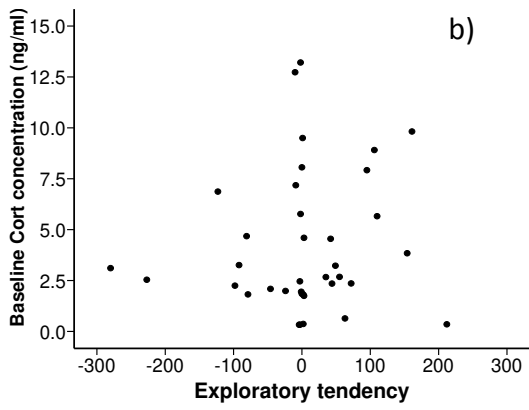
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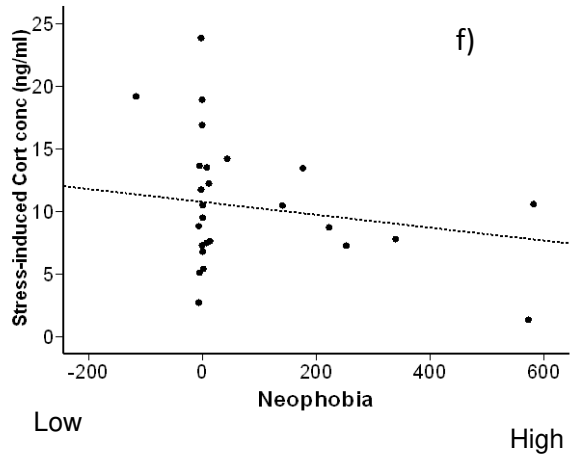
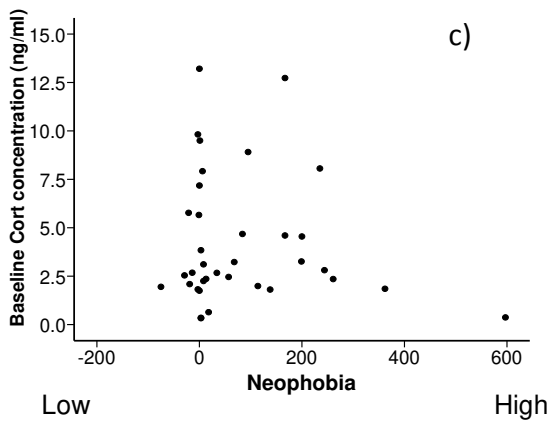


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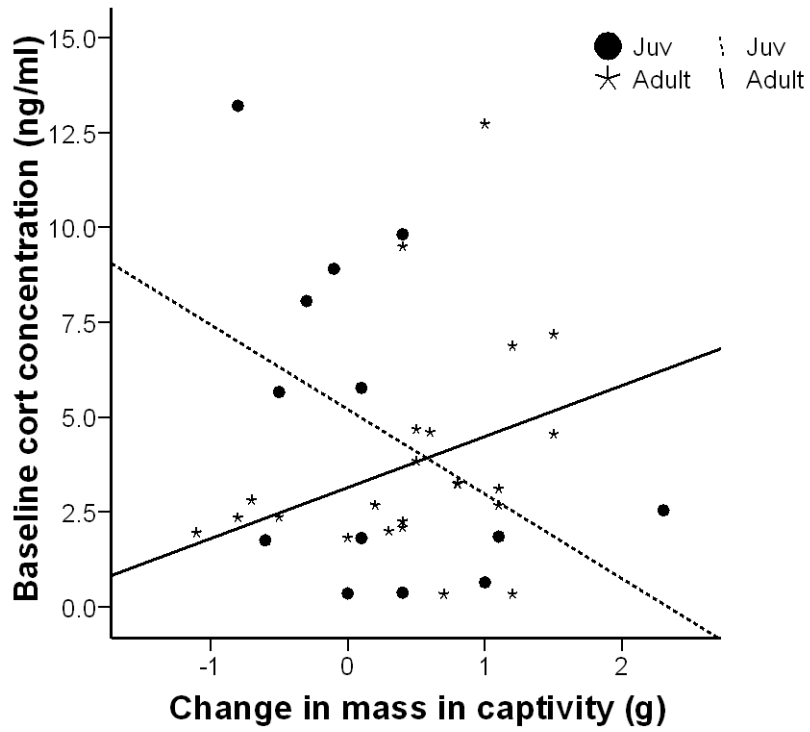


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2 Figure 2



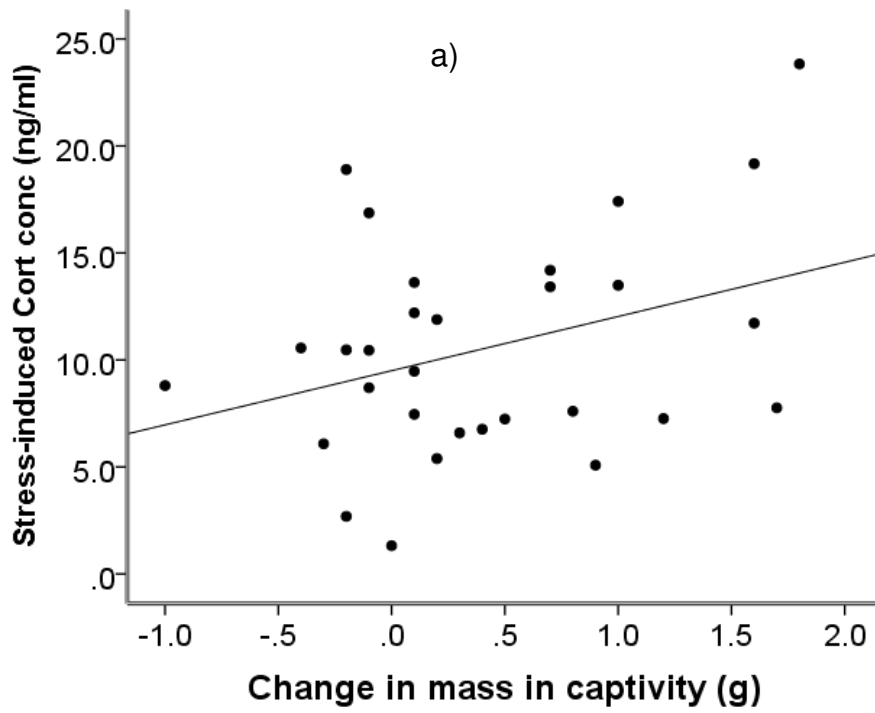
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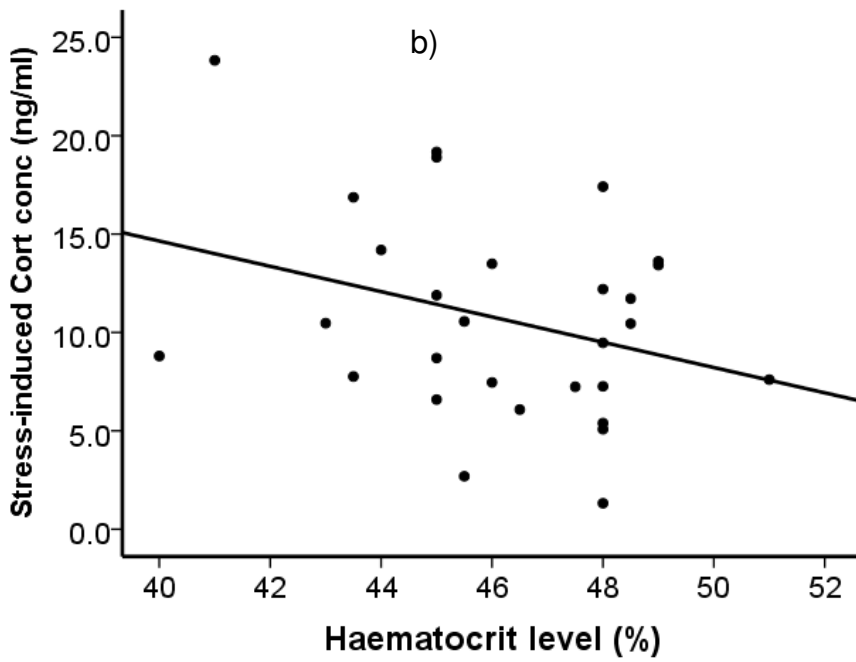
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1 Figure 3

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1 SUPPLEMENTARY MATERIAL

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9 (b)

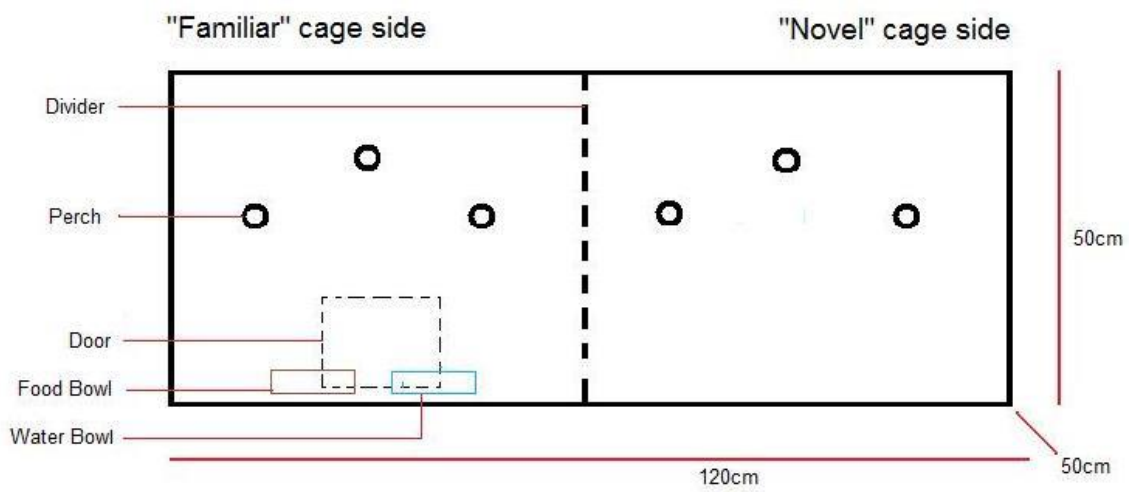
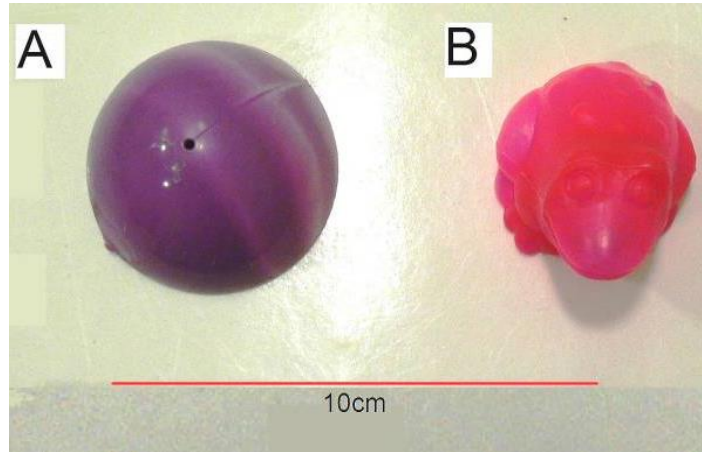


Figure S1: Apparatus used during the captive exploration trials (a) Photo of cage set up. (d) Schematic of cage set up; the food was removed 1hr and water bowl 30min before the start of the trial, via the door.

1
2 (a)



3
4
5 (b)



6
7
8 **Figure S2:** The apparatus used for neophobia trial. (a) A photo of the novel objects used in the
9 neophobia trial: A: half a purple rubber ball, B: a plastic pink frog. (b) A photo of a blue tit
10 approaching the novel object in a food bowl (photo credits: Katherine Herborn).
11

12
13