The possible effects of extreme winters on the barn owl (*Tyto alba* Scop., 1769) population in Hungary: survival, physiology and population genetics

PhD Thesis

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Introduction

The barn owl (*Tyto alba*) evolved under a tropical and subtropical climate. The recent distribution of this owl is however rather cosmopolitan and it presently occupies habitats that can be characterized by more extreme continental weather conditions. One of the most serious natural mortality factors is the harsh winter (long lasting snow cover and freezing conditions) that occur under a continental climate regime, as exists across Hungary (Altwegg *et al.* 2003, Altwegg *et al.* 2006). With the onset of global climate change (it is proven that the frequency and amplitude of winter extremes are increasing in Central Europe, Bartholy *et al.* 2007) it is very likely that the population dynamics of Hungarian barn owls will be affected.

The barn owl populations can be characterized by a high mortality rate in central Europe (Taylor 1994). The thermo-neutral zone of the species is relatively high, between 25-33 °C, and barn owls start catabolise their protein tissues before they would have used all the adipose reserves (Thouzeau *et al.* 1999). This species is unable to deposit as much fat as other European owl species (*Strigidae*) of the same size (5,3-6,6% vs. 9-12,3%, Piechocki 1960). The high body mass at the age of 40-50 days of the owlets is due to the high water content of the tissues, not to the body fat reserves (Durant *et al.* 2008). The thermo-insulation capacity of the plumage is slightly poorer then of the other strigid owl species.

Consequently, barn owls can less cope with harsh winters than other owl species of the same size. They can survive a shorter period of starvation and the constant high body temperature costs lot of energy. These result in both higher winter mortality and in greater extent of population fluctuation. The barn owl can compensate the high mortality rate with high reproductive allocation (second brood, more but smaller eggs, high number of fledglings). In the present study I addressed the question of the likely consequences of harsh winters on the Hungarian barn owl population knowing that this species evolved under warm climate, expanded its distribution all over the world, and that changing climate can present a real challenge to survival where continental climate conditions exist. I investigated this subject from the aspects of survival, physiology (endocrinology) and population genetics.

1) The effect of nest-sites: A common method of barn owl conservation is the placement of nest-boxes in church towers. Despite the usefulness of nest-boxes, several studies have shown that there may be associated disadvantages and that nest-boxes may even act as ’ecological traps’. In theory, if one of the applied conservation methods results in lower survival, this can cause population stagnation or decline and may be ineffective in helping

population recovery after a population crash. Hence, I compared the survival rate of owlets hatched in nest-boxes with those hatched in the more natural environment of church towers.

2) Physiological aspects: Thyroid hormones (THs) regulate growth and development, basal metabolic rate, and most importantly from the present viewpoint, they play critical roles in the maintenance of constant body temperature. For this reason we carried out experiments to determine barn owl thyroid patterns and the function in thermoregulation using thyrotropin releasing hormone (TRH) provocation test - the interrelation of age, gender and the patterns of the two thyroid hormones such as thyroxin (T4) and 3,3',5-triiodothyronine (T3) in wild barn owls during two breeding seasons. The responses of the thyroid functions were also investigated by measuring the THs content after a TRH challenge.

3) Population genetics aspects: Barn owl populations undergo serious population crashes from time to time as a consequence of extreme continental climate conditions. These natural population fluctuations raise the question of whether or not population genetical consequences exist, e.g. bottle-neck effect. To investigate this question we first tested and characterised universal bird and owl-specific microsatellite primer pairs. Using a set of 24 polymorphic microsatellite primer pairs, we described the allele-diversity of the Hungarian population after a well documented population decline in 2002-2003.

1. The effect of nest-sites on the survival-time of owlets

Introduction: Nest-site reduction has played a significant role in the decline of Barn Owl populations throughout Europe and North America. Techniques of nest-site augmentation, involving the provision of nest-boxes, have been widely used in a range of species of conservation concern, including falcons, eagles, parrots, owls and cavity-nesting ducks. A common method of Barn Owl conservation is the placement of nest-boxes on church towers. Despite the usefulness of nest boxes, several studies have shown that there may be associated disadvantages and that nest boxes may even act as ''ecological traps''. The purpose of this research was to compare the survival rate of owlets hatched in nest-boxes with those hatched in the more ''natural'' environment of church towers.

Methods: We examined an 8-year period between 1995 and 2003. The Hungarian Bird Ringing Centre provided the data. Data covered the entire territory of Hungary and included all details according to the European Bird-ringing Schemes (EURING), such as ring number, date, body condition, circumstances, latitude and longitude. However, there was no information on the type of nest-site (e.g. nest-box, spire, barn, tree-cavity) as ringers are not required to report this. We therefore contacted each ringer personally and managed to collect reliable data on the origin of 8,130 ringed individuals. From the 8,130 specimens 437 were recovered and 297 of the 437 were marked as owlets. The various nest-sites (church towers with and without nest-boxes) were distributed randomly within the total sample. Data were analysed by survival time analysis (STA), which is appropriate where data are collected over time by repeatedly observing uniquely identified individuals (Fox 2001).

Results:

1.1 Fewer juveniles were ringed in nest boxes than in church towers, but the proportion of recoveries among birds marked as owlets did not differ between the two types of brood sites.

1.2 Birds ringed as owlets and hatched in nest boxes were found dead in a greater ratio than those hatched in church towers.

1.3 The difference in surviavl-time was most pronounced in the first year of life, after owlets became independent from their parents.

1.4 The hatching time did not have any impact on survival pattern of owlets.

Discussion: The main finding of this study is that the short-term survival of the owlets from nest-boxes is lower than that of owlets hatched in the more natural church towers during the parent independent period of their first year (50–385 days). This result implies that when the owlets leave the vicinity of the parental nest and begin their independent life, their chance of survival, to an extent, depends upon the breeding place of their parents. Even though nest-box installation is traditionally thought to be the most effective Barn Owl conservation method throughout Europe (Taylor 1994), and is clearly better than having no nesting opportunities at all, our results suggest that this method of protection has major disadvantages. It is therefore a realistic hypothesis to conclude that Barn Owl nest-boxes act as ‘‘ecological traps’’ in the sense that when man suddenly alters the environment, formerly reliable cues might no longer be associated with adaptive outcomes, which can cause reduced survival or reproduction (Schlaepfer et al. 2002). If a significant proportion of the population breeds in nest-boxes, which can negatively affect the survival of the owlets, the decreased survival can lead to population instability or decline.

2. Characterization of the thyroid hormones

Introduction: Thyroid hormones (THs) regulate growth and development, basal metabolic rate, and most importantly from the present viewpoint, they play critical roles in the maintenance of constant body temperature (Decuypere *et al.* 2005). Under the climatic conditions of the Carpathian Basin the functional characteristics of the adenohypophysis–thyroid gland axis are supposed to be critical factors of metabolic adaptation, particularly in the winter period, before the nesting season. For this reason we carried out experiments to determine barn owl thyroid patterns and the function in thermoregulation using thyrotropin releasing hormone (TRH) provocation test - the interrelation of age, gender and the patterns of the two thyroid hormones such as thyroxin (T4) and 3,3',5-triiodothyronine (T3) in wild barn owls during two breeding seasons. The responses of the thyroid functions were also investigated by measuring the THs content after a TRH challenge.

Methods: Experiment 1) Blood samples were collected from wild barn owls where the birds nested, in June – August 2004 and 2005. A total of 145 individuals (22 adults, 123 juveniles) were involved in this study with TRH stimulation test. Experiment 2) Before the nesting season – at the beginning of March 2005 – 11 injured female owls were weighed and transferred indoors from their outdoor aviary. Birds were kept under their thermoneutral temperature and divided into two groups. The experiments lasted for 8 days. First, owls were acclimated to the respective indoor conditions for two days and then Group1 was submitted to cold temperature whereas Group2 to warm conditions for 3 days (Period 1). At the end of day 5, weighing, TRH stimulation test and blood sampling were carried out and we changed the groups between the two rooms (Period 2). Blood samples (500-800 µl) were taken from the brachial vein after that each bird received 20 µg of TRH (thyrotropin releasing hormone acetate iv. To determine the TRH-challenged T4 and T3 response exactly 60 minutes after the TRH injection a second blood sample had been drawn.

Results:

2.1 The age had a significant impact on the plasma T4 level: owlets younger than 51 days had the highest concentration of T4 in the blood, whereas the adults had the lowest one

2.2 Among the adults, males had significantly higher T4 plasma level than females

2.3 Neither the age, nor the gender showed significant difference in the basal T3 level

2.4 Neither TRH, nor the gender affected the concentration of plasma T4 after a TRH treatment

2.5 TRH treatment elevated significantly T3 plasma level in every age and sex categories

2.6 Higher T3 concentration could be measured in captured birds which were kept in cold, comparing to those, which were placed under warm temperature conditions. Difference in T4 level was not detected.

2.7 TRH treatment increased plasma T3 level regardless the temperature, however, TRH did not affect T3 plasma concentration.

2.8 Birds kept in cold showed a lower sensitivity towards TRH treatment

Discussion: The basal T4 plasma level showed a characteristic altricial pattern in the three age categories. Owlets younger than 51 days had higher T4 plasma concentration than their elder siblings, and this profile was independent of sex. T3 profile of the owlets did not show consistent pattern with age or sex. The effect of TRH on T4 hormone level is not in accordance with the findings of earlier studies. Neither in the owlets nor in adult males we could not find any significant change in the TRH stimulated T4 plasma level but there was a significant increase in adult females. Each group responded to TRH treatment with an elevated serum T3 level, which is a consistent result with the conclusions of other experiments. T3 response was much more pronounced both under cold (around 10 °C) and warm (around 20 °C) conditions, whereas T4 response ranged so widely that we could not point out any significant change in it. Basal T3 plasma level was significantly higher in birds exposed to cold temperature, and they responded to TRH treatment with a lower plasma T3 elevation than the birds kept in a warm chamber. This pattern, however, cannot be explained by increased food intake, but is in agreement with the fact that enhanced T3 level may account for higher avUCP mRNA expression, which results in higher heat production on the cell level (Raimbault *et al.* 2001, Collin *et al.* 2005). From the results it is concluded that altering T3 plasma level plays a significant role in cold-induced thermoregulation.

3. Population genetics

Introduction: Barn owl populations undergo serious population crashes from time to time as a consequence of extreme continental climate conditions. These natural population fluctuations raise the question of whether or not population genetical consequences exist, e.g. bottle-neck effect. To investigate this question we first tested and characterised universal bird and owl-specific microsatellite primer pairs. Using a set of 24 polymorphic microsatellite primer pairs, we described the allele-diversity of the Hungarian population after a well documented population decline in 2002-2003.

Methods: Blood samples were collected from 58 unrelated barn owl individuals belonging to a single population inhabiting the counties of Baranya and Somogy in the southwest of Hungary. In total, 190 avian microsatellite loci were assessed for utility in the barn owl. Of the 190 microsatellite loci tested in the barn owl, 56 were originally isolated from six Strigidae owl species. (1) PCR (Polymerase Chain Reaction) optimization. (2) Testing the polymorphism of the loci on 5-10 individuals. (3) Sequencing of an allele of the polymorphic loci. (4) Redesigning those primers which showed deviation from Hardy-Weinberg equilibrium or null-allele frequency. (5) Analysing recent genetic bottleneck effect on the population using the genotype data with the software BOTTLENECK (90% SMM/10% IAM). (6) Simulation of diffuse and intensive bottle-neck effect on the dataset with program GENELOSS.

Results:

3.1 The population genetics parameters did not reveal any proofs of decline in genetical diversity.

3.2 The Wilcoxon test of the „Two-Phased Model” did not show any evidences for population bottleneck when 58 individuals were used

3.3 Changing the parameters of the „Two-Phased Model” (increasing the proportion if IAM steps) significant bottleneck effect could be detected.

3.4 The L-shape distribution test did not show any significant bias from equilibrium populations

Discussion: The 20 polymorphic loci (including those amplified with newly designed primers) displayed two to 26 alleles when tested in 56–58 barn owls. Observed heterozygosity varied between 0.30 and 0.85. We could not show out any sign of loss of genetic diversity as we compared the population genetics parameters (such as Ho, He, HWE, null-allele, PIC values) of the Hungarian population with those of other populations. Bottleneck program could not reveal any significant population genetical consequences of recent bottle-necks. Our results, however, depends on the proportion of the applied various microsatellite mutation models (SMM vs. IAM). As the mutational mechanisms of microsatellite loci are not perfectly clarified so far we cannot be certain of the extent of type II error (we cannot detect any significant effect even though there is one). If we accept, that there are no genetical consequences of population bottle-neck in the Hungarian barn owl population, we must recon that the effective population size is not affected by the reduction of the population. It means that 58 individuals of barn owls from SW Hungary represent that allele pool and level of heterozigosity as a population on which the drift has no impact. The Geneloss simulations showed that 250 breeding pairs, which have the experienced allele diversity can keep the number of alleles and level of heterozigosity even if this population is exposed to either intensive or diffuse bottleneck-effect (8,05 → 7,2 and 0,71 → 0,69). The 250 breeding pair for SW Hungary is a realistic number as the whole population estimated is about 1000 breeding pairs in Hungary.

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