

# Behavioural responses to handling stress in the Great Tit: within-individual consistency and the effect of age, sex and body condition

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Gábor Markó, Manuel Azcárate, Gergely Hegyi, Gábor Herceg, Miklós Laczi, Gergely Nagy, Juan Carlos Señar, János Török, László Zsolt Garamszegi 2013. Behavioural responses to handling stress in the Great Tit: within-individual consistency and the effect of age, sex and body condition. – Ornis Hungarica 21(1): 12–25.

**Abstract** In birds, individuals may show different behavioural and physiological responses when handling, and such variation may be related to individual differences in antipredator strategies. We performed a pilot study in both breeding and wintering populations of the Great Tit (*Parus major*), and we characterised three typical behavioural traits during a standard ringing procedure in captured birds. We assessed between-individual variations in breath rate, pecking rate and the number of distress calls displayed in response to handling, and also calculated the within-individual variation of these traits by repeated behavioural measurements. We found that these behaviours were consistently displayed within individuals (with repeatability varying between 0.44 and 0.82), and there was also some modest correlation between them (e.g. breath rate covaried with the number of distress calls). Furthermore, using multivariate linear models assessing a role of some potential predictors we found that a considerable amount of between-individual variation can be explained by sex and age differences and also by variation in body condition. However, the magnitude and direction of these relationships was inconsistent across seasons. Our results are in line with previous findings that several consistent behavioural traits measured during human handling could reflect individual specific antipredator strategy, but some confounding effects cannot be ruled out. Hence, our preliminary results require careful interpretation, and further studies are needed to assess the exact magnitude by which different behavioural traits are inter-related.

Keywords: acute stress response, animal personality, coping style, repeatability

**Összefoglalás** A madarak az emberi jelenlétre adott egyedi viselkedési és fiziológiai válaszai tükrözik a ragadozók elleni stratégiákban megnyilvánuló egyedi különbségeket. Elővizsgálatunkban egy természetes populációból származó költő és telető széncinege (*Parus major*) egyedeket fogtunk meg és vizsgáltunk a gyűrűzéseink alkalmával. A morfológiai bélyegek lemérésén túl, három jellegzetes viselkedést is dokumentáltunk: a légzési rátát, a csipési rátát és a leadott vészjelzések számát. A madarak viselkedésének ismételt mérése lehetővé tette, hogy a viselkedési bélyegeknél kiszámoljuk az egyeden belüli varianciáját. Eredményül azt kaptuk, hogy ezek a viselkedési bélyegek konzisztens egyeden belüli mintázatot mutatnak (a repetabilitás 0,44 és 0,82 között változva), valamint a vizsgált egyedeknél mért viselkedési bélyegek mérsékelt módon korrelálnak egymással (pl.: légzési ráta együtt mozog a leadott vészjelzések számával). Többváltozós lineáris modellek segítségével kimutattuk, hogy az egyedek között fennálló viselkedési változatosságot számos lehetséges változó közül az ivar, az életkor valamint a kondíció magyarázza a legnagyobb mértékben, bár mind a feltárt kapcsolatok erőssége, mind pedig azok iránya szezonfüggő mintázatot mutatott (tavaszi költés, ill. téli kóborlás). Eddigi eredményeink alapján elmondható, hogy az emberi kezelésekre konzisztens egyedre jellemző válaszokat kaptunk néhány viselkedési bélyegben. Ezen bélyegek együttesen tükrözhetik az egyedre jellemző ragadozó ellen mutatott egyedi viselkedési különbségeket is, bár ez utóbbi kapcsolatot eddig még nem vizsgáltuk. Jelen munkában bemutatott előzetes eredményeket az összefüggések további tisztázásáig fenntartásokkal kell kezelni.

Kulcsszavak: akut stresszválasz, állati személyiség, repetabilitás

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## Introduction

Individuals apply consistent behavioural and physiological responses to cope with environmental challenges, and adaptation at both levels includes several traits and their complex interactions. At the behavioural level, the consistent individual strategy is manifested as behavioural correlations and the involved traits usually form behavioural syndromes (Bell 2007). At the physiological level, consistency becomes transparent in the associations between the components of stress machinery, which ultimately define individual coping styles along the reactive and proactive axes (Koolhaas *et al.* 1999). Behavioural syndromes and coping styles are not independent of each other, because both have evolved for the same or similar function and it is likely that a mechanistic link exists between the two levels (i.e. hormone levels determine behavioural activity) (Coppens *et al.* 2010, Koolhaas *et al.* 2010). Recognizing the importance of consistent individual responses that are integrated within complex physiological and behavioural processes, it has been proposed that individual strategies become expressed in several levels (from physiology to life history) (Buchanan 2000, Réale *et al.* 2010). Given that most of the environmental challenges are non-predictable in nature, a range of individual strategies are maintained within the wild populations of animals.

One of the most important environmental stimuli to which each individual has to react somehow is the appearance of life-threatening predators. Individuals of many species develop several forms of antipredator strategies, in which they apply morphological, physiological or behavioural means to reduce the chances of being predated (Sih *et al.* 2003, Bell 2005, Liker & Bókony 2009). These defence mechanisms are expressed in two different contexts, first to avoid the incidence of being captured and then to increase the probabilities of escape if already caught (Laiolo *et al.* 2004, Laiolo *et al.* 2009). Selection acting in one context does not necessarily have consequences for selection acting in the other. Importantly, consistent individual responses might be prevalent in both contexts, resulting in that different individuals apply different behavioural and physiological strategies when a predator threatens or catches them. Some choose to freeze in this situation, while others rather attempt to fight (Eilam 2005). The freezer strategy can be beneficial if it deceivingly suggests to the predator that the prey is of low quality (e.g. unhealthy or dead). On the contrary, fighting can be advantageous if it incurs injury costs to the predator. As with other non-predictable environmental changes, an individual cannot foresee what type of predator attack it suffers during its life, thus not only one advantageous escape behaviour is selected for. Hence, one can observe an

entire distribution of freezing and fighting strategies in natural prey populations.

The combined physiological and behavioural reaction to a capture event often results in the increase in heart and breath rates, the production of distress calls and intense fighting behaviour (e.g. pecking, biting, kicking, wriggling). Elevated heart and breath rates can generally reflect the incurred levels of fear as perceived by any prey in risky contexts (Axelrod & Reisine 1984, Carere & van Oers 2004, Deviche *et al.* 2010). Therefore, such traits could be good indicators of acute stress response in both freezer and fighter individuals. Accordingly, it has been shown in different taxa, such as in the Three-spined Stickleback (*Gasterosteus aculeatus*) (Bell *et al.* 2010) and in Great Tit *Parus major* (Carere & van Oers 2004, Torné-Noguera *et al.* in press), that the presence of predator or handling by human positively affects respiratory rate. However, active vocalization and attempting to struggle with the predator should be typical in individuals that apply fighter strategy (Brodie 1978, Perrone 1980). In an experimental study on several species caught by mist-net, the potential functions of distress calls were tested in a predatory context (Conover 1994). In most of the species investigated, distress call was only emitted when captured by human observers.

Distress calls of birds may have evolved to function as a reliable pursuit deterrent trait of individuals that can be used in both the within- and between-species context (Laiolo *et al.* 2004). This vocal signal could also serve as startling the predators to loose its grip and to make it possible for the prey to escape, warning conspecifics about the danger or calling for help (Woodland *et al.* 1980, Högstedt 1983). Moreover, intense pecking behaviour of bird preys can not on-

ly affect the energy and time investment of the predator, but could also increase their accidental injuries from the pugnacious fight responses of preys with active escape behaviour. Human presence or the effect of temporary handling during the standard ringing and measuring protocols could also generate acute stress response or escape behaviour in birds (Møller 2008, Fucikova *et al.* 2009). Therefore, in addition to breath rate, the amount of distress calls and pecking rate during the human manipulation of birds have been widely used as standard measure of the degree by which individuals respond behaviourally and psychologically to a capture by predator (e.g. see: Romero 2004).

In this correlative paper, we report a pilot study on free living Great Tit, in which we aimed at establishing the behavioural ecological roles of escape behaviour. We captured individuals during breeding and wintering with different ecological and social contexts, and estimated breath rate, the number of distress calls and pecking rate upon handling. Most of the individuals were measured more than once within the same capture session, while others were recaptured in another sampling day, thus we could determine the repeatability of traits. We predicted that if consistent behavioural responses to handling stress exist, we would detect significant repeatability for each trait. Furthermore, such consistency may be manifested in the form of correlation between traits reflecting a similar phenomenon, thus we also predicted relationship between the behavioural variables. However breath rate may have another meaning than distress call or pecking rate, as the former reflect the levels of the experienced acute stress, while the latter signify the willingness to fight against the predator. Accordingly, we also expected

weaker relationships for breath rate than for the other two traits. We also investigated sex, age and body condition in relation to the assessed behavioural responses. We hypothesized that these traits could be potentially important determinants of how individuals respond to a capture event, as these predictors likely describe inter-individual variation in experience, life history and physiology. Previous studies (e.g. Laiolo *et al.* 2009) showed that sex, age, and condition differences can affect antipredator behavioural responses, which might potentially reflect the degree of stress-susceptibility. Finally, we also predicted that estimates of antipredator and acute stress responses would vary between different ecological contexts as experienced during the favourable breeding and the harsh wintering seasons. The possible reason could be that both periods may require dissimilar individual energetic investment, but underlying ecological and social contexts are also different.

## Materials and Methods

### Study site and capturing protocol

Capturing protocols and behavioural tests were carried out in our study plots where a nest-box network had been established for the long-term ecological monitoring of hole-nest breeding passerines in the Pilis Mountains, nearby Pilisszentlászló, Hungary (47°43'N, 19°01'E). During the breeding season, the artificial nest-boxes ('B' type: Lambrechts *et al.* 2010) are occupied mainly by the non-migratory Great Tit and the migratory Collared Flycatcher (*Ficedula albicollis*). Breeding birds were caught by conventional nets-box traps when they feed their young at the 8th-12th days after hatch-

ing. During winter, a vagrant population (not identical with the breeding population) is present in our field sites. In this season, we provide sunflower seeds at two feeding stations, around which we regularly perform capturing protocols using mist-nets. Wintering birds were tested between November and December in 2012, while breeding birds were measured between May and June in 2013. Both conventional nets-box traps and mist-nests were checked regularly within 15-25 minutes. Upon capture, we recorded catch date, catch time, time spent in the bird bag between capture and handling, and time of behavioural tests.

### Behavioural tests

We performed measurements on escape behaviour within 5 min after capture. We have characterised 3 simple behavioural variables that are known to reflect individual stress response and/or fighting ability (Carere & van Oers 2004, Fucikova *et al.* 2009, Laiolo *et al.* 2009). First, breath rate (BR) was used to estimate the degree of acute physiological stress experienced during handling (Carere & van Oers 2004, Torné-Noguera *et al.* in press). We counted the number of respiratory movements within a given time (winter: 60 sec, spring: 15 sec), while holding the wings fixed and also ensuring reduced disturbance by visual and sound cues. Given that we used different timeframes for the two periods, we brought winter BR data into the scale of 15 sec by dividing the number of breaths by 4. Second, following BR measurements, we estimated pecking rate (PR) and distress calls (DC) by counting the number of pecks and calls that were emitted during the next 15 sec of handling (Fucikova *et al.* 2009, Laiolo *et al.* 2009). PR refers to the number of pecks against a straight fin-

ger positioned at 2-3 cm from the beak of the focal bird while holding by their legs. DC was simply the number of vocalized distress or alarm calls that we observed during the same PR trial. DC was only measured during spring, thus information on this trait is unavailable for the wintering period. Most birds (spring: N=27; winter N=11) were assayed 2 or 3 times at the same capturing session ( $45 \pm 23$  SD min apart) that allowed calculating within individual repeatability. The recapture rate of measured individuals within the same season was very low. Altogether 4 birds were recaptured in another day during winter, and we could obtain multiple measurements for 1 individual only during spring.

### Morphological traits

Morphological measurements were carried out after the behavioural tests by a standardized protocol as follows. Body mass was measured with a Pesola spring balance (to the nearest 0.1 g) and tarsus length to reflect body size by using a sliding calliper (to the nearest 0.1 mm). Sex and age (first-year or older) were determined according to the typical plumage coloration (Svensson 2002). Individuals were then labelled by numbered rings and released. None of the birds died during the manipulations by hand.

To describe individual body condition, we calculated index of scaled body mass (SBM) based on the allometric relationship between body mass (BM) and tarsus length (TL) following Peig and Green (2009, 2010). This approach relies on the following formula:

$$SMB_i = BM_i \times \left( \frac{TL_0}{TL_i} \right)^{B_{SMA}}$$

where  $BM_i$  stands for body mass of individual  $i$ ,  $TL_0$  is the population mean of tarsus length (20.16 mm in our sample),  $TL_i$  is the tarsus length of individual  $i$ , while  $B_{SMA}$  is the slope of a regression line that originates from a reduced major axis regression between body mass and tarsus length after the log-transformation of data and exclusion of obvious outliers (1.735 in our sample). SMB index was standardized across seasons by using z-transformed values that bring the data into a common scale with 0 mean and unit variance.

### Statistical analyses

Prior to analyses, all variables were checked for normality, and statistical transformations were applied if appropriate.

Repeatability was calculated as the ratio of between-individual variance and total variance. Such variance components were obtained from a Generalised Linear Mixed Model that included individual identity as a random factor (Nakagawa & Schielzeth 2010). To control for seasonal effects and also for the fact that some birds with multiple measurements were assayed in different days while others within the same capturing session, we also added season and date as covariates. To determine the statistical significance of the estimated repeatabilities, we performed a permutation test, in which we randomly shuffled the data and examined the probability of finding the true repeatability values within the distribution of permuted values reflecting random chances. To determine the confidence intervals around estimates, we applied a parametric bootstrap, in which data were simulated based on the estimated model parameters, and then the same model was fitted again to estimate repeatability. This procedure was

Season	Sex	BR Mean $\pm$ SE, n	PR Mean $\pm$ SE, n	DC Mean $\pm$ SE, n
<b>Spring</b>	Female	29.00 $\pm$ 0.99, 14	3.93 $\pm$ 0.90, 14	1.79 $\pm$ 0.98, 14
	Male	26.44 $\pm$ 1.06, 16	5.69 $\pm$ 1.63, 16	5.81 $\pm$ 1.72, 16
	Total	27.63 $\pm$ 0.75, 30	4.87 $\pm$ 0.97, 30	3.93 $\pm$ 1.08, 30
<b>Winter</b>	Female	37.42 $\pm$ 1.34, 6	8.54 $\pm$ 2.41, 6	
	Male	35.26 $\pm$ 1.15, 24	1.95 $\pm$ 0.46, 24	
	Unidentified	35.92 $\pm$ 1.88, 3	0.67 $\pm$ 0.42, 3	
	Total	35.71 $\pm$ 0.88, 33	3.03 $\pm$ 0.70, 33	
<b>Repeatability</b>		r = 0.502 (0.291/0.667), P < 0.001	r = 0.448 (0.222/0.643), P = 0.002	r = 0.842 (0.728/0.912), P < 0.001

**Table 1.** Descriptive statistics and repeatability (r) of three behavioural traits measured in response to handling by human in Great Tit during the wintering and breeding seasons. The repeatability of BR and PR were calculated from both spring and winter samplings, while data for the calculations of DC repeatability obtained from only spring sampling. The significance of repeatability estimates was determined by random permutation. In brackets, the 95% confidence intervals are given (lower bound/upper bound) that were calculated by parametric bootstrap (see materials and methods). In each trait, we provide the season and sex specific means  $\pm$  standard errors with their sample sizes (n)

**1. táblázat** A táblázat a széncinegék humán kezelésre adott viselkedési bélyegeinek leíró statisztikáit és repetabilitását (r) mutatja be a költési és a téli időszakban. Míg a vészjelzések repetabilitásának kiszámolásához csak tavaszi adatok álltak a rendelkezésünkre, addig a légzési és csípési ráta repetabilitás értékének meghatározásához mindkét időszak mintavételéből származó adatokat fel tudtuk használni. A repetabilitás szignifikancia szintjét random permutációval határoztuk meg. A zárójelben feltüntetett 95%-os konfidencia intervallum alsó és felső határértékeit parametrikus bootstrap módszerrel számoltuk ki (ld. Anyag és módszer). Minden viselkedési bélyegnél megadtuk a szezononon belüli, ivaronkénti várható értéket és feltüntetettük a standard hibával és a hozzájuk tartozó elemszámmal (n)

	Breath rate	Pecking rate	Distress call
<b>Breath rate</b>	–	Rspring = 0.137, N = 30 P = 0.464	Rspring = -0.394, N = 30 P = 0.028
<b>Pecking rate</b>	Rwinter = 0.322, N = 32 P = 0.052	–	Rspring = 0.215, N = 30 P = 0.244

Table 2. Correlations (R) of three behavioural traits measured in response to handling by human in Great Tit during the wintering and breeding seasons. Correlations are given as Spearman rank correlations with their sample size (N) and their significance level (P)

3. táblázat A táblázat a széncinegék humán kezelésre adott viselkedési bélyegeinek egymással alkotott korrelációit (R) mutatja be a költési és a téli időszakban. A korrelációk értékeit Spearman-féle rang korrelációval számoltuk ki, amelyek mellett a táblázatban feltüntetjük a minta méretét (N) és a szignifikancia szintjét (P) is

Predictor variables	Winter						Spring								
	BR			PR			BR			PR			DC		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
<b>Sex</b>	0.104	1, 22	0.750	2.981	1, 21	0.084	<b>4.601</b>	1, 23	<b>0.043</b>	<b>8.441</b>	1, 24	<b>0.004</b>	<b>16.72</b>	1, 21	<b>&lt;0.001</b>
<b>SBM</b>	<b>5.181</b>	<b>1, 22</b>	<b>0.032</b>	<b>11.88</b>	<b>1, 21</b>	<b>&lt;0.001</b>	0.001	1, 23	0.974	2.636	1, 24	0.104	<b>12.43</b>	1, 21	<b>&lt;0.001</b>
<b>Age</b>	0.681	2, 22	0.517	<b>6.881</b>	<b>1, 21</b>	<b>0.032</b>	0.597	1, 23	0.448	<b>7.115</b>	<b>1, 24</b>	<b>0.008</b>	0.485	1, 21	0.486
<b>Date of capture</b>	0.913	1, 22	0.350	0.052	1, 21	0.820	0.749	1, 23	0.396	2.193	1, 24	0.139	<b>4.707</b>	1, 21	<b>0.030</b>

Table 3. The effects of predictor variables (Sex, SBM/body condition, Age and Date of capture) on three behavioural traits displayed in response to handling in Great Tit during the wintering and breeding seasons. Effects originate from Generalized Linear Models with the corresponding behavioural traits as predictors. Significant statistical outputs highlighted in bold

3. táblázat A táblázatban a széncinegék humán kezelésre adott viselkedési válaszait mutatják be a különböző magyarázó változók (ivar, testi kondíció, életkor és megfogás dátuma) függvényében a költési és a téli időszakban. A mért változók hatásait az egyes viselkedési bélyegeknél Altalanosított Lineáris Modell segítségével külön-külön számoltuk ki. A szignifikáns összefüggéseket felkövér betűtípussal emeltük ki

repeated 1000 times and the range of simulated repeatabilities was used to describe confidence range (5% and 95% quantile).

To check for the association between behavioural traits, we carried out Spearman's rank correlations. Generalised Linear Models were used to investigate the effect of the considered predictors on the behavioural response variables. Sex, age, SBM and date of capturing were entered in these models as predictors. Due to the limitation of sample sizes we did not consider interactions between variables (*Table 1*). The model for BR fulfilled criteria for Gaussian distribution, while for models on PR and DC we used Poisson distribution. For transparency, and to avoid the confounding effects of different scales used for some variables, statistical models were run separately for each seasons.

All statistical analyses were performed in the *R* statistical environment (version 2.12.2) (2012).

## Results

### Repeatability and correlation of behavioural traits

We found that each behavioural trait, BR, PR and DC, showed repeatability of intermediate to high magnitude (*sensu* Cohen 1988) that could be statistically differentiated from zero (*Table 1*).

When assessing the correlation between traits (summarized in *Table 2*), we detected that more stressed individuals performed significantly less alarm calls in the breeding season. A positive, but only marginally significant relationship was found between BR and PR during winter indicating that individuals under higher stress levels picked

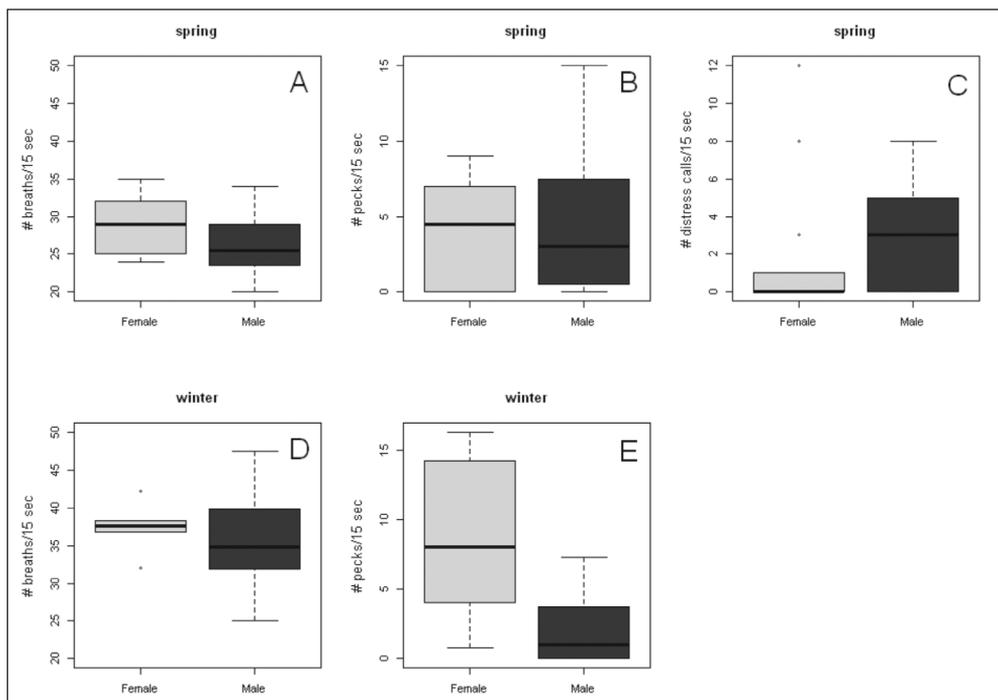
more frequently than the calmer individuals as revealed by their lower BR. On the contrary, this association was not detectable for birds tested in the breeding season even at a more permissive significance criterion ( $\alpha = 0.1$ ). We did not find significant relationship between the DC and PR during the breeding period.

### Predictors of escape behaviour

The outputs from the statistical models designed to test the effects of the considered predictor variables are given in *Table 3*. The general patterns emerging in most of these models were that behavioural traits showed variation between sexes and along body condition (SBM). BR and PR showed a sex-dependent pattern among seasons: in both traits, females displayed a significantly higher rate than males in spring, while these relationships were not detectable in winter (*Figure 1*). In contrast, DC was significantly higher in males comparing to females in spring. For DC, we only had data for the breeding season, during which we detected differences between sexes: males fought more than females, contrary to the relationships of the previous measurements (*Figure 1*). SBM was a significant predictor of BR and PR in winter and DC in spring (*Figure 2*). Furthermore, age was related significantly to PR in the spring sample only indicating that juveniles maintaining higher pecking activity against the investigator's finger than adults (*Figure 3*).

## Discussion

We investigated both the within- and between-individual co-variations of three simple behavioural traits, which were hy-



**Figure 1.** The significant relationships between sex (females: light grey, males: dark grey) and three behavioural traits measured in response to handling by human in Great Tit (see Table 3 for statistics). The Whisker box shows the most important information about the measured groups (data range: dashed Whiskers, interquartile range: square, median: black line). A: number of breaths per 15sec (spring); B: number of pecks per 15sec (spring); C: number of distress calls per 15sec (spring); D: number of breaths per 15sec (winter); E: number of pecks per 15sec (winter)

**1. ábra** A Whiskers diagramok az ivar (tojó: világosszürke, hím: sötétszürke) és a széncinegék human kezelésére adott, három viselkedési bélyegei közötti szignifikáns kapcsolatát mutatja be (a statisztikáért lásd 3. táblázatot), ahol leolvasható a teljes adattartomány (szaggatott vonal), az interkvartilis tartomány (tégllalap) és a medián (vízszintes vastag vonal). A: légzések száma 15sec alatt (tavasz); B: csípések száma 15sec alatt (tavasz); C: vészjelzések száma 15sec alatt (tavasz); D: légzések száma 15sec alatt (tél); E: csípések száma 15sec alatt (tél)

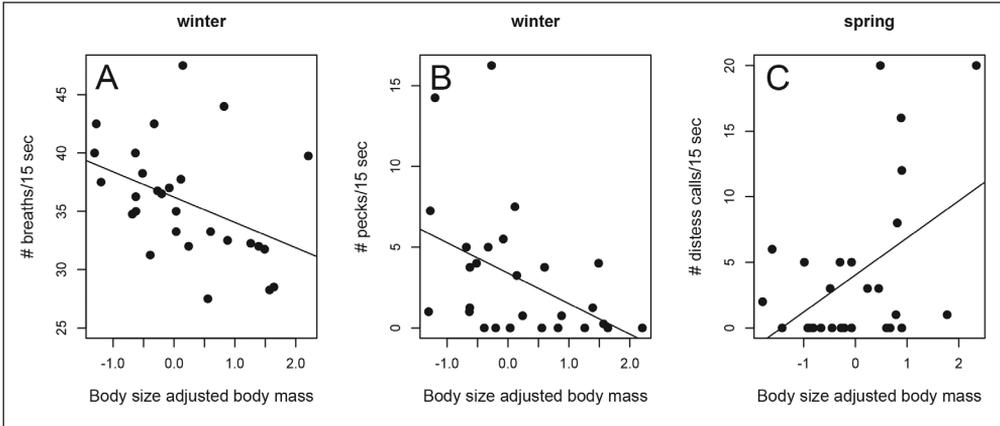


Figure 2. The significant relationships between body condition (body size corrected body mass) and three behavioural traits measured in response to handling by human in Great Tit (see Table 3 for statistics). A: number of breaths per 15sec (winter); B: number of peaks per 15sec (winter); C: number of distress calls per 15sec (spring)

2. ábra Az ábra a kondíció (testméretre korrigált testtömeg) és a színcinegék humán kezelésre adott, három viselkedési bélyegei közötti szignifikáns kapcsolatát mutatja be (a statisztikáért lásd 3. táblázatot). A: légzések száma 15sec alatt (tél); B: csipések száma 15sec alatt (tél); C: vészjelzések száma 15sec alatt (tavasz)

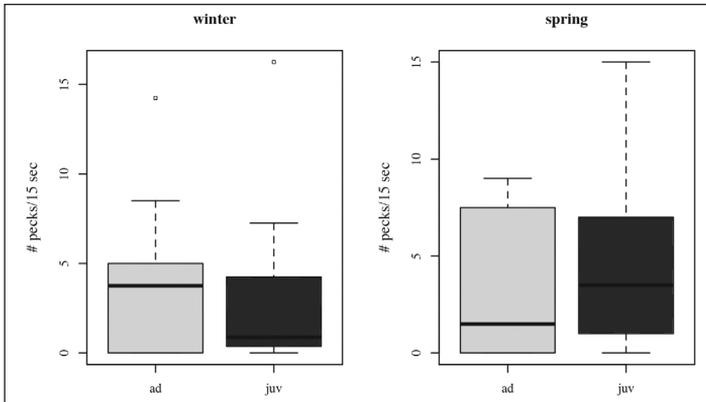


Figure 3. The significant relationships between age (adult: light grey, juvenile: dark grey) and pecking rate of Great Tit in response to handling by human in spring and winter (see Table 3 for statistics). The Whisker box shows the most important information about the measured groups (data range: dashed Whiskers, interquartile range: square, median: black line). A: number of peaks per 15sec (winter); B: number of peaks per 15sec (spring)

3. ábra Színcinegék életkora (felnőtt: világosszürke, fiatal: sötétszürke) és humán kezelésre adott tavaszi és téli csipési rátájának szignifikáns kapcsolata a téli és a tavaszi időszakban (a statisztikáért lásd 3. táblázatot), ahol leolvasható a teljes adattartomány (szaggatott vonal), az interkvartilis tartomány (téglalap) és a medián (vízszintes vastag vonal). A: csipések száma 15sec alatt (tél); B: csipések száma 15sec alatt (tavasz)

pothesised to reflect the behavioural and physiological components of antipredator defence in free-living Great Tit. We found evidence for consistent within-individual variation of the same traits and also for the correlation of different behavioural traits. Furthermore, we also assessed the effect of the most important predictors that could potentially affect the between-individual variance of traits. The corresponding analyses revealed that sexes differ in the expression of most of the investigated traits, while body condition is also an important predictor. The detected patterns of these measurements showed slight variations between the sampled seasons.

We found that the significance and magnitude of repeatabilities are in line with patterns that are typical for behavioural and physiological traits (Bell *et al.* 2009). Repeatability significantly larger than zero implies that individuals systematically differ in the level of trait expression thus they display BR, DC and PR in an individual-specific manner. That is, some of them consistently respond to predation event with elevated respiration rate and fighting activity, while others respond with smaller activity. Whether the existence of such axis reflects individual differences in how they differ in responding to predation along the freeze and fight scale requires further investigation. Importantly, despite their significance, the modest repeatabilities suggest that a considerable within-individual variation exist in these traits, which could allow individuals to adjust their response with some flexibility depending on the environment (Bell *et al.* 2009, Coppens *et al.* 2010).

The correlations between behavioural traits measured in the same predatory context may indicate that individual antipreda-

tor strategies are manifested in a multi-trait dimension. In particular, if an individual has a high BR has an apparently reduced DC in spring (although, we could not know anything about winter due to the lack of available DC data from this season). Furthermore, BR tended to positively correlate with PR in the winter sample. Therefore, the covariance structure is sensitive to the period considered, which may suggest a weak, if any, role for the existence of strict physiological constraints that would force behavioural syndromes (Bell 2005). In addition, the fact that there was not an overwhelming relationship between all traits implies that some of the variation in these behaviours occurs independently of each other, thus they not necessarily reflect the same thing. On the other hand, the correlated components may be explained by the existence of behavioural syndromes (Bell 2007, Sih *et al.* 2012).

We detected that the expression of most traits occurs in a sex-dependent way. However, the direction of these relationships varied considerably across traits and seasons. In spring, sex-dependence was observed for all physiological and behavioural variables contrary to the winter sample when such differences were not detectable. Hence, it is difficult to conclude anything about a generally applicable sex-specific adaptive response to predators. At least, our results are in line with a previous finding suggesting that the behavioural response to predictable environmental challenges may differ between males and females (Laiolo *et al.* 2009, Bókony *et al.* 2012). Such differences may arise because the cost/benefit balance of different behavioural responses can vary from one sex to the other, as females generally invest more efforts into the reproduction than males. In addition, such

sex-dependence may be more emphasized during the breeding season than during winter when the environmental challenges occur in a more balanced manner, which may explain our findings with respect to seasonal differences.

Body condition also emerged as a considerable predictor of some traits (Laiolo *et al.* 2009, Peig & Green 2009, 2010). For example, birds in better condition had lower BR and PR during winter (*Figure 2*). Therefore, we could obtain some evidence for that, individuals accumulating more fat during winter experience less acute stress response and demonstrate less fighting upon handling. Furthermore, the direction of the relationship seems to be the opposite in more favourable conditions, as during breeding better body condition allows more active behavioural responses against predators (Laiolo *et al.* 2004, Atwell *et al.* 2012). Breeding individuals in better body condition could emit more alarm calls (*Figure 2*), which might reflect that only individuals in better condition could elevate considerably their chances of escaping a predator. These patterns may signify that the prevailing ecological conditions have a strong effect on the determination of the acute stress responses and the escape behaviour, which are mediated through potentially different constraints mechanisms.

In general, we refrain ourselves from

making strong conclusions from this pilot study that is based on correlative data and limited sample sizes. However, our results add to the growing literature on consistent behaviours and its complex interplay with physiological stress that help individuals to cope with the unpredictable environments in which they live (Coppens *et al.* 2010). The findings also suggest that the measured variables can be used as proxies of the stress response of birds only with great care, i.e. when several confounders that mediate within-individual variation are held constant. This pilot study may be useful for designing more targeted experiments in similar topics in the future.

## Acknowledgements

We thank to the members of the Behavioural Ecology Group for their assistance during the fieldwork. This study was supported by funds from the Spanish government within the frame of the ‘Plan Nacional’ program (ref. no. CGL2012-38262 and CGL2012-40026-C02-01), the Hungarian Scientific Research Fund (grants K75618, K101611 and 105517), TÁMOP-(4.2.1./B-09/1-KMR-2010-0005 and 4.2.2./B-10/1-2010-0023), Erdők a Közjóért Alapítvány, Pilis Park Forestry and Eötvös Loránd University.

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