Use of Captive Bred Passerines to Monitor Human Disturbance Using Corticosterone Metabolites

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Abstract

Given that human-wildlife conflicts are an everyday issue, we propose the use of captive bred birds to determine the effects that environmental acoustic disturbance may have on small passerines. We used greenfinches and located them at three rural and one urban site and collected faeces to measure corticosterone metabolites using enzyme immunoassays. We found that birds at the urban site excreted higher amounts of corticosterone metabolites than those at the natural sites, but that some natural sites also had high levels of corticosterone when noise levels were high. We conclude that captive bred individuals can be used to monitor sites where there may be possible effects of disturbance on wild individuals.

Keywords: Glucocorticoids, Disturbance, Non-invasive methods, Greenfinch, Carduelis chloris

1. Introduction

The interaction between humans and wildlife more often than not, leads to a negative effect for wildlife in general. It is important to monitor how human activities affects the local fauna in order to determine appropriate compensatory or mitigation measures. Several attempts have been made to establish the reaction of a species to human disturbance, ranging from behavioural responses (Burger & Gochfeld, 1993; Sirot, 2006), breeding success (Jakubas, 2005; Pearce-Higgins et al., 2007; Sandvik & Barrett, 2001), mate choice (Rogers et al., 2006; Swaisgood, 2007), flight initiation distance (Beale & Monaghan, 2004; Burger, 2003; Manor & Saltz, 2005), immunocompetence (Amo et al., 2006; Martin et al., 2006), glucocorticoids (Mostl & Palme, 2002; Palme et al., 2005; Walker et al., 2005a), and cardiac response (De Villiers et al., 2006; Nimon et al., 1996; Walker et al., 2005b). Most of the methods mentioned above require invasive techniques or may not reflect the real situation

(Gill et al., 2001). Out of all these methods, we used glucocorticoids as an indicator of stress in captive bred individuals.

Levels of corticosterone, the single best hormonal indicator of stress in birds, increase following stressful events (Harvey et al., 1984; Mostl & Palme, 2002). The measurement of this hormone to estimate the stress of an individual can help us to determine if that individual is under constant disturbance and how different levels of disturbance produce different corticosterone levels (Walker et al., 2005a). The presence of glucocorticoids can be assayed from avian blood plasma only a few minutes after a stressful event (Mostl & Palme, 2002), however physical restraint to collect such samples is itself a known stressor (Silverin, 1998; Touma & Palme, 2005). Non-invasive methods of sample collection overcome the dilemma of handling and caging of the individual, as they do not require the individual to be captured, and this may be particularly useful for wild animals, especially elusive species such as the large felids and in studies with a focus on adrenal stress hormones.

Steroids are metabolized mainly in the liver and excreted via the urine and faeces. Therefore, measured amounts of steroid hormone metabolites in droppings reflect an event that occurred a certain time ago, which allows the separation of the treatment and sampling phases of the experiment (Palme, 2005). Concentrations of those metabolites in faeces reflect serum concentrations of corticosterone in blood allowing the non-invasive, non-stressful determination of corticosterone production as demonstrated for several species of birds (Touma & Palme, 2005). Additionally, collection of droppings enables continuous monitoring from the same individual, even over long periods, with minimal disturbance of its activities and social environment. Glucocorticoids are useful as they help an individual to overcome a stressful situation, such as escaping from predators (Bokony et al., 2009). However, if the individual is under stress for longer periods, the continuous presence of glucocorticoids can be detrimental in several ways, from weight loss, to immunosupression or reduced breeding success and ultimately death (Hood et al., 1998; Walker et al., 2006). It was observed that stress hormones levels in faeces reflect stress hormones levels in blood, so it is a reliable method for analysing the effect of human disturbance from a physiological point of view .

With regard to birds, changes in behavioural responses to high levels of anthropogenic acoustic disturbance have been observed, such as singing at higher pitch (Slabbekoorn & Peet, 2003) or reduced breeding success (Slabbekoorn & Ripmeester, 2008). On the other hand, no studies have addressed whether a given level of environmental acoustic disturbance would be reflected in stress hormone levels. The present study aims to test differences in stress hormone levels in captive-bred individuals of a wild species of bird while they are kept in cages at different locations and subjected to different levels of environmental acoustic disturbance.

The use of captive-bred birds, instead of individuals captured from the wild, to conduct this type of study is preferable as keeping wild birds in cages will induce an undetermined quantity of extra stress hormones that could lead to bias in the results (Piersma & Ramenofsky, 1998).

In our study, captive-bred greenfinches (Carduelis chloris), a widespread Palaearctic passerine species commonly found in both rural and urban habitats, were used. Birds were housed at four different sites, one in the centre of Venice, Italy, and three in natural protected areas at the border of the Venice lagoon. Noise disturbance resulted to be of a comparable level in Venice as well in two out three of the rural sites, while at the other site acoustic disturbance was greater due to building activities conducted adjacent to the protected areas to regulate tidal flow inside the lagoon.

The novel part of our experiment is the development of a tool to implement monitoring protocols in disturbed areas. We considered that measurement of corticosterone metabolites (hereafter CM) hormones in captive bred individuals could be useful to predict the effect of disturbance to wild populations.

2. Methods

2.1 Experimental setting

Four sites were selected for this study on the basis of their environmental acoustic conditions, one of which was in the centre of Venice (urban site) and the remaining three were rural sites. At the urban site, outside of the crowded areas of Venice, Italy, birds had to face the everyday environmental noise level of a city where car traffic is absent. The rural sites (Nat1, Nat2 and Nat3) were situated at each of the three inlets that border the Venice lagoon, where a flood barrier system is currently being constructed (2004-2012) to control exceptionally high tides. These rural sites were located in areas included in the "Natura 2000" European ecological network, with vegetation dominated by Populus alba and Pinus sp. and shrubs such as Rubus sp. and Eleagnus angustifolia, as well as a complex bird community. So they are strictly comparable from an ecologic point of view (vegetation cover, human disturbance, land use), but at the Nat3 site noise disturbance was greater due to the actual building activities carried out at the time of the study.

The ambient acoustic levels measurements were provided by University of Ferrara and by the environmental office of Venice city council. Noise measurements were obtained using a Bruel & Kjær phonometer model 2260 fitted with a Bruel & Kjær microphone model 4189 and with measurements recorded every minute. Means ± standard error at all four sites are reported in Table 1.

A total of 11 birds were obtained from authorized dealers (FOI, Italian captive-breeders Federation). Birds were singly housed in wire net cages, with no visual contact with the others. Each cage measured 40x40x60 cm and had two perches each as well as two feeders (with Canary grass, Phalaris canariensis) and two bottles of water. Cages were located on sites protected from exposure to weather and had a protective roof and shadowing netting. Cages were also protected from predators with a metallic grid covering all sides of the cages.

During the whole period birds were fed ad libitum and fresh water was constantly provided. At the beginning of experimental trials, birds were relocated from the location of their rearing to a common place at the urban site for three weeks to habituate them to the new cages. We then randomly assigned three individuals to each of the three rural sites, and left two at the urban site. They were left four days for habituation to each site before we took the first sample. Although there is no evidence of a decrease in cortiscosterone metabolites after the dropping has been excreted, we selected the freshest dropping sample from each cage every three days until three samples had been obtained from each individual. Once collected, the dropping samples were kept in a freezer at -20º C until CM analysis was performed. The birds were then taken back to the common urban place until the next experimental trial started. Three trials were carried out, commencing in June, September and October 2007. Birds were located at a different site in each trial.

At the rural sites, bird cages were positioned inside the enclosed ground of the building sites for the barrier system, far from any external activity and as close as possible to natural habitats. At the urban site, cages were located in a court surrounded by high buildings (3 stories) inside the university department, where only the authors had access. At the end of the experiment, birds were returned to the breeder.

2.2 Stress hormones extraction and analysis

For extraction, droppings (0.05 g) were diluted with 0.2 ml water in a microcentrifuge tube vortexed for 10 sec and, after adding 0.3 ml methanol, vortexed for a further 15 min using an Eppendorf vortex. Subsequently, the tubes were centrifuged (14000rpm, 1 min). An aliquot of the supernatant (0.2 ml) was transferred into a new tube and diluted with 0.1 ml assay buffer.

To select the assay for the experiment nine randomly selected samples were extracted and assayed using four enzyme immunoassays which are group specific for 11ß-hydroxyetiocholanolone (Frigerio et al., 2004), 11-oxoetiocholanolone (Möstl et al., 2002) corticosterone (Palme & Möstl, 1997) and tetrahydrocorticosterone (Quillfeldt & Mostl, 2003) All assays showed the same trend but the assay for 11-oxoetiocholanolone showed highest values and was therefore selected for analysing all samples. (The antibody for this assay was raised in rabbits against 5ß-androstane-3 α -ol-11,17-dione CMO:BSA, as label 5ß-androstane-3α-ol-11,17-dione CMO linked to biotinyl-3,6,9-trioxaundecanediamin (Fa. Pierce, New York, USA) by a mixed anhydride reaction was used.)

2.3 Data analysis

To confirm that individuals did not intrinsically differ in their stress hormone levels, the variance in the hormone assay data was tested for homogeneity using a Levene's test. We tested for differences between sites using a General Linear Model (Tabachnick & Fidell, 1996), with corticosterone metabolites as dependent variable, site as fixed factor and noise levels as random factor. When analysing the effects of acoustic disturbance we used mean daily noise levels recorded at each site.

3. Results

Comparing data from the three trials obtained in the urban site, we observed a statistically homogeneous trend (Levene's test $P=0.06$) indicating that there were comparable noise levels during test times, no exceptionally disturbing events and that the individuals reacted similarly.

Stress hormones levels of the individuals at the urban site were higher than at the rural sites except for site Nat3 (Table 1). There were no differences between sites Nat1 and Nat 2 in the level of CM; the hormone levels measured remained constant during the three trials (Levene's test $P=0,787$ for Nat1 and $P=0.722$ for Nat2) and they were below the hormone levels found at the urban site. Conversely, higher levels of CM were measured at site Nat3 compared to the other rural sites ($P < 0.05$ for Nat1 and $P < 0.01$ for Nat2).

Specifically in the trial carried out in June, we found that the urban and Nat1 sites had higher levels of stress hormones (Table 2, $P<0.001$ in all cases). Subsequently, in the second trial (September) we found that, again, birds in the urban site had higher levels of stress hormones but that they were lower than at site Nat3. Differences in stress hormone levels between these two sites (urban and Nat3) and sites Nat1 and Nat2 were significant ($P<0.01$). During the 3rd trial, we found that the birds at site Nat3 had higher CM levels, while birds at the urban site showed hormone levels similar to the individuals at sites Nat1 and Nat2. Differences in hormone levels between Nat3 and all the other rural sites were significant ($P<0.05$), but there was no difference in hormone level between Nat3 and the urban site ($P>0.05$).

We found that only site had an effect on stress hormone levels (One-way ANOVA for corticosterone metabolites $F_{3,105} = 3.53$, $P< 0.01$), while noise level (24 hr) was only marginally excluded from the model ($F_{1,105} = 3.4$, $P> 0.06$). There was, however, a strong correlation between noise levels and site, with site Nat3 showing higher noise levels compared to the urban site and the other two natural sites ($P< 0.001$ in all cases).

The correlation between acoustic level (diurnal noise only) and stress hormone levels resulted to be statistically significant with an increase of hormone levels at increasing acoustic levels ($r= 0.323$, $P< 0.01$), while nocturnal noise did not affect the stress levels ($r= 0.167$ $P= 0.15$). Also, we observed that the day of the week had an effect, although not significant, on the stress hormones levels; CM levels were higher on working days compared to resting days (Sunday; Figure 1).

4. Discussion

Our results indicate that the effects of a chronic acoustic stressor are measurable in captive-bred indivuduals using corticosterone metabolites in droppings. Similar results have been obtained from other small passerines, but analising corticosterone metabolites in blood (Alexandrov et al., 2001; Tilgar et al., 2010; Tilgar et al., 2009). We also observed that males and females react in a similar way to stress. The use of CM levels in droppings as an indicator of stress is reliable, given that the data were consistent among the three performed trials. Furthermore, the results of this study show that, as expected, the levels of CM found in greenfinch droppings correlated with the amount of noise that the birds were exposed to.

Looking at the results obtained from the rural sites, it turns out that at site Nat3 individuals are likely to excrete the highest amount of CM in their droppings. In accordance with mean noise levels recorded at that site, it can be concluded that the level of stress hormone is directly dependent on the noise level. The levels of acoustic disturbance were lower at sites Nat1 and Nat2 since different building activities were carried out during the trials and glucocorticoid levels were low.

It is possible that wild bird communities will present similar levels of stress if their habitat has similar levels of noise disturbance. This will be especially important if individuals cannot relocate (e.g. if they are in the middle of a reproductive event or if an alternative site is not locally available (Gill et al., 2001). The use of caged passerines to estimate the stress levels of a bird community may constitute a useful tool for managing the activities that can impact on bird welfare. This is a fact of paramount importance for the management of protected areas and for ecological networks such as the European “Natura 2000”.

It was unexpected that a high level of CM was produced by birds from the urban site given that noise levels were similar to or less than those recorded at sites Nat1 and Nat2. There may be many factors responsible for this finding, such as being on an inner court, instead of on open spaces as in the other sites, the presence of large number of rats (Rattus norvegicus) in the city, but those directly linked to urban life deserve, in our opinion, particular attention, as cities and towns are habitats far from any found in nature where life conditions may induce in individuals different forms of stress responses (Scheuerlein et al., 2001; Ylonen et al., 2006). Urban-settlement by birds (as well as other organisms) is a widespread and increasingly active process in which the species colonize man-made spaces directly or through different forms of adaptation or even of pre-adaptation (Diamond, 1987). A markedly broader environmental tolerance may predispose some bird species to thrive in urban habitats, as estimated by Bonier et al. (2007). The mechanisms that mediate such adaptative capabilities are poorly understood but they probably reflect changes in physiology and behaviour (Moller, 2008) as a consequence of a high phenotypic plasticity and rapid power of prior evolution.

Some variation in the stress hormone levels may be influenced by other environmental variables, such as temperature, oestrus cycle or food type (Touma & Palme, 2005), but it has also been concluded that, despite these possible biases, stress hormone analysis still produces reliable results (Lane, 2006; Sheriff et al., 2010). In our study all experiments were carried outside of the breeding season, so alteration by reproductive activity can be dismissed. Furthermore, the type of food was constant for all birds during the whole study and, therefore, differences in stress hormone levels are not likely to be influenced by this factor. Temperature may also influence results, such that individuals in colder conditions exhibit higher hormone levels if they are not adapted to such temperatures or temperature changes are drastic (Goymann et al., 2006);nevertheless we carried out the experiments in early summer (June) and after summer (September and October), when temperatures are high, but likely not so high as to create discomfort to this bird species in its natural habitat.

Although our sample size was low due to difficulties of finding more individuals of captive-bred greenfinches, we propose the use of captive bred individuals to monitor how human disturbance may affect resident populations, without the need to constrain wild-born individuals to caging.

It would be interesting to repeat this kind of study in different disturbance conditions, with different animal taxa in order to have a wider range of information to assess how environmental disturbance affects the metabolic status and consequently welfare of natural animal communities.

Animal welfare implications

From the results obtained in this study, we propose the use of captive bred individuals to estimate the effects of disturbance on free wild birds, without constraining the natural populations to unneeded disturbance or caging.

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References

Alexandrov, L. I., Korneeva, E. V., & Golubeva, T. B. (2001). Increasing selectivity of defense behavior in the ontogeny of pied flycatcher nestlings. *Zhurnal Vysshei Nervnoi Deyatelnosti Imeni I P Pavlova*, 51(1), 110-113.

Amo, L., Lopez, P., & Martin, J. (2006). Nature-based tourism as a form of predation risk affects body condition and health state of podarcis muralis lizards. *Biological Conservation*, 131(3), 402-409. http://dx.doi.org/10.1016/j.biocon.2006.02.015

Beale, C. M., & Monaghan, P. (2004). Behavioural responses to human disturbance: A matter of choice? *Animal Behaviour*, 68, 1065-1069. http://dx.doi.org/10.1016/j.anbehav.2004.07.002

Bokony, V., Lendvai, A. Z., Liker, A., Angelier, F., Wingfield, J. C., & Chastel, O. (2009). Stress response and the value of reproduction: Are birds prudent parents? *American Naturalist*, 173(5), 589-598. http://dx.doi.org/10.1086/597610

Bonier, F., Martin, P. R., & Wingfield, J. C. (2007). Urban birds have broader environmental tolerance. *Biology Letters*, 3(6), 670-673. http://dx.doi.org/10.1098/rsbl.2007.0349

Burger, J. (2003). Personal watercraft and boats: Coastal conflicts with common terns. *Lake and Reservoir Management*, 19(1), 26-34. http://dx.doi.org/10.1080/07438140309353986

Burger, J., & Gochfeld, M. (1993). Tourism and short-term behavioral responses of nesting masked, red-footed, and blue-footed boobies in the Galapagos. *Environmental Conservation*, 20(3), 255-259. http://dx.doi.org/10.1017/S0376892900023043

De Villiers, M., Bause, M., Giese, M., & Fourie, A. (2006). Hardly hard-hearted: Heart rate responses of incubating northern giant petrels (*Macronectes halli*) to human disturbance on sub-antarctic Marion island. *Polar Biology*, 29(8), 717-720. http://dx.doi.org/10.1007/s00300-006-0137-2

Diamond, A. W., Schreiber, R. L., Attenborough, D., & Prestt, I. (1987). *Save the birds*. London: Cambridge University Press.

Frigerio, D., Dittami, J., Möstl, E., & Kotrschal, K. (2004). Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male greylag geese (*Anser anser*). *General and Comparative Endocrinology*, 137(1), 29-36. 10.1016/j.ygcen.2004.02.013

Gill, J. A., Norris, K., & Sutherland, W. J. (2001). Why behavioural responses may not reflect the population consequences of human disturbance? *Biological Conservation*, 97, 265-268. http://dx.doi.org/10.1016/S0006-3207(00)00002-1

Goymann, W., Trappschuh, M., Jensen, W., & Schwabl, I. (2006). Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in european stonechats. *Hormones and Behavior*, 49(5), 644-653. http://dx.doi.org/10.1016/j.yhbeh.2005.12.006

Harvey, S., Phillips, J. G., Rees, A., & Hall, T. R. (1984). Stress and adrenal function. *Journal of Experimental Zoology*, 232, 633-645. http://dx.doi.org/10.1002/jez.1402320332

Hood, L. C., Boersma, P. D., & Wingfield, J. C. (1998). The adrenocortical response to stress in incubating magellanic penguins (*Spheniscus magellanicus*). *Auk*, 115(1), 76-84.

Jakubas, D. (2005). Factors affecting the breeding success of the grey heron (*Ardea cinerea*) in northern poland. *Journal of Ornithology*, 146(1), 27-33. http://dx.doi.org/10.1007/s10336-004-0051-8

Lane, J. (2006). Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Animal Welfare*, 15(4), 331-342.

Manor, R., & Saltz, D. (2005). Effects of human disturbance on use of space and flight distance of mountain gazelles. *Journal of Wildlife Management*, 69(4), 1683-1690. http://dx.doi.org/10.2193/0022-541X(2005)69[1683:EOHDOU]2.0.CO;2

Martin, J., de Neve, L., Polo, V., Fargallo, J. A., & Soler, M. (2006). Health-dependent vulnerability to predation affects escape responses of unguarded chinstrap penguin chicks. *Behavioral Ecology and Sociobiology*, 60(6), 778-784. http://dx.doi.org/10.1007/s00265-006-0221-1

Moller, A. P. (2008). Climate change and micro-geographic variation in laying date. *Oecologia*, 155(4), 845-857. http://dx.doi.org/10.1007/s00442-007-0944-3

Mostl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, 23, 67-74. http://dx.doi.org/10.1016/S0739-7240(02)00146-7

Möstl, E., Maggs, J. L., Schrotter, G., Besenfelder, U., & Palme, R. (2002). Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications*, 26(2), 127-139. http://dx.doi.org/10.1023/A:1014095618125

Nimon, A. J., Schroter, R. C., & Oxenham, R. K. C. (1996). Artificial eggs: Measuring heart rate and effects of disturbance in nesting penguins. *Physiology & Behavior*, 60(3), 1019-1022. http://dx.doi.org/10.1016/0031-9384(96)00079-0

Palme, R. (2005). Measuring fecal steroids: Guidelines for practical application. *Annals of the New York Academy of Sciences*, 1046(1), 75-80. http://dx.doi.org/doi:10.1196/annals.1343.007

Palme, R., & Möstl, E. (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Zeitschrift Fur Saugetierkunde-International Journal of Mammalian Biology*, 62, 192-197.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., & Möstl, E. (2005). Stress hormones in mammals and birds - comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples Trends in comparative endocrinology and neurobiology, 1040, 162-171. http://dx.doi.org/10.1196/annals.1327.021.

Pearce-Higgins, J. W., Finney, S. K., Yalden, D. W., & Langston, R. H. W. (2007). Testing the effects of recreational disturbance on two upland breeding waders. Ibis, 149, 45-55. http://dx.doi.org/10.1111/j.1474-919X.2007.00644.x

Piersma, T., & Ramenofsky, M. (1998). Long-term decreases of corticosterone in captive migrant shorebirds that maintain seasonal mass and moult cycles. *Journal of Avian Biology*, 29(2), 97-104. http://dx.doi.org/10.2307/3677186

Quillfeldt, P., & Mostl, E. (2003). Resource allocation in wilson's storm-petrels *Oceanites oceanicus* determined by measurement of glucocorticoid excretion. *Acta Ethologica*, 5, 115-122. http://dx.doi.org/10.1007/s10211-003-0074-9

Rogers, D. I., Piersma, T., & Hassell, C. J. (2006). Roost availability may constrain shorebird distribution: Exploring the energetic costs of roosting and disturbance around a tropical bay. *Biological Conservation*, 133(2), 225-235. http://dx.doi.org/10.1016/j.biocon.2006.06.007

Sandvik, H., & Barrett, R. T. (2001). Effect of investigator disturbance on the breeding success of the black-legged kittiwake. *Journal of Field Ornithology*, 72(1), 30-42.

Scheuerlein, A., Van't Hof, T. J., & Gwinner, E. (2001). Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, 268(1476), 1575-1582. http://dx.doi.org/10.1098/rspb.2001.1691

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010). Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? *General and Comparative Endocrinology*, 166(3), 614-619. http://dx.doi.org/10.1016/j.ygcen.2009.12.017

Silverin, B. (1998). Stress responses in birds. *Poultry and Avian Biology Reviews*, 9(4), 153-168.

Sirot, E. (2006). Social information, antipredatory vigilance and flight in bird flocks. *Animal Behaviour*, 72, 373-382. http://dx.doi.org/10.1016/j.anbehav.2005.10.028

Slabbekoorn, H., & Peet, M. (2003). Ecology: Birds sing at a higher pitch in urban noise - great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature*, 424(6946), 267-267. http://dx.doi.org/10.1038/424267a

Slabbekoorn, H., & Ripmeester, E. A. P. (2008). Birdsong and anthropogenic noise: Implications and applications for conservation. *Molecular Ecology*, 17(1), 72-83. http://dx.doi.org/10.1111/j.1365-294X.2007.03487.x

Swaisgood, R. R. (2007). Current status and future directions of applied behavioral research for animal welfare and conservation. *Applied Animal Behaviour Science*, 102(3-4), 139-162. http://dx.doi.org/10.1016/j.applanim.2006.05.027

Tabachnick, B. G., & Fidell, L. S. (1996). Using multivariate statistics. Needham Heights, MA: Allyn & Bacon.

Tilgar, V., Saag, P., & Moks, K. (2009). Development of stress response in nestling pied flycatchers. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology*, 195(8), 799-803. http://dx.doi.org/10.1007/s00359-009-0452-5

Tilgar, V., Saag, P., Kulavee, R., & Mand, R. (2010). Behavioral and physiological responses of nestling pied flycatchers to acoustic stress. *Hormones and Behavior*, 57(4-5), 481-487. http://dx.doi.org/10.1016/j.yhbeh.2010.02.006

Touma, Chadi, & Palme, Rupert. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. *Annals of the New York Academy of Sciences*, 1046(1), 54-74. http://dx.doi.org/doi:10.1196/annals.1343.006

Walker, B. G., Boersma, P. D., & Wingfield, J. C. (2005a). Field endocrinology and conservation biology. *Integrative and Comparative Biology*, 45(1), 12-18. http://dx.doi.org/10.1093/icb/45.1.12

Walker, B. G., Boersma, P. D., & Wingfield, J. C. (2005b). Physiological and behavioral differences in magellanic penguin chicks in undisturbed and tourist-visited locations of a colony. *Conservation Biology*, 19(5), 1571-1577. http://dx.doi.org/10.1111/j.1523-1739.2005.00104.x

Walker, B. G., Boersma, P. D., & Wingfield, J. C. (2006). Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology*, 20(1), 146-154. http://dx.doi.org/10.1111/j.1523-1739.2006.00271.x

Ylonen, H., Eccard, J. A., Jokinen, I., & Sundell, J. (2006). Is the antipredatory response in behaviour reflected in stress measured in faecal corticosteroids in a small rodent? *Behavioral Ecology and Sociobiology*, 60(3), 350-358. http://dx.doi.org/10.1007/s00265-006-0171-7

Table 1. Mean, range and standard error for stress hormones levels in the four sites. On the column of the right hand side, mean ± S.E noise levels are provided

Site	Stress hormones levels			Noise (dB)
	Mean	Range	S.E.	Mean ± S.E.
Urban	1.37	0.77-1.82	0.03	56.0 ± 0.5
Nat1	1.23	0.67-1.86	0.05	58.45 ± 0.62
Nat2	1.19	0.69-1.50	0.03	54.19 ± 1.5
Nat3	1.41	0.97-1.90	0.06	67.14 ± 1.47

Table 2. Mean ± SE for stress hormones levels for the four sites and for each trial

Site	Nat1		Nat2		Urban		Nat3	
Trial	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
June	1.11	0.08	1.44	0.09	1.42	0.04	1.16	0.06
September	1.26	0.04	0.97	0.07	1.31	0.07	1.66	0.09
October	1.21	0.04	1.28	0.06	1.37	0.08	1.52	0.02

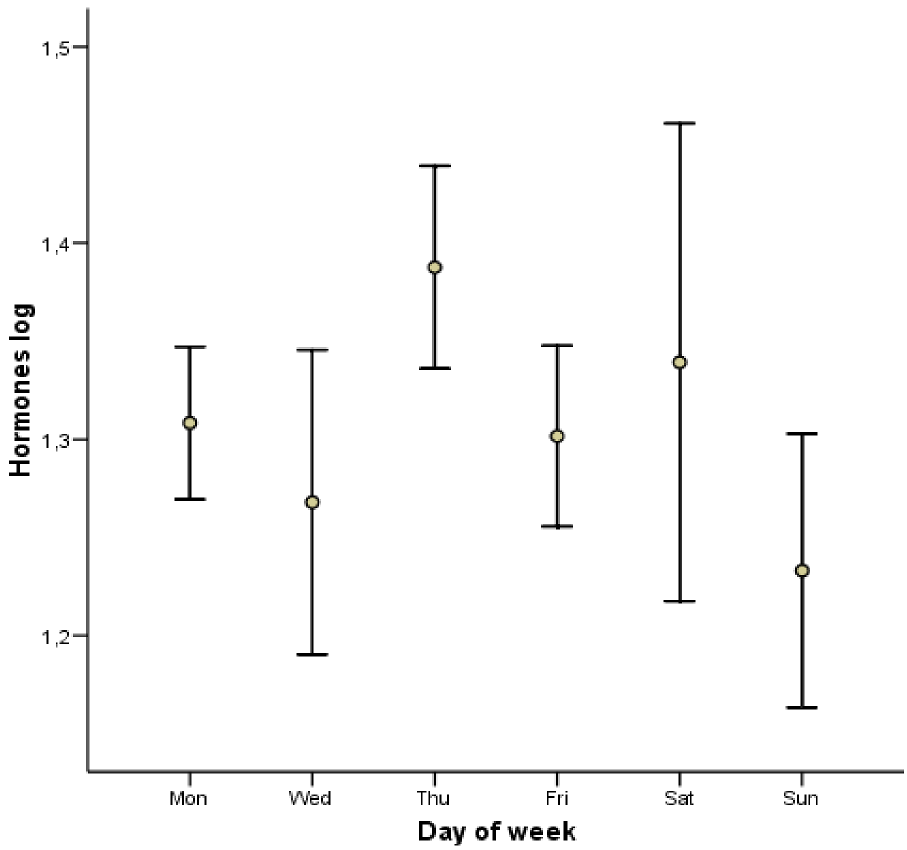

Figure 1. Mean stress hormones levels according to day of the week. It can be observed that during workdays stress hormones levels are higher, compared to Sunday (Saturday is also a working day in this area)