**Universidade de Aveiro** Departamento de Ambiente
**2005**

**Rute Alexandra Pais Costa**

# Avifauna como Bioindicador de Poluição Atmosférica por Metais Pesados

**Universidade de Aveiro** Departamento de Ambiente
**2005**

**Rute Alexandra Pais Costa**

# Avifauna como Bioindicador de Poluição Atmosférica por Metais Pesados

dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Gestão Ambiental, Materiais e Valorização de Resíduos, realizada sob a orientação científica do Dr. Amadeu Soares, Professor Catedrático do Departamento de Biologia da Universidade de Aveiro.

**o júri**

presidente

Prof. Dr. João António Labrincha Batista
professor associado com agregação da Universidade de Aveiro

Prof. Dr. Amadeu Mortágua Velho da Maia Soares
professor catedrático da Universidade de Aveiro

Prof. Dr. José Victor de Sousa Vingada
professor auxiliar da Escola de Ciências da Universidade do Minho

**agradecimentos**

A realização do presente trabalho resultou do envolvimento e ajuda de muitas pessoas a quem gostaria de agradecer.

Em primeiro lugar, agradeço ao meu Orientador Científico neste trabalho, Professor Doutor Amadeu Soares.

Agradeço ao pessoal da Mata de Quiaios: João Ferreira, Jorge Vaqueiro, Pedro Rodrigues, Laura Cerqueira, Marisa Ferreira, Catarina Eira, pela disponibilidade na ajuda da colocação das caixas-ninho e das visitas de rotina às mesmas.

Agradeço em especial ao José Vingada e ao João Petronilho pela constante disponibilidade, sugestões e orientação que muito contribuíram para realização desta dissertação.

À minha família e amigos pela compreensão e apoio demonstrados.

**palavras-chave**

Poluição Atmosférica, Metais Pesados, Penas de Aves.

**resumo**

O presente trabalho engloba uma série de estudos cujos objectivos são a avaliação da avifauna florestal como bioindicador de metais pesados e o potencial impacto da poluição na biologia de reprodução da avifauna.
Os estudos decorreram em três zonas de amostragem que foram seleccionadas em função do tipo de indústria existente nas proximidades, de forma a criar diferentes cenários de contaminação: Mata Nacional de Quiaios, Figueira da Foz (MQ) – zona sem indústria; Mata Nacional do Urso, Figueira da Foz (MU) – zona com influência da indústria da pasta e papel; e Complexo Químico de Estarreja (EST) – zona de poluição crónica por mercúrio.
Na MQ e MU monitorizámos e comparámos a ecologia reprodutora do Chapim-real, e recolhemos penas das crias para análise de metais pesados. Na MQ e EST foi apenas feita a recolha de penas de Melro-preto *Turdus merula*, Toutinegra-de-barrete-preto *Sylvia atricapilla* e Pisco-de-peito-ruivo *Erithacus rubecula*, capturados em redes, para posterior análise de metais pesados.
A avaliação da biologia de reprodução de Chapim-real decorreu na primavera de 2003 e 2004, com a utilização de caixas-ninho. MU apesar de ser uma área mais pobre em alimento foi a que apresentou valores reprodutivos mais elevados, tendo mesmo mais um ovo e mais um juvenil voador por ninho do que MQ. No entanto MQ tem uma maior taxa de ocupação. Apesar de a taxa de ocupação por Chapim-real ser semelhante em ambas as áreas, a taxa de ocupação total (todas as espécies) é muito superior na MQ. Mecanismos de competição inter-específica parecem influenciar de forma mais significativa a biologia reprodutora do Chapim-real do que a qualidade do território. Estes resultados sugerem que a reprodução próximo de uma zona industrial, não afecta directamente o Chapim-real, mas pode afectar outras espécies que também utilizam as caixas-ninho para se reproduzirem.
A recolha das penas para avaliação da contaminação por metais pesados decorreu durante a primavera de 2003. Na comparação das penas de crias de Chapim-real entre MQ e MU detectámos valores de mercúrio significativamente superiores na MU. Para todos os outros elementos estudados as concentrações não apresentaram diferenças significativas entre as áreas. Estes resultados sugerem que as penas de crias de Chapim-real são um método vantajoso para avaliar a presença de alguns metais pesados como o mercúrio, mas a espécie não pode ser considerada como monitora mas sim como sentinela, já que os valores de contaminação são contrários aos valores de performance reprodutiva.
Na comparação entre as áreas MQ e EST e as espécies Melro-preto, Pisco-de-peito-ruivo e Toutinegra-de-barrete-preto, detectámos valores significativamente superiores para todas as espécies em EST. O Melro-preto apresentou valores significativamente superiores para cádmio e níquel, o Pisco-de-peito-ruivo para cádmio, chumbo, zinco e mercúrio, e a Toutinegra-de-barrete-preto para cádmio, zinco e mercúrio. Para todos os outros elementos estudados as concentrações não apresentaram diferenças significativas entre as áreas. Entre espécies também houve diferenças nos níveis de acumulação dos contaminantes. O Pisco-de-peito-ruivo acumulou significativamente mais metais que as outras duas espécies e apresentou diferenças entre áreas. Estes resultados sugerem que a utilização de redes e penas de Pisco-de-peito-ruivo são um método credível na avaliação da poluição por metais pesados.

**keywords** Atmospheric Pollution, Heavy Metals, Bird Feathers.

**abstract**

The present work includes a series of studies which the main gold's were the evaluation of birds as bioindicators of heavy metal pollution and the possible influence of pollution in their breeding biology.
Studies were carried out in three areas selected because of the type of industry in the surroundings, to create different sets of contaminations: Mata Nacional de Quiaios (MQ), Figueira da Foz – area with no industry; Mata Nacional do Urso (MU) – area with the influence of paper industry; Complexo Químico de Estarreja (EST) – area with chronic pollution by mercury.
In MQ and MU we studied the breeding biology of the Great Tit *Parus major* and collected nestling bird feathers. In MQ and EST we just collect feathers from Blackbird *Turdus merula*, Blackcap *Sylvia atricapilla*, Robin *Erithacus rubecula* (resident passerines) caught in mist nets.
The evaluation of the breeding biology of the Great Tit occurred during the spring of 2003 and 2004, using nest boxes. Although MU being a poorer feeding area, the breeding performance was higher, having one more egg and one more fledgling per pair than in MQ. However, MQ had a higher occupation rate for all species. Occupation rate by Great Tit was similar in both areas. Interspecific competition mechanisms seem to be more important in the breeding biology of the Great Tit than territory quality. Therefore, the results provide evidence that breeding close to an industrial complex does not influence directly the Great Tit, but it may affect others species that also breed in nest boxes.
Feathers were collected during the spring of 2003 and analysed to evaluate heavy metal pollution. In MQ and MU we analysed the feathers of nestlings Great Tit. Mercury concentration was significantly higher in MU. For all other elements considered, concentrations did not differ significantly between MQ and MU. These results suggest that the use of Great Tit nestlings' feathers is a useful method to evaluate the presence of heavy metals, such as mercury, in the forest environment. But the specie can not be considered biomonitor because Great Tit presented a better reproductive performance in the polluted area. On the other hand it can be considered as sentinel specie.
In MQ and EST we found significantly higher levels in EST for all species. Blackbird presented significantly higher values of cadmium and nickel, Robin of cadmium, lead, zinc and mercury, and Blackcap of cadmium, zinc and mercury compared with MQ. For all other elements considered, concentrations did not differ significantly between MQ and EST. Differences in metal levels between species were found, Robin accumulated more metals presenting differences between areas. These results suggest that the use of mist nets and Robin feathers are a reliable method when using birds to evaluate the presence of heavy metals.

# ÍNDICE

## INTRODUÇÃO GERAL E OBJECTIVOS

A poluição atmosférica tem-se cada vez mais tornado um problema em expansão no ultimo século, afectando significativamente os ecossistemas terrestres, alterando a estrutura física e/ou química dos habitats tornando-os menos apropriados para as espécies de vida selvagem (Pitelka, 1994; Eeva, 1996 e Newton, 1998).

Um caso especifico é a poluição por metais pesados. Os metais, com especial destaque para o mercúrio, são bioacumuláveis, persistentes no meio e a partir de determinadas concentrações tornam-se tóxicos. Podem acumular-se nos organismos, particularmente nos que habitam próximo de áreas industrializadas (Fowler, 1990) e a partir de determinados níveis podem causar diminuição do sucesso reprodutivo e da sobrevivência das espécies selvagens (Esselink *et al*, 1995; Eeva e Lehikoinen, 1996).

O mercúrio é um metal pesado que ocorre no ambiente por processos naturais como a actividade vulcânica e processos de volatilização do metal a partir da superfície terrestre, mas ocorre também por emissões antrópicas. As principais fontes de contaminação de mercúrio são (Abreu, 1996):

- Indústria do cloro,
- Indústria do papel e pasta de papel,
- Indústria mineira e refinarias,
- Indústria das tintas,
- Laboratórios, hospitais e aplicações dentais,
- Estações de tratamento de águas residuais,
- Pesticidas,
- Combustíveis fósseis,
- Produção de cimento,
- Indústria química no geral,
- Indústria de componentes eléctricos,
- Incineradoras.

Devido à ecotoxicidade do mercúrio e de outros metais pesados a monitorização e avaliação dos seus níveis é deveras importante.

A utilização de organismos vivos como bioindicadores de contaminação por metais pesados apresenta diversas vantagens (Burger, 1993; Burger *et al*, 1994; Eens *et al*, 1999):

- Os níveis de contaminação são superiores nos tecidos biológicos em relação a amostras de água ou sedimentos;
- Organismos sensíveis;
- Fáceis de amostrar;
- Permitem amostragem não destrutiva;
- Permitem monitorizar áreas de diferentes tamanhos;
- Têm uma aplicação global;
- Têm uma vantagem económica;
- Permite registar gamas de concentrações em espécies utilizadas para consumo humano identificando níveis potencialmente perigosos a saúde pública.

No presente estudo utilizámos penas de quatro espécies de passeriformes residentes para avaliar os níveis de contaminação por metais pesados. As penas de aves são indicadores já bastante utilizados em estudos de biomonitorização (Hahn *et al*, 1993) porque acumulam metais pesados na altura da formação das penas (primeiras penas e período de muda) e também por ser um método não invasivo e de fácil amostragem, já que uma pequena amostra pode ser retirada de uma ave viva sem causar danos à ave (Burger, 1993).

Os metais pesados combinam-se com os grupos de queratina à medida que a pena cresce e está ligada à corrente sanguínea por meio de pequenos vasos sanguíneos. Depois da pena estar completamente formada, os vasos sanguíneos atrofiam e a pena torna-se fisiologicamente separada da ave (Denneman e Douben, 1993). Os metais acumulados na pena permanecem extremamente resistentes a demais alterações.

As aves de rapina e aves marinhas têm sido as espécies mais utilizadas nestes estudos, no entanto, a maioria destas espécies é bastante móvel, alimentando-se numa área geográfica extensa e variável, tornando-se difícil a sua monitorização e a determinação exacta do local de contaminação (Burger e Gochfeld, 1997).

Mais recentemente, vários estudos têm verificado que pequenos passeriformes como Chapim-real e o Chapim-azul são bons indicadores de contaminação local, já que estas aves são residentes na maioria das populações e com uma área de alimentação limitada e pouco variável (Cramp e Perrins, 1993). Além disso, estas espécies utilizam buracos nas árvores para se reproduzirem e facilmente utilizam ninhos artificiais (caixas-ninho), podendo assim uma população reprodutora rapidamente estabelecer-se e facilmente ser monitorizada, em quase qualquer área de interesse (Eens *et al*, 1999).

Este trabalho tem como objectivos:

- Avaliação da sensibilidade da avifauna florestal como bioindicadores da contaminação por mercúrio e outros metais pesados;
- Avaliação dos níveis de contaminação em função do tipo de fonte poluente;
- Relação dos níveis de contaminação com parâmetros populacionais e ecológicos (taxa de reprodução, sucesso reprodutivo, taxa de crescimento de juvenis, etc).

Para cumprir os objectivos foram delineados os seguintes trabalhos:

- Monitorização, avaliação e comparação da biologia de reprodução do chapim-real em duas zonas: Rural *vs* Industrial;
- Utilização de penas de crias de chapim-real como bioindicador de metais pesados;
- Utilização de penas de passeriformes adultos como bioindicador de metais pesados.

Este estudo desenrolou-se em três zonas de amostragem, que foram seleccionadas em função do tipo de indústria existente nas proximidades, de forma a criar diferentes cenários de contaminação:

- MQ - Mata Nacional de Quiaios (Fig.2) – zona sem indústria:

Situada no limite norte do Concelho da Figueira da Foz é uma zona rural incluída na zona de protecção especial sítio Natura 2000 – Dunas de Mira (Natura 2000 PTCON055). É essencialmente uma mata de pinheiro, *Pinus pinaster,* com 70 a 80 anos de idade, onde o único factor de perturbação será possivelmente a agricultura nos terrenos adjacentes à mata.

- MU - Mata Nacional do Urso (Fig.2) – zona com influência da indústria da pasta e papel:

Situada no limite sul do Concelho da Figueira da Foz é uma mata de pinheiro, *Pinus pinaster*, com 70 a 80 anos de idade. Nesta área existem duas fábricas de pasta e papel e uma estação de tratamento de águas residuais (ETAR).

A zona de amostragem situa-se na proximidade de uma das fábricas de pasta e papel. Esta fábrica teve um início de actividade em 1967 e durante anos os efluentes líquidos seguiam em vala aberta para o mar (Vala do Estremal) (Celulose Billerud, sem data). Só nos anos 90 foi construído um exutor submarino que faz as descargas a várias milhas da costa. Ao longo desta tubagem (na zona de terra) existem pequenas "chaminés" com a função de libertação de vapores (Fig.1).

O processo de fabrico é a digestão contínua Kraft com prehidrólise e as matérias-primas são sulfato de sódio, soda cáustica, cloro, cloreto de sódio, ácido sulfúrico, enxofre, calcareo, fuel-oil, hipoclorito de sódio e dióxido de cloro (Celulose Billerud, sem data).

Fig.1- "Chaminé" existente ao longo do exutor submarino da Fábrica de Pasta de Papel, na Mata Nacional do Urso.

- EST - Complexo Químico de Estarreja (Fig.2) – zona de poluição crónica com mercúrio:

Situado no Concelho de Estarreja, a cerca de 1Km para norte do perímetro urbano da vila, inclui uma série de empresas produtoras de compostos químicos como anilinina, mononitrobenzeno, nitrato de amónio, adubos compostos, resinas sintéticas, isocianatos, ácido sulfúrico, cloro e soda cáustica.

A nossa especial atenção é a fábrica de cloro e soda cáustica, que é uma das indústrias com maiores consumos e simultaneamente uma das maiores fontes emissoras de mercúrio para o ambiente (Ferreira, 1993). Esta fábrica teve um início de actividade em 1950 (inicialmente ligada ao sector têxtil) e até 1975 os efluentes líquidos seguiam em vala aberta (Vala de São Filipe) para a Ria de Aveiro. Só então foi construída uma conduta fechada.

O processo de fabrico, utilizando células com cátodo de mercúrio, baseia-se na decomposição electrolítica de uma solução de salmoura com obtenção de cloro, soda cáustica e hidrogénio, verificando-se uma perda de 150 a 250g de mercúrio por cada quilograma de cloro produzido (Ferreira, 1993; Abreu, 1996). Na última década este processo produtivo tem sido gradualmente substituído por tecnologias menos poluentes, como a tecnologia de membranas ou células de diafragma (Ferreira, 1993).

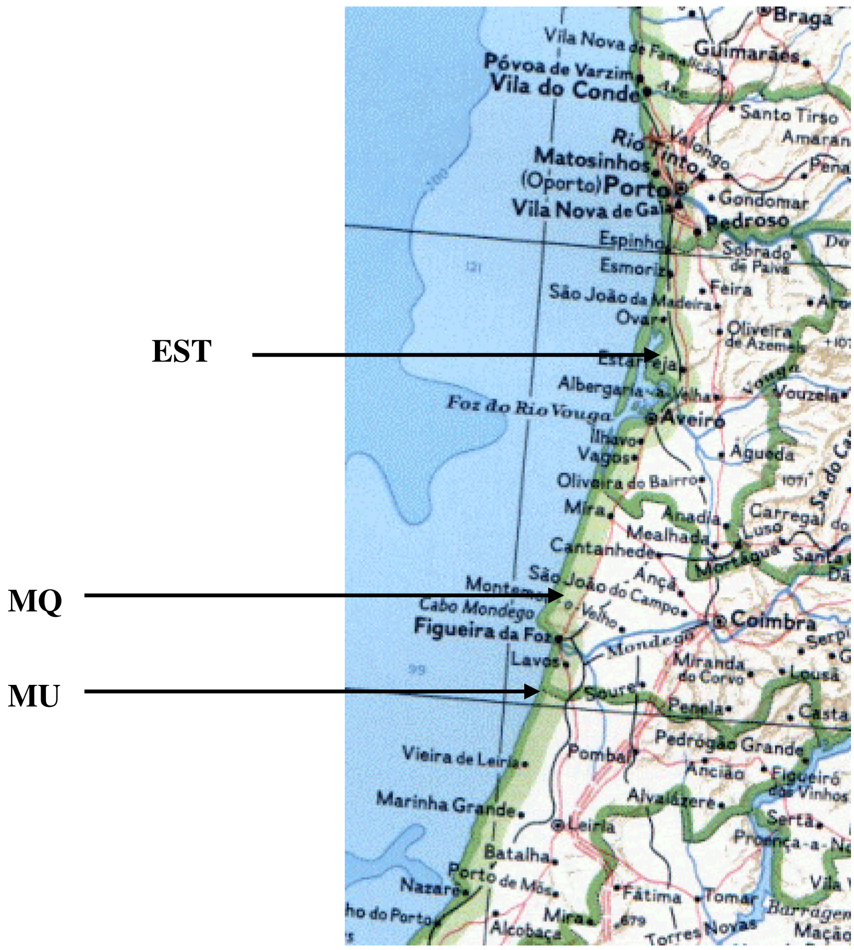


Fig.2- As três zonas de estudo: EST – Estarreja, MQ – Mata Nacional de Quiaios, MU – Mata Nacional o Urso.

A estratégia de amostragem definida foi:

Na Mata Nacional de Quiaios e na Mata Nacional do Urso foram instalados 3 pontos de amostragem de 4 hectares cada. Cada ponto de amostragem foi constituído por 36 caixas ninho para aves florestais segundo uma grelha de 6X6 com espaçamento de 40 metros.

Durante o período de reprodução estes locais foram monitorizados de forma a avaliar a taxa de colonização, ecologia reprodutora, taxa de crescimento de juvenis, estrutura populacional dos adultos e recolha de penas.

Em Estarreja (por impossibilidade de colocação de caixas ninho) a amostragem foi feita por capturas em redes japonesas de malha fina, conhecidas por "mist nets" (6 redes de 12 metros). O mesmo método foi utilizado na Mata Nacional de Quiaios para que os termos de comparação fossem os mais correctos.

Esta dissertação está definida em 4 partes:

Este primeiro capítulo onde se faz uma introdução do tema e onde se propõem os objectivos deste trabalho. Um segundo capítulo, onde se discutem de maneira acessível os resultados e algumas conclusões retiradas dos documentos apresentados nos anexos I, II e III. No terceiro capítulo encontram-se as referências bibliográficas utilizadas neste trabalho. No capítulo final, estão incluídos 3 anexos constituídos por documentos resultantes deste trabalho que serão de seguida submetidos para publicação, sendo que o primeiro anexo já se encontra publicado.

## CONSIDERAÇÕES FINAIS

**Biologia de reprodução do Chapim-real MU *vs* MQ** (Anexo I e II)

Apesar da proximidade geográfica e da semelhança de habitat das duas áreas existem claras diferenças na biologia de reprodução do Chapim-real.

A MU é claramente uma zona mais pobre ao nível de recursos alimentares, já que em 2004 existiam significativamente menos invertebrados no solo que na MQ. Com estes valores poderíamos pensar que em MQ os chapins teriam um maior sucesso e performance reprodutivos, já que muitos estudos mostram que os recursos alimentares são o factor que mais influencia a reprodução em aves e uma maior disponibilidade alimentar permite um maior número de ninhadas bem sucedidas (Martin, 1987; Rytkonen e Orell, 2001). No entanto neste estudo as melhores performances (dimensão da postura, dimensão da ninhada, nº de juvenis voadores, condição física dos juvenis) foram conseguidas na MU. De facto na MU em média cada casal colocou mais um ovo e teve mais uma cria que na MQ, apesar de as taxas reprodutoras (eclosão, sobrevivência e sucesso reprodutivo) terem sido semelhantes.

Uma das explicações para este facto poderá ser a densidade populacional pois, apesar da taxa de ocupação das caixas-ninho por Chapim-real ter sido semelhante nas duas áreas, a taxa de ocupação das caixas-ninho por todas as espécies foi significativamente superior na MQ. Poderíamos considerar que esta diferença se devesse à maior disponibilidade de buracos naturais na MU, já que conforme descrito por Fidalgo (1988) a ausência de buracos naturais em áreas de pinhal é um factor limitante na reprodução da família *Paridae*. No entanto, depois de analisadas, as áreas foram consideradas semelhantes e a atracção demonstrada pelos chapins para com as caixas-ninho instaladas (a colonização das caixas começou uma semana depois da instalação) realça que na MU também não existem buracos naturais disponíveis. De facto, esta densidade populacional mais elevada na MQ é um factor relevante já que normalmente a densidade está negativamente correlacionada com o tamanho da ninhada (Perrins e McCleery, 1989; Wiggins, 1998; Martins, 1999; Both, 2000). Estes resultados podem ser explicados se considerarmos que a competição inter-especifica é um factor mais relevante que a qualidade do território, o que leva a que performances reprodutivas sejam superiores num território pobre quando comparado com um território onde existe elevada competição.

Esta mesma consideração pode também explicar o início de postura mais prematuro na MU em relação à MQ. Na MU a competição é menor e por isso os chapins facilmente escolhem o seu ninho e rapidamente iniciam a reprodução. Por outro lado na MQ, onde a competição é elevada, os chapins além de terem de encontrar um ninho disponível têm também de despender tempo a defende-lo, o que faz com o início da reprodução seja mais tardio. Este atraso pode ter implicações importantes na performance reprodutiva (Forsman, 1998; Dubiec, 2001). Segundo Naef-Daenzer *et al* (2001) tanto um prematuro início de reprodução como uma boa condição física das crias aumentam a probabilidade de sobrevivência, e segundo Perrins e McCleery (1989) um início tardio da reprodução afecta a dimensão da ninhada. Num estudo realizado por estes autores a dimensão média da ninhada diminuiu cerca de 0.07 ovos por cada dia de atraso em relação à data média da postura. Isto vem de encontro ao nosso estudo, onde MQ tem uma data média de postura mais tardia e também uma dimensão média da ninhada menor que MU.

Além do Chapim-real, as outras espécies que ocuparam caixas-ninho neste estudo foram o Chapim-preto *Parus ater*, Chapim-de-poupa *Parus cristatus*, Trepadeira-comum *Certhia brachydactyla* e Chapim-azul *Parus caeruleus* (somente na MQ). Todavia a ocupação por estas espécies foi significativamente inferior na MU. Poderíamos pensar que estas espécies ocorrem em menor número na MU, no entanto ambas as áreas têm valores de diversidade semelhantes, o que nos faz questionar quais os motivos da menor diversidade reprodutiva na MU. Alguns dos motivos poderão ser os parcos recursos alimentares da MU e a incapacidade destas espécies competirem com uma espécie maior como o Chapim-real. No entanto, temos também de considerar a qualidade ambiental e sendo a MU uma zona industrial existe uma grande probabilidade que esta qualidade seja bem menor que a da MQ. Segundo Newton (1998) os poluentes atmosféricos podem alterar a estrutura física e/ou química dos habitats tornando-os menos adequados para certas espécies. De facto alguns estudos salientam que os invertebrados do solo são significativamente afectados pela poluição atmosférica (Flousek, 1989; Newton, 1998). Assim a baixa disponibilidade de invertebrados detectada na MU é indicadora da baixa disponibilidade de recursos alimentares para aves insectívoras, dando ênfase à relação entre poluição e baixa qualidade de habitat. Além disso, espécies como o Chapim-preto, Chapim-de-poupa e Trepadeira-comum que são mais pequenas que o Chapim-real e por conseguinte com uma maior taxa metabólica, poderão estar mais expostos e serem mais sensíveis à poluição (Root, 1990; Eens *et al*, 1999). Também a dieta mais restrita em insectos, ao contrário do Chapim-real que tem um maior consumo de sementes e frutos durante o Outono e Inverno (Cramp e Perrins, 1993), pode contribuir para uma maior acumulação de contaminantes. Assim, a menor densidade reprodutiva da MU pode estar relacionada com uma menor qualidade ambiental desta área.

Este estudo sugere que o Chapim-real não é afectado directamente pela poluição, mas outras espécies que também nidificam em caixas-ninho podem ser mais sensíveis. Todavia o Chapim-real é por vezes a única espécie disponível em áreas contaminadas com densidades adequadas para efectuar estudos desta natureza.

**Análise das penas das crias de Chapim-real MU *vs* MQ** (Anexo III)

Um dos objectivos deste estudo era comparar as concentrações de metais pesados (cobre, zinco, mercúrio, cádmio, níquel e chumbo) nas penas de crias de Chapim-real entre a área industrial MU e a área de referência MQ.

Todos os elementos foram detectados nas penas excepto chumbo na MU. Os níveis de mercúrio foram significativamente superiores na MU, mas os níveis de zinco e cobre não tiveram diferenças significativas entre áreas, todavia foram ligeiramente superiores na MQ. Em alguns estudos que avaliaram a concentração de metais em penas de crias de Chapim-real (Dauwe *et al*, 2000; Janssens *et al*, 2002) os níveis de cobre e zinco também não tiveram diferenças significativas entre a área de referência e a área poluída. Estes autores sugerem que as penas de crias de Chapim-real não podem ser utilizadas como biomonitores para zinco e cobre porque elas podem não reflectir adequadamente a carga corporal das crias. Mecanismos homeostáticos (ex. excreção pelos excrementos) podem ainda controlar adequadamente os níveis destes metais nos tecidos nesta fase precoce de maturidade (Dauwe *et al*, 2000).

Para os elementos cádmio, níquel e chumbo muitos dos valores foram abaixo do limite de detecção, o que reduziu drasticamente o tamanho da amostra, e por isso só foram comparadas as percentagens de detecção de cada elemento em cada área. Estas percentagens foram superiores na MQ.

Num estudo conduzido por Dauwe *et al* (2000) os valores de cádmio também foram superiores na área de referência. Uma explicação poderá ser o facto de o cádmio estar geralmente fortemente ligado a metalotioninas no rim e por isso normalmente não estar disponível para incorporação nas penas (Elliot e Scheuhammer, 1997).

A maior presença de níquel e chumbo na MQ pode ser explicada pela existência nos arredores da MQ de campos agrícolas, onde normalmente são usados fertilizantes, e uma estrada nacional de elevado tráfico.

Uma surpresa foi a não detecção de valores de chumbo na MU. Segundo a EPER – European Pollutant Emission Register, em 2002 as emissões de metais produzidas pela fábrica de pasta de papel existente na MU eram níquel e chumbo. No entanto, no nosso estudo foram os valores de mercúrio que foram significativamente superiores na MU em relação à MQ.

O mercúrio é um elemento preocupante porque é bioacumulável, é persistente no meio, e a partir de determinadas concentrações torna-se tóxico. O mercúrio acumula-se no ar, água e sedimentos e é posteriormente incorporado pelos produtores e daí ao longo da cadeia alimentar, sendo por isso potencialmente perigoso para a saúde humana.

A elevada presença de mercúrio na MU pode ser o resultado da bioacumulação de emissões passadas, quando ainda não havia preocupações ambientais e legislação para este tipo de emissões, ou pode ser o resultado de emissões actuais da fábrica onde possivelmente o processo produtivo ainda liberte mercúrio. De qualquer forma é um caso preocupante e que deve ser regularmente monitorizado considerando os níveis de toxicidade do mercúrio.

Em conclusão este estudo sugere que as penas de crias de Chapim-real são um método vantajoso para avaliar a presença de metais pesados com a excepção do cádmio, zinco e cobre.

**Chapim-real como espécie monitora, indicadora ou sentinela**

A avaliação da utilização de penas de crias de Chapim-real como bioindicador de metais pesados era também um dos objectivos deste trabalho.

Segundo Beeby (2001) existem três tipos de espécies que podem de diferentes formas avaliar sinais de poluição: espécies monitoras, que medem o impacto pela diminuição da sua performance; espécies indicadoras, que o impacto é avaliado pela sua ausência ou abundância; e espécies sentinelas, que são insensíveis aos poluentes (pelo menos dentro dos valores normalmente encontrados no meio ambiente – poluição crónica), ou seja a sua performance e abundância não são afectadas, mas mostram uma correspondência directa entre acumulação nos tecidos e níveis ambiente e o sinal de poluição pode ser quantificado por uma área / intervalo de tempo.

No nosso estudo quando analisámos as penas de crias de Chapim-real concluímos que elas eram um método vantajoso para avaliar a presença de metais pesados, todavia não podemos dizer que a espécie é uma boa monitora ou indicadora, já que os valores de sucesso e performance reprodutivo foram inversos aos valores de contaminação, ou seja na MU onde os níveis de contaminação foram significativamente superiores a performance reprodutiva foi mais elevada. No entanto, se analisarmos os parâmetros descritos por Beeby na tabela 6, podemos considerar o Chapim-real como uma espécie sentinela, já que ela satisfaz quase todos os parâmetros enunciados.

Tabela6- Características "ideais" das espécies sentinelas (adaptado de Beeby, 2001).

| |
|---|
| Rápido equilíbrio com a fonte de contaminação |
| Um relação linear com a fonte de contaminação no que se refere aos limites de poluição encontrados no meio ambiente |
| A relação entre tecidos e poluentes deve ser a mesma em todos os locais de estudo |
| Espécies abundantes das quais um número largo pode ser retirado sem que haja alteração na estrutura etária ou outro efeito significativo sobre a população. |
| Fácil detecção e determinação da idade |
| Conhecimentos extensos sobre a fisiologia da espécie, incluindo o efeito da idade, tamanho, sexo, época e actividade reprodutiva na assimilação do poluente |
| Tempo de vida longo – permitindo a integração do poluente durante longos períodos |
| Sedentária ou com um home-range limitado ou bem definido |
| Conhecer detalhadamente as fontes de entrada do poluente no organismo |

O Chapim-real é uma espécie residente predominantemente florestal, com grande expansão por toda a Europa, sendo um óptimo representante da avifauna florestal. Alimenta-se principalmente de insectos e por isso ocupa um lugar elevado na cadeia alimentar. Nidifica preferencialmente em cavidades, e devido à escassez destas na mata, facilmente ocupa as caixas-ninho e por isso populações reprodutoras podem facilmente ser monitorizadas (Cramp e Perrins, 1993; Eens *et al*, 1999; Dauwe *et al*, 2000). É uma espécie ubíqua e abundante, e por vezes a única espécie de passeriformes florestais disponível em densidades adequadas em áreas poluídas. As crias de Chapim-real são também facilmente monitorizadas porque elas são relativamente insensíveis a perturbações no ninho (Janssens *et al*, 2002). Outra vantagem no uso de crias é o facto de elas estarem restringidas ao ninho por um determinado período de tempo e serem alimentadas por recursos recolhidos nas proximidades do ninho, podendo assim os possíveis sinais de poluição serem quantificados numa área restrita e intervalo de tempo definido (Furness, 1993).

Podemos considerar que as outras espécies que também nidificaram nas caixas-ninho, mas em menor número na MU, sejam potencialmente espécies indicadoras, no entanto mais estudos são necessários para fazer correctamente esta avaliação.

**Análise das penas de Melro, Pisco e Toutinegra EST *vs* MQ** (Anexo III)

Um dos objectivos deste estudo era comparar as concentrações de metais pesados (cobre, zinco, mercúrio, cádmio, níquel e chumbo) nas penas de três espécies de passeriformes residentes, Melro-preto (*Turdus merula*), Pisco-de-peito-ruivo (*Erithacus rubecula*) e Toutinegra-de-barrete-preto (*Sylvia atricapilla*) entre a zona do complexo químico de Estarreja (EST) e a área de referência MQ.

EST é uma zona de poluição crónica por mercúrio. Em 1988 as emissões de mercúrio para os efluentes líquidos eram de 8.5mg/l/ano (Tavares, 1995) e, segundo a EPER, em 2002 eram de 7.61kg/ano. Como seria de esperar as três espécies estudadas apresentaram valores de contaminação por mercúrio mais elevados em EST comparando com MQ (apesar de no Melro-preto as diferenças não serem significativas).

Os valores de cádmio também foram significativamente superiores em EST para todas as espécies. Apesar do cádmio não estar geralmente disponível para incorporação nas penas (Elliot e Scheuhammer, 1997), neste estudo ele foi consistentemente detectado nas três espécies, sugerindo a existência de um nível muito elevado de cádmio no meio ambiente. A área é claramente dominada pela a agricultura e a presença de uma fábrica de adubos compostos em EST pode ajudar a explicar estes níveis de contaminação, já que o cádmio pode ter origem em actividades antrópicas como o uso de adubos fosfatados (EPER).

Os valores de zinco e chumbo também foram mais elevados em EST para todas as espécies comparativamente com a MQ (apesar de nem todas as espécies apresentarem diferenças significativas).

Quase todos os elementos demonstraram a mesma direccionalidade nas três espécies, ou seja, valores mais elevados em EST do que na MQ. Apenas o níquel e o cobre tiveram diferentes acumulações entre as espécies e áreas. Estas diferenças nos níveis de acumulação entre espécies e áreas podem ser o resultado das diferentes dietas, estratégias alimentares e taxas metabólicas das espécies estudadas. Considerando que o Melro-preto se alimenta especialmente de minhocas, seria de esperar que esta espécie acumulasse mais metais. Todavia, no nosso estudo foi o Pisco-de-peito-ruivo a espécie que acumulou mais metais, apresentado diferenças entre áreas. O Pisco-de-peito-ruivo é essencialmente insectívoro e alimenta-se no solo, é uma espécie relativamente pequena e por isso apresenta uma maior taxa metabólica (Root, 1990; Eens *et al*, 1999) comparando com o Melro-preto, o que pode ser responsável por uma maior acumulação de metais. A Toutinegra-de-barrete-preto também é uma espécie insectívora e pequena, no entanto, alimenta-se sobretudo em árvores, reduzindo assim a exposição aos metais comparando com as espécies que se alimentam no solo.

Os resultados deste estudo sugerem que o Pisco-de-peito-ruivo é uma espécie eficiente para ser utilizada em estudos de monitorização de metais pesados. Além disso, em zonas de habitat fragmentado como é o caso de EST, onde a utilização de caixas-ninho não é possível, a utilização de redes e penas de Pisco-de-peito-ruivo são um método credível na avaliação da poluição por metais pesados.

## REFERÊNCIAS BIBLIOGRÁFICAS

Abreu SN. 1996. Modelação da dispersão de mercúrio no Esteiro de Estarreja. Dissertação de Mestrado. Universidade de Aveiro.

Appelquist H, Asbrik S, Drabaek I. 1984. Mercury monitoring: mercury stability in bird feathers. Mar Pollut Bull 15(1): 22-25.

Bairlein F. 1994. Manual of field methods. European-African songbird migration network. Institut fur Vogelforschung, Wilhelmshaven.

Beeby A. 2001. What do sentinels stand for? Environmental Pollution 112: 285-298.

Both C. 2000. Breeding territory size affects fitness: an experimental study on competition at individual level. Journal of Animal Ecology 69: 1021-1030.

Burger J. 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev Environ Toxicol 5: 203-311.

Burger J, Gochfeld M. 1997. Age differences in metals in the blood of Herring (*Larus argentus*) and Franklin's (*Larus pipixcan*) gulls. Arch Environ Contam Toxicol 33: 436-440.

Burger J, Veitch CR, Gochfeld M. 1994. Locational differences in metal concentrations in feathers of Australasian Gannet (*Morus serrator*) in New Zealand. Environ Monit Assess 32: 47-57.

Celulose Billerud SARL. Sem data. Elementos sobre a fábrica de cellulose da Leirosa. Biblioteca Municipal da Figueira da Foz.

Cramp S, Perrins CM. 1993. The birds of Western Paleartic, Vol VII. Oxford Univ. Press, Oxford.

Dauwe T, Bervoets L, Blust R, Pinxten R, Eens M. 2000. Can excrement and feathers of nestling song birds be used as a biomonitor for heavy metal pollution? Arch Environ Contam Toxicol 39: 541-546.

Denneman WD, Douben PET. 1993. Trace metals in primary feathers of the barn owl (*Tyto alba guttattus*) in the Netherlands. Environmental Pollution 82: 301-310.

Dubiec A. 2001. Seasonal decline in health status of Great Tit (*Parus major*) nestlings. Can J Zool 79: 1829-1833.

Eens M, Pinxten R, Verheyen RF, Blust R, Bervoets L. 1999. Great and Blue Tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety 44: 81-85.

Eeva T. 1996. Direct and indirect effects of air pollution on two hole-nesting bird species. Annales Universitatis Turkuensis. Vol 83: 1-11.

Eeva T, Lehikoinen E. 1996. Growth and mortality of nestlings Great Tits (*Parus major*) and Pied Flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 108: 631-639.

Elliot JE, Scheuhammer AM. 1997. Heavy metal and metallothionein concentrations in seabirds from the Pacific coast of Canada. Mar Pollut Bull 34: 794-801.

EPER. European Pollutant Emission Register. http://www.eper.cec.eu.int (cited 2/Mar/05).

Esselink H, van der Geld FM, Jager LP, Posthuma-Trumpie GA, Zoun PEF, Baars AJ. 1995. Biomonitoring heavy metals using the barn owl (*Tyto alba guttattus*): sources of variation especially relating to body condition. Arch Environ Contam Toxicol 28: 471-486.

Ferreira MMSI. 1993. Mercúrio em solos da área do Complexo Químico de Estarreja. Dissertação de Mestrado. Universidade de Aveiro.

Fidalgo LGZ. 1988. Contribuição para o estudo da biologia de reprodução dos parídeos nidificantes na Reserva Natural das Dunas de S. Jacinto. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Flousek J. 1989. Impact of industrial emissions on bird populations breeding in mountain spruce forests in central Europe. Ann Zool Fenn 26: 255-263.

Forsman J. 1998. Heterospecific attration and food resources in migrants' breeding patch selection in northern boreal forest. Oecologia 115: 278-286.

Fowler SW. 1990. Critical review of selected heavy metal and chlorinated hydrocarbon concentrations in the marine environment. Mar Environ Res 29: 1-64.

Furness RW. 1993. Birds as monitors of pollutants. In: Furness RW, Greenwood JJD (eds) Birds as monitors of environmental change. Chapman and Hall, London, p 86.

Hahn E, Hahn K, Stoepler M. 1993. Bird feathers as bioindicators in areas of the German Environmental Specimen Bank – bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. The Science of the Total Environment 139/140: 259-270.

Janssens E, Dauwe T, Bervoets L, Eens M. 2002. Inter and intraclutch variability in heavy metals in feathers of Great tit nestlings (*Parus major*) along a pollution gradient. Arch Environ Contam Toxicol 43: 323-329.

Martin TE. 1987. Food as a limit on breeding birds: a life-history perspective. Annu Rev Ecol Syst 18: 453-487.

Martins T. 1999. Contribuição para o conhecimento da biologia da reprodução do Chapim-real e do Chapim-azul num montado de sobro do Baixo Alentejo. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Naef-Daenzer B, Widmer F, Nuber M. 2001. Differencial post fledging survival of Great and Coal Tits in relation to their condition and fledging date. Journal of Animal Ecology 70: 730-738.

Newton I. 1998. Population Limitation in Birds. Academic Press.

Perrins CM, McCleery RH. 1989. Laying dates and clutch size in the Great Tit. Wilson Bulletin 101(2): 236-253.

Pitelka LF. 1994. Air polution and terrestrial ecosystems. Ecol. Appl. 4: 627-628.

Root M. 1990. Biological monitors of pollution. Bioscience 40: 83-86.

Rytkonen S, Orell M. 2001. Great tits, *Parus major*, lay too many eggs: experimental evidence in mid-boreal habitats. Oikos 93: 439-450.

Tavares CMOF. 1995. Contaminação por Hg do solo e plantas dos campos marginais do Esteiro de Estarreja. Dissertação de Mestrado. Universidade de Aveiro.

Tenreiro PJQ, Carvalho CMM, Alves HMD, Martins JC, Fernandes CN. 2001. Contribuição para o estudo da biologia de reprodução dos Parídeos na Região Centro de Portugal. III Congresso de Ornitologia da Sociedade Portuguesa para o Estudo das Aves. SPEA, Castelo Branco.

Wiggins D. 1998. Island biogeography and the reproductive ecology of great tits *Parus major*. Oecologia 115: 478-482.

# BREEDING BIOLOGY OF THE GREAT TIT *Parus major* IN TWO MARITIME PINE *Pinus pinaster* FORESTS IN THE REGION OF FIGUEIRA DA FOZ (Beira Litoral, Portugal)

## ABSTRACT

During the spring of 2003 some aspects of the breeding biology of the Great Tit *Parus major* were studied in two maritime pine (*Pinus pinaster)* forests of Figueira da Foz, using nest boxes. One study site was located in Mata Nacional do Urso (MU), close to an industrial area and the other was located in Mata Nacional de Quiaios (MQ), a forested area with no direct influence of industrial pollution. The total occupation rate of nest boxes for all species was significantly higher in MQ, but the nest-boxes occupation rates by Great tit were similar in both areas. The number of fledglings per pair, hatching success, survival rate and breeding success were similar between the two study areas. However, clutch size and brood size were marginally significantly larger in MU. Therefore, the results provide evidence that breeding close to an industrial complex does not influence directly the Great tit, but it may affect others species that also breed in nest boxes. Interspecific competition mechanisms seem to be more important in the breeding biology of the Great Tit, which might dissimulate the effects induced by pollution.

## INTRODUCTION

Air pollution has become a widespread problem in this last century, affecting significantly terrestrial ecosystems, changing the physical or/and chemical structure of habitats and making them less suitable for wildlife species (Pitelka, 1994; Eeva, 1996; Newton, 1998). Environmental pollution studies made during the last few decades have largely concerned the toxic levels of pollutants in the environment and organisms. Other studies have dealt with the effects of pollutants in the abundance of sensitive species (Eeva, 1996). Small insectivorous passerines are considered to be good candidates for such studies in terrestrial ecosystems. In addition to being ubiquitous, easily studied, and high in the food chain, they are considered good biomonitors due to their high metabolic rate (Root, 1990; Eeva, 1996).

Among passerines, some species of the *Paridae* family are ideal for ecological studies because they easily occupy nest boxes, reach sexual maturity in a relative short time period and are good and important representatives of forest bird community (Fidalgo, 1988). Therefore, the Great Tit *Parus major* was the subject species selected for the present study. Numerous studies on the breeding biology of the Great tit have been carried throughout Europe, (e.g. VanBalen, 1973; Lindén, 1988; Cramp and Perrins, 1993). However, in Portugal such studies are scarce and basically restricted to those published by Fidalgo (1988), Martins (1999) and Tenreiro *et al* (2001). Studies related with the use of *Paridae* as pollutions-sensitive species are even scarcer among the actual Portuguese research community.

So, the aim of this study was to increase the knowledge of different aspects of the breeding biology of the Great tit in Portugal, in view of the potential impact of the proximity of an industrial complex, upon the species breeding performance.

**STUDY AREA AND METHODS**

The study was carried out in two maritime pine *Pinus pinaster* forests located in Figueira da Foz County, in the central coast of Portugal, during the Great Tit breeding season of 2003. One area was located in Mata Nacional do Urso (MU), close to an industrial complex and the other was located in Mata Nacional de Quiaios (MQ), a forested area without the direct influence of industrial pollution. The North part of MU is influenced by the airborne emissions originated mainly in paper mill factories and study plots are located at a maximum distance of 1 Km from the industrial area. MQ is a classified area included in Dunas de Mira (Natura 2000 PTCON055). There is a 20 km distance between study areas and sampling plots were chosen according to their resemblance. Both study areas were established in man-made plantations with 70-80 years old, dominated by *Pinus pinaster* and *Pinus pinea*, with a tree density varying from 666 to 1066 individuals per ha. Shrub layer is dominated by *Myrica faya*, *Halimium halimifolium*, *Cytisus scoparius*, *Ulex* spp, *Cistus* spp and *Acacia* spp. In each study area, nest boxes (n=108) were equally distributed along three quadrangular 4 ha sampling plots. The distance between boxes was 40m.

Nest boxes were checked from the 25th of February till the 27th of July. In the beginning of the breeding season each site was visited once per week. After the first signs of nest occupation, the visits started to be planned in function of the working schedule that was needed for each nest-box. In each visit, hatching date, clutch size, brood size and number of fledglings were registered for each box. Finally, it was possible to calculate the hatching success (% of eggs that hatched successfully excluding clutches predated), survival rate (number of fledglings/numbers of hatched eggs) and breeding success (number of fledglings/numbers of eggs laid). Nests where egg laying was initiated, whether successfully or not, were considered as occupied nests. In order to estimate the average clutch date, dates were converted to number of days, assuming day 1 to be the 16th of March and day 85 to be the 23rd of May.

Because the nest boxes weren't checked every day, some dates were estimated by admitting that Great tits lay one egg per day and have an average incubation period of 12 days (Fidalgo, 1988).

Wing length (Svensson, 1992), fat score (0-8 scale according to Kaiser, 1993) and muscle score (0-3 according to Barlein, 1994) of all nestlings were measured at the age of 15 (± 1) days after eclosion. These birds were weighted with a spring scale, and marked with aluminium rings, provided by Central Nacional de Anilhagem.

Data collected was statistically analysed to detect significant differences between study sites and all results are presented as an average ± SD. A chi-square analysis with Yates correction was used to compare occupation rates (Zar, 1999). Clutch size, brood size, number of fledglings per pair, survival rate and breeding success were compared using a T-test for unequal variances, after checking for normal distribution (Zar, 1999). Hatching success, fat score and muscle score were compared using a Mann Whitney U-test, because data was not normally distributed (Zar, 1999).

## RESULTS

Mata Nacional de Quiaios (MQ)

In this area 40 nest boxes were occupied (considering all the species that breed in the boxes), corresponding to an occupation rate of 37.04%. Great Tit occupied 20 of the total of colonized boxes, an occupation rate of 18.52% (Fig.1). The other species occupying the boxes were Coal Tit *Parus ater*, Crested Tit *Parus cristatus*, Blue Tit *Parus caeruleus* and Short-toed Treecreeper *Certhia brachydactyla*.


Fig.1 - Occupation rate of the nest boxes in both study areas, MQ and MU. The total occupation rate refers to all species that occupied the nest boxes (MQ: n=40 nests; MU: n=16 nests); the occupation rate by Great Tit refers only to nest boxes occupied by the specie Great Tit (MQ: n=20 nests; MU: n=15 nests).

In relation to Great Tit, the laying of the first and last eggs occurred on the 29th of March and 8th of June, respectively, and the first and last hatching occurred on the 18th of April and 26th of June, respectively. Average clutch date was 36.25 ± 22.1 days. Clutch size varied between 3 and 9 eggs (6.05±1.47), brood size between 2 and 7 nestlings (5.2±1.14), and, the number of fledglings per pair between 1 and 7 (4.2±1.77) (Fig.2). In this area the mean hatching success recorded was 88.25% (±0.19), survival rate was 80.62% (±0.25), and breeding success corresponded to 71.35% (±0.28) (Fig.3). The average weight of the nestlings was 15,84g (±2.3, n=87), the average fat score was 1.49 (±0.91, n=87) and the average muscle score was 1.88 (±0.67, n=87) (Fig.4).

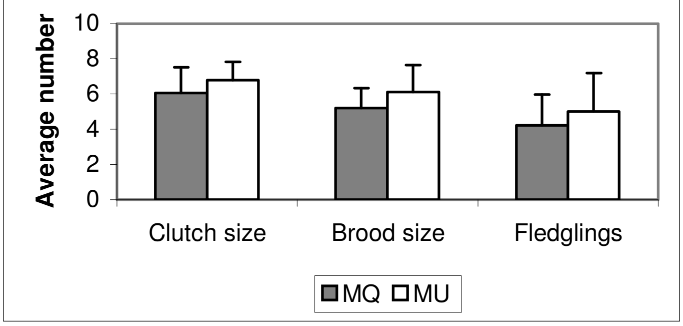


Fig.2 - Clutch size (mean ±SD), Brood size (mean ±SD) and number of fledglings per pair (mean ±SD) of Great Tit in the two study areas (MQ: n=20 nests; MU: n=15 nests).

Mata do Urso (MU)

In this area there were 16 occupied nest boxes (considering all the species that breed in the boxes), corresponding to an occupation rate of 14.81%. Great Tit occupied 15 of the total colonized boxes leading to an occupation rate of 13.89% (Fig.1). Crested Tit was the other registered species, with only one breeding pair.

The laying of the first and last eggs occurred on the 16th of March and 2nd of June, respectively, and the first and last hatching occurred on the 8th of April and 19th of June, respectively. Average clutch date corresponds to 35.71 days (± 28.01). Clutch size varied between 5 and 8 eggs (6.8±1.01), brood size varied between 2 and 8 nestlings/nest (6.13±1.51) and the number of fledglings per pair varied between 0 and 7 (5±2.17) (Fig.2). Hatching success was 91.31% (±0.20), while survival rate and breeding success were 83.84% (±0.29) and 75.63% (±0.32), respectively (Fig.3). Average weight of nestlings was 16.8g (±1.37, n=64), average fat score was 1.72 (±1.02, n=47) and average muscle score was 1.98 (±0.46, n=44) (Fig.4).


Fig.3 - Hatching success (mean ±SD), survival rate (mean ±SD) and breeding success (mean ±SD) of Great Tit in the two study areas (MQ: n=20 nests; MU: n=15 nests).

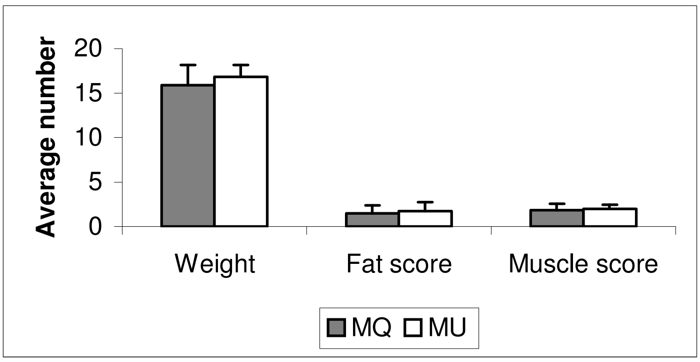


Fig.4 - Weight (mean ±SD; n=151 young), fat score (mean ±SD; n=134 young) and muscle score (mean ±SD; n=131 young) of Great Tit nestlings, at the age of 15 (± 1) days, in the two study areas.

Comparison between the study areas

In both study areas the breeding season of the Great Tit lasted from the 10th of March (when the first nest started to be built) to the 11th of July (when the last young abandoned the nest). Despite the geographical proximity and the habitat similarity of both study areas, the beginning of the egg laying period started 13 days earlier in MU. However, no significant differences were detected when comparing the average clutch date (Table 1). Comparing occupation rates between areas, it was possible to find that the total occupation rate was significantly higher in MQ, although the Great Tit occupation rate was similar (Table 1).

Breeding parameters were, on average, larger in MU, although it was not possible to find any significant differences. Only when referring to clutch size and brood size differences could be classified as marginally significant (near P=0.05) (Table 1).

Table1 – Comparison between the two study areas of total occupation rate, Great Tit occupation rate, clutch size, brood size, number of fledglings per pair, hatching success, survival rate, breeding success, nestlings weight, nestlings fat score and nestlings muscle score.

| | **MQ** | **SD** | **MU** | **SD** | **$\chi 2$** | **T** | **U** | **P** |
|---|---|---|---|---|---|---|---|---|
| **Total occupation rate** | 37.04% | - | 14.81% | - | 7.391 | - | - | **0.007** |
| **Great tit occupation rate** | 18.52% | - | 13.89% | - | 0.362 | - | - | **0.055** |
| **Clutch date** | 36.25 | 22.11 | 35.71 | 28.01 | - | 0.06 | - | 0.953 |
| **Clutch size** | 6.05 | 1.47 | 6.8 | 1.01 | - | 2.024 | - | **0.084** |
| **Brood size** | 5.2 | 1.14 | 6.13 | 1.51 | - | 1.89 | - | **0.069** |
| **Fledglings per pair** | 4.2 | 1.77 | 5 | 2.17 | - | 1.167 | - | 0.254 |
| **Survival rate** | 80.62% | 0.25 | 83.84% | 0.29 | - | 0.341 | - | 0.736 |
| **Breeding success** | 71.35% | 0.28 | 75.63% | 0.32 | - | 0.411 | - | 0.645 |
| **Nestlings weight** | 15.84g | 2.30 | 16.80g | 1.37 | - | 3.086 | - | **0.002** |
| **Hatching success** | 88.25% | 0.19 | 91.31% | 0.20 | - | - | 128.5 | 0.475 |
| **Nestlings fat score** | 1.49 | 0.91 | 1.72 | 1.03 | - | - | 2002 | 0.529 |
| **Nestlings muscle score** | 1.88 | 0.67 | 1.98 | 0.46 | - | - | 1751 | 0.365 |

## DISCUSSION

In spite of the geographical proximity and habitat similarity of both areas, the use of nest boxes by passerines and reproduction parameters of Great tit do not follow the same patterns. The number of species occupying nest boxes differs between MU and MQ. This difference may be related with the density of the other breeding species (except for Great Tit). A lower breeding species density in MU would explain the significantly lower occupation rate detected in this study. However, further census data (in terms of abundance) are not available in order to test this hypothesis but, even though all species occur in both areas (except Blue Tit, which only occurs in MQ), they did not use the nest boxes in MU. According to Fidalgo (1988), the absence of natural holes in coastal pine areas is one of the limitation factors in *Paridae* reproduction. Therefore, a potential higher availability of natural holes in MU would result in a lower use of the artificial nests. However, habitats in both areas were found to be similar and the desirability of Great Tits towards recently installed boxes (box colonization started within one week after installation) emphasises that natural holes are also not available in MU. Based on these results, it is possible to say that the diversity of reproductive passerines in artificial boxes is lower in the area influenced by the industrial complex even though a causal connection cannot be detected. Thorough studies on this matter are necessary, especially considering that, as stated by Noordwijk (1990), Eeva and Lehikoinen (1996) and Eeva (1996), few studies have dealt with the consequences of pollution in the diversity of forest birds and took a global approach of the problem. With this preliminary data and with the studied parameters, results obtained indicates that Great Tit is not directly affected because of breeding close to an industrial complex, which may be related with the Great tit being the largest of the nest boxes breeding birds and thus the species with the lowest metabolic rate as previously reported by Eens *et al* (1999) and Root (1990). Eens *et al* (1999) referred to Great and Blue Tit as indicators of heavy metal contamination and Root (1990) analysed a set of possible biological monitors of pollution. These authors proposed that smaller species (like Blue Tit), due to their higher metabolic rate, tend to become more exposed and sensitive to pollution. Another possible explanation is a more diverse feeding ecology, with a higher consumption rate of seeds and fruits during autumn and winter (Cramps and Perrins, 1993) thus contributing to a lower accumulation of contaminants.

According to Newton (1998), atmospheric pollutants can alter the physical or chemical structure of habitats, making them less suitable for certain species. Therefore, the lower bird diversity in MU may be related with a less suitable environment in this area.

The early laying date detected in MU could be related with a weaker interspecific competition in this area as a result of lower densities of other nest box breeding species. Because of that, territory and nest selection was easier in MU.

The same interspecific competition phenomena may explain the lower (although not significantly different) breeding parameters found in MQ. Martins (1999) found similar results with higher density values leading to lower breeding parameters. Another result supporting the importance of interspecific competition in the breeding ecology of the Great Tit is the fact that in MU there is almost one more egg per nest and one more nestling per box.

Despite the initial higher reproduction efforts in MU, breeding success becomes similar in both areas, suggesting that in MU Great Tit should lay one extra egg to achieve the same breeding success in MQ. It is possible to conclude that the lower competition in MU allows Great Tit to invest more in reproduction (higher egg number). However, because of the potentially pollution-induced habitat degradation, the initial higher investment in reproduction is not translated into a higher number of fledglings in the end of the reproductive season.

Data collected indicated a higher nestling physical condition in MU. The significantly different average weight supports interspecific competition as an important mechanism influencing the breeding ecology of the Great Tit (Minot 1981, Newton 1998). These results suggest that there was a direct competition for food and space in MQ for the Great Tit as opposed to the occurred in MU where Great Tit occupied almost all nest boxes (only one box with Crested Tit) while in MQ the boxes were occupied by four species other than Great Tit.

The results obtained put in evidence that Great Tit does not seem to be directly influenced by pollution and other nest box breeding species might be more sensitive. However, Great Tit is sometimes the only forest passerine species available in contaminated areas suiting density values required to allow the use of a bird as a biosensor. Finally, this study emphasises the need for further research in order to identify variables that would help to clarify ecological effects of pollutants upon wildlife.

**ACKNOWLEDGEMENTS**

Authors are grateful for the support of Eng. António Grácio, Co-ordinator of Núcleo Regional do Corpo Nacional da Guarda Florestal da Beira Litoral. We thank João Ferreira and Laura Sequeira who dedicated their time to help us in the visits to the nest-boxes. We also thank Ricardo Tomé and Catarina Eira for the value comments made during the revision.

**REFERENCES**

Bairlein F. 1994. Manual of field methods. European-African songbird migration network. Institut fur Vogelforschung, Wilhelmshaven.

Cramp S, Perrins CM. 1993. The birds of Western Paleartic, Vol VII. Oxford Univ. Press, Oxford.

Eens M, Pinxten R, Verheyen RF, Blust R, Bervoets L. 1999. Great and Blue Tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety 44: 81-85.

Eeva T. 1996. Direct and indirect effects of air pollution on two hole-nesting bird species. Annales Universitatis Turkuensis. Vol 83: 1-11.

Eeva T, Lehikoinen E, Nurmi J.1994. Effects of ectoparasites on breeding sucess of Great Tits (*Parus major*) and Pied Flycatchers (*Ficedula hypoleuca*) in an air pollution gradient. Can. J. Zool. Vol. 72: 624-635.

Eeva T, Lehikoinen E. 1996. Growth and mortality of nestlings Great Tits (*Parus major*) and Pied Flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 108: 631-639.

Fidalgo LGZ. 1988. Contribuição para o estudo da biologia de reprodução dos parídeos nidificantes na Reserva Natural das Dunas de S. Jacinto. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Kaiser A. 1993. A new multi-category classification of subcutaneous fat deposits of songbirds. Journal of Field Ornithology 62: 246-255.

Lindén M. 1988. Reproductive trade-off between first and second clutches in the Great Tits *Parus major*: an experimental study. Oikos 51: 285-290.

Martins T. 1999. Contribuição para o conhecimento da biologia da reprodução do Chapim-real e do Chapim-azul num montado de sobro do Baixo Alentejo. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Minot EO. 1981. Effects of interspecific competition for food in breeding Blue and Great Tits. J. Anim. Ecol. 50: 375-385.

Newton I. 1998. Population Limitation in Birds. Academic Press.

Noordwijk AJ. 1990. The effects of forest damage on caterpillars and their effect on the breeding biology of the great tit, an overview. *In Population Biology of Passerine Birds* (Ed. Blondel J, Gobler AG, Lebreton JD, McCleery RH). Springer-Verlag, Berlin: pp 215-222.

Pitelka LF. 1994. Air polution and terrestrial ecosystems. Ecol. Appl. 4: 627-628.

Root M. 1990. Biological monitors of pollution. Bioscience 40: 83-86.

Svensson L. 1992. Identification Guide to European Passerines. 4th ed. Fingraf AB, Sweden.

Tenreiro PJQ, Carvalho CMM, Alves HMD, Martins JC, Fernandes CN. 2001. Contribuição para o estudo da biologia de reprodução dos Parídeos na Região Centro de Portugal. III Congresso de Ornitologia da Sociedade Portuguesa para o Estudo das Aves. SPEA, Castelo Branco.

VanBalen JH. 1973. A comparative study of the breeding ecology of the Great Tit *Parus major* in different habitats. Ardea 61: 1-93.

Zar JH. 1999. Biostatistical analysis. Prentice-Hall.

# THE BREEDING BIOLOGY OF THE GREAT TIT IN FIGUEIRA DA FOZ, PORTUGAL


## ABSTRACT

During the spring of 2003 and 2004 some aspects of the breeding biology of the Great Tit *Parus major* were studied in two maritime pine forests (*Pinus pinaster)* of Figueira da Foz (Centre of Portugal). One study site was located in Mata Nacional do Urso (MU), in the vicinity of an industrial area and the other was located in Mata Nacional de Quiaios (MQ), a rural area with no direct influence of industrial pollution. Although the total occupation rate of nest boxes was significantly higher in MQ, the nest-box occupation rates obtained for the Great Tit did not present significant differences among areas. Moreover, other parameters such as hatching success, survival rate and breeding success did not present significant differences between study areas. However, clutch size, brood size and the number of fledglings per pair were significantly larger in MU. Additionally, in MU the laying period occurred significantly earlier.

Interspecific competition mechanisms seem to be more important than territory quality in the breeding biology of the Great Tit. Therefore, the results provide evidence that breeding close to an industrial complex does not directly influence the Great Tit, but it may affect others species that also breed in nest boxes.


## INTRODUCTION

Breeding birds in the same population often vary in their breeding parameters, as reported by several studies carried throughout Europe (Seki, 1998). However, the reasons for these differences are yet difficult to explain. There are several factors such as the territory quality or intraspecific competition that can influence breeding performance (Forsman, 1998; Seki, 1998).

The Great Tit (*Parus major*) breeds in all kinds of woodland, eats mainly insects and seeds, and nests in cavities (Cramp and Perrins, 1993). Because they cannot excavate their own cavities, which are considered a limiting resource, they readily occupy man-made nest boxes to reproduce, thus facilitating research on this species (Eens *et al*, 1999). They are also good and important representatives of a forest bird community (Fidalgo, 1988).

The aim of this study was to increase the knowledge on the breeding biology of the Great Tit in Portugal by detecting differences in the breeding performance between study areas. This work is integrated in a larger project that studies the effects of air and soil pollution on several terrestrial wildlife species in order to assess their potential as sentinel species.

## STUDY AREA AND METHODS

The study was carried out in two maritime pine forests (*Pinus pinaster*) located in Figueira da Foz, in the central coast of Portugal, during the breeding seasons of 2003 and 2004. One area was located in Mata Nacional do Urso (MU), in the vicinity of an industrial compound and the other was located in Mata Nacional de Quiaios (MQ), a rural area included in the Natura 2000 site “Dunas de Mira” (PTCON055). There is a 20 km distance between study areas. Both study areas were established in 70 to 80 year-old, man-made plantations, of *Pinus pinaster* and *Pinus pinea* presenting a shrub layer dominated by *Myrica faya*, *Halimium halimifolium*, *Cytisus scoparius*, *Ulex* spp, *Cistus* spp and *Acacia* spp. In each study area, nest boxes (n=108) were equally distributed along three quadrangular 4 ha sampling plots. The distance between boxes was 40m.

Nest boxes were checked regularly in order to gather information about hatching date, clutch size, brood size and number of fledglings, hatching success (% of eggs that hatched successfully excluding clutches predated), survival rate (number of fledglings/numbers of hatched eggs) and breeding success (number of fledglings/numbers of eggs laid). Some of the hatching dates were estimated bearing in mind that Great Tits lay one egg per day and have an average incubation period of 12 days (Fidalgo, 1988). Nests where egg laying was initiated, whether successfully or not, were considered as occupied nests.

Wing length (Svensson, 1992), fat score (Kaiser, 1993) and muscle score (Barlein, 1994) of all nestlings were measured at the age of 15 (± 1) days after eclosion. These birds were weighed with a spring scale, and marked with aluminium rings, provided by Central Nacional de Anilhagem.

Bird diversity was calculated using the Shannon Diversity Index, from data gathered using a point survey method, where species and individuals were accounted during five minutes (Bibby *et al*, 1992). A total of 24 points were estimated in each sampling plot.

Arthropod availability was assessed in both areas by scanning the soil and counting invertebrates larger than 1 mm, during five minute periods. Samples were obtained by randomly sampling 6 transects in each plot producing a total of 18 points in each study area (Cooper and Whitmore, 1990; Carrascal *et al*, 1998).

A chi-square analysis with Yates correction was used to compare occupation rates (Zar, 1999). Clutch size, brood size, number of fledglings per pair, survival rate, breeding success, nestling weight, diversity index and average arthropod were compared using a t-test for unequal variances, after checking for normal distribution (Zar, 1999). Hatching success, fat score and muscle score were compared using a Mann Whitney U-test (Zar, 1999).

## RESULTS

MQ presented a significantly ($\chi^2$=9.01, P=0.0027) higher rate of nest box occupation (all species) when compared with MU (figure 1). In fact, 94 nests were occupied in MQ while only 63 nests were occupied in MU. However, the occupation rate obtained for Great Tit did not differ between areas ($\chi^2$=0.013, P=0.91). Apart from Great Tit, other breeding species were detected in both study areas, i.e., Coal Tit *Parus ater*, Crested Tit *Parus cristatus* and Short-toed Treecreeper *Certhia brachydactyla*, and in MQ it was also possible to detect Blue Tit *Parus caeruleus*.

In both years egg laying, first and late broods started earlier in MU, with significant differences in all cases (see table 1) except for the first brood of 2003, which did not differ among areas.

The average clutch size, average brood size and average number of fledglings per pair of Great Tit differed (t-test, all P<0.01) among areas (table 2).

However, in spite of the higher breeding parameters exhibited in MU, the hatching success, the survival rate and the breeding success were not significantly different (t-test, all P>0.05) among the studied areas (see table 2).


Fig.1- Occupation rate in both study areas, MQ and MU. The total occupation rate refers to all species occupying nest boxes (n=157); the occupation rate by Great Tit refers only to nest boxes (n=102) occupied by the specie *Parus major.*

Table1. Comparison between average clutch date of MQ and MU in both study years (2003: day1=16$^{th}$ of March; 2004: day1=13$^{th}$ of March) using a t-test.

| | 2003 | | | | 2004 | | | |
|---|---|---|---|---|---|---|---|---|
| | **MQ** | **MU** | **t** | **P** | **MQ** | **MU** | **t** | **P** |
| **First clutch** | 26.94 | 21 | 0.8962 | 0.3893 | 33.31 | 22.05 | 3.969 | **0.0004** |
| **Late clutch** | 82.33 | 69 | 3.430 | **0.0415** | 79 | 69.46 | 3.705 | **0.0035** |

The physical condition parameters of nestlings were measured at 15 (±1) days of age when a significant difference was detected in fat score values registered for both areas (see table 2).

In 2004, we also measured arthropod availability. The amount of invertebrates in the soil was significantly higher in MQ (table 3).

The bird diversity, calculated by the Shannon Diversity Index (MQ: H=0.52 and MU: H=0.45), did not present significant differences between areas (t=0.9267, P=0.374).

Tab.2 – Comparison between the two study areas of total clutch size, brood size, number of fledglings per pair, hatching success, survival rate, breeding success, nestlings weight, nestlings fat score and nestlings muscle score. Weight values are expressed in grams, t refers to the statistic of t-test, U refers to the statistic of Mann Whitney test.

| | **MQ** | **SD** | **MU** | **SD** | **t** | **U** | **P** |
|---|---|---|---|---|---|---|---|
| **Clutch size** | 5.70 | 1.46 | 6.85 | 1.05 | 4.299 | - | **0.0001** |
| **Brood size** | 5.00 | 1.20 | 6.25 | 1.30 | 4.785 | - | **0.0001** |
| **Fledglings per pair** | 3.72 | 1.62 | 4.83 | 2.03 | 2.877 | - | **0.0051** |
| **Survival rate** | 74.80% | 0.27 | 77.32% | 0.30 | 0.4254 | - | 0.6716 |
| **Breeding success** | 66.85% | 0.27 | 70.95% | 0.30 | 0.6939 | - | 0.4896 |
| **Hatching success** | 90.21% | 0.15 | 91.79% | 0.15 | - | 952 | 0.5195 |
| **Nestlings weight** | 15.47 | 2.42 | 15.92 | 2.38 | 1.819 | - | 0.0698 |
| **Nestlings fat score** | 1.36 | 0.91 | 1.61 | 1.01 | - | 14440 | **0.0429** |
| **Nestlings muscle score** | 1.92 | 0.63 | 1.84 | 0.56 | - | 15020 | 0.2327 |

Tab.3 - Comparison between the two study areas of the average invertebrates in the soil obtained during five minutes search transects.

| | **MQ** | **MU** | **t** | **P** |
|---|---|---|---|---|
| **Arachnids** | 10.5±0.56 | 4.11±0.51 | 8.466 | 0.0001 |
| **Coleoptera** | 3.56±0.41 | 0.94±0.26 | 5.332 | 0.0001 |
| **Others** | 5.06±0.49 | 3.17±0.41 | 2.950 | 0.0058 |

## DISCUSSION

In spite of the similarity and proximity of the study areas, three major differences were detected: (1) the higher total occupation rate in MQ, (2) the earlier laying date in MU, and (3) the significantly lower food availability in MU.

Many studies have shown that food resources are one of the most important factors influencing reproduction in birds and a higher availability of resources allows for more successful breeders (Martin, 1987; Rytkonen and Orell, 1993). However, in the present study the best performances (one more egg and one more fledgling per pair than in MQ) were achieved in the area presenting the lowest availability of food resources and a lower environmental quality, considering its industrial surroundings.

It is known that population density is usually negatively correlated with clutch size (Perrins and McCleery, 1989; Wiggins, 1998; Martins, 1999; Both, 2000). Therefore, the present results may be explained if we consider intraspecific competition to be a much stronger factor than territory quality, leading to a more successful breeding performance in a poorer territory than that found in a good territory with competition.

The same notion might explain the earlier hatching date detected in MU. In this area, where competition is low, tits easily choose their breeding sites and soon start to breed. On the other hand, in MQ competition forces tits to spend more time searching and maintaining a breeding site thus producing a delay in the breeding period, with important implications to the breeding performance (Forsman, 1998; Dubiec, 2001). Furthermore, according to Naef-Daenzer *et al* (2001) both early breeding and high fledging mass increase survival and according to Perrins and McCleery (1989) late breeding affects clutch size. These authors showed that clutch size declines by almost 0.07 eggs for each 1-day delay in the mean laying date. This is in accordance with the present study where, in spite of the similar surviving rates, MQ had a later hatching date and a lower clutch size in comparison to MU.

The remaining question refers to the lower reproductive density found in MU when comparing to MQ, considering that both areas present similar species richness. Reasons why some species fail to reproduce may be related with the lack of food resources and their inability to compete with larger species such as Great Tit. However, the effect of a poor environmental quality of the area should be taken into account. According to Newton (1998), atmospheric pollutants can alter the physical or chemical structure of habitats, making them less suitable for certain species. Previous studies evidenced that soil invertebrates are significantly affected by atmospheric pollution (Flousek, 1989; Newton, 1998). Therefore, the lower arthropod abundance detected in the polluted area indicates a lower availability of food resources for insectivorous birds, thus emphasising the relationship between pollution and low habitat quality.

Besides Great Tit the other breeding species present in both areas were Coal Tit *Parus ater*, Crested Tit *Parus cristatus* and Short-toed Treecreeper *Certhia brachydactyla*, but in MU the nest occupation rate was much lower. Because of their smaller size and higher metabolic rates they tend to become more exposed (Root, 1990; Eens *et al*, 1999) and could indeed be more sensitive (Root, 1990; Eens *et al*, 1999). Thus, the lower reproductive density in MU may be related with a less suitable environment in this area.

## ACKNOWLEDGEMENTS

Authors are grateful for the support of Eng. António Grácio, Coordinator of Núcleo Regional do Corpo Nacional da Guarda Florestal da Beira Litoral. We thank João Ferreira, Laura Cerqueira, Jorge Vaqueiro and Pedro Rodrigues for their help during the fieldwork. We also thank Catarina Eira for the value comments made during the revision.

## REFERENCES

Bairlein F. 1994. Manual of field methods. European-African songbird migration network. Institut fur Vogelforschung, Wilhelmshaven.

Bibby CJ, Burgess ND, Hill DA. 1992. Bird Census Techniques. Ed. Academic Press.

Both C. 2000. Breeding territory size affects fitness: an experimental study on competition at individual level. Journal of Animal Ecology 69: 1021-1030.

Carrascal LM, Senar JC, Mozetich I, Uribe F, Domenech J. 1998. Interactions among environmental stress, body condition, nutritional status, and dominance in Great Tits. The Auk 115(3): 727-738.

Cooper RJ, Whitmore RC. 1990. Arthropod sampling methods in ornithology. Studies in Avian Biology 13: 29-37.

Cramp S, Perrins CM. 1993. The birds of Western Paleartic, Vol VII. Oxford Univ Press, Oxford.

Dubiec A. 2001. Seasonal decline in health status of Great Tit (*Parus major*) nestlings. Can J Zool 79: 1829-1833.

Eens M, Pinxten R, Verheyen RF, Blust R, BervoetsL. 1999. Great and Blue Tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety 44: 81-85.

Fidalgo LGZ. 1988. Contribuição para o estudo da biologia de reprodução dos parídeos nidificantes na Reserva Natural das Dunas de S. Jacinto. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Forsman J. 1998. Heterospecific attration and food resources in migrants' breeding patch selection in northern boreal forest. Oecologia 115: 278-286.

Flousek J. 1989. Impact of industrial emissions on bird populations breeding in mountain spruce forests in central Europe. Ann Zool Fenn 26: 255-263.

Kaiser A. 1993. A new multi-category classification of subcutaneous fat deposits of songbirds. Journal of Field Ornithology 62: 246-255.

Martin TE. 1987. Food as a limit on breeding birds: a life-history perspective. Annu Rev Ecol Syst 18: 453-487.

Martins T. 1999. Contribuição para o conhecimento da biologia da reprodução do Chapim-real e do Chapim-azul num montado de sobro do Baixo Alentejo. Relatório de estágio. Faculdade de Ciências da Universidade de Lisboa.

Naef-Daenzer B, Widmer F, Nuber M. 2001. Differencial post fledging survival of Great and Coal Tits in relation to their condition and fledging date. Journal of Animal Ecology 70: 730-738.

Newton I. 1998. Population Limitation in Birds. Academic Press.

Perrins CM, McCleery RH. 1989. Laying dates and clutch size in the Great Tit. Wilson Bulletin 101(2): 236-253.

Root M. 1990. Biological monitors of pollution. Bioscience 40: 83-86.

Rytkonen S, Orell M. 2001. Great tits, *Parus major*, lay too many eggs: experimental evidence in mid-boreal habitats. Oikos 93: 439-450.

Seki S. 1998. Caterpillar abundance in the territory affects the breeding performance of great tit *Parus major minor*. Oecologia 114: 514-521.

Svensson L. 1992. Identification Guide to European Passerines. 4th ed. Fingraf AB, Sweden.

Wiggins D. 1998. Island biogeography and the reproductive ecology of great tits *Parus major*. Oecologia 115: 478-482.

Zar JH. 1999. Biostatistical analysis. Prentice-Hall.

# THE USE OF PASSERINE FEATHERS TO EVALUATE HEAVY METAL POLLUTION IN CENTRAL PORTUGAL


## ABSTRACT

During the spring of 2003, two sampling strategies were applied in order to evaluate metal contamination in passerine bird feathers. One strategy was the use of nest boxes and nestling Great Tits in two forest habitats, MU – an industrial area and MQ the reference site. Feathers were collected from 15-day (±1) old nestlings when they were still on the nest. The other strategy was the use of mist nets to capture adult Blackbird (*Turdus merula*), Robin (*Erithacus rubecula*) and Blackcap (*Sylvia atricapilla*) in a fragmented non-forested habitats (where the use of nest boxes is not possible) like the industrial area EST and compared with the reference site MQ. The captured birds were measured, ringed and the two outermost tail feathers were collected. Feathers were put in a sterile, metal-free plastic eppendorf tubes and stored at -20ºC until analysis. Feathers were analysed by ICP-AES and CVAA to evaluate the heavy metal contamination of the industrial areas, MU and Est. Mercury concentration was significantly higher in MU. For all other elements considered, concentrations did not differ significantly between MQ and MU.

In EST Blackbird presented significantly higher values of cadmium and nickel, Robin presented significantly higher values of cadmium, lead, zinc and mercury, and Blackcap presented significantly higher values of cadmium, zinc and mercury compared with MQ. For all other elements considered, concentrations did not differ significantly between MQ and EST. Differences in metal levels between species were found, Robin accumulated more metals presenting differences between areas.

In conclusion, this study suggests that the use of Great Tit nestlings' feathers is a useful method to evaluate the presence of heavy metals, such as mercury, in the forest environment. Also, in fragmented habitats where the use of nest boxes is not possible, the use of mist nets and Robin feathers are a reliable method when using birds to evaluate the presence of heavy metals.

## INTRODUCTION

Heavy metals are recurrent waste products of industrial activities and their emission often results in the contamination of the surrounding environment (Eeva and Lehikoinen, 2000). Bird feathers have been used intensively in numerous biomonitoring studies as indicators for heavy metal pollution (Hahn *et al*, 1993; Denneman and Douben, 1993). Heavy metals are sequestered in the sulfhydryl groups of keratin as feathers grow. Once the feather growth is complete, the blood supply atrophies and the metal contents of the feather remain extremely resistant to further change (Appelquist *et al*, 1984; Burger, 1993). Moreover, the procedure used to obtain feathers is non-invasive, because a small sample can be plucked from a live bird without causing harm (Burger, 1993). Most studies have been carried out on birds of prey (Denneman and Douben, 1993; Esselink *et al*, 1995; Jager *et al*, 1996) and on seabird species (Kim *et al*, 1996). However, the majority of these species are very mobile, feeding over a wide geographical area, making it difficult to determine where contaminants were acquired (Burger and Gochfeld, 1997). On the other hand, passerines are mainly non-migratory, residents and hence forage in small ranges (Cramp and Perrins, 1993) thus implying that contamination will originate from a limited area, making them suitable for identifying local pollution (Burger, 1993; Furness, 1993).

Although used to a much lesser extent, nestling birds are also potential good monitors for terrestrial point-source pollution (Burger and Gochfeld, 1993; Burger, 1996). Metal levels in nestling feathers may reflect local pollution levels because metal contamination occurs in a clearly defined time period and its source is limited to the parent's foraging area (Furness, 1993).

In the present study, we analysed the feathers of nestling Great Tits (*Parus major*) and the feathers of three adult resident passerine bird species, Blackbird (*Turdus merula*), Robin (*Erithacus rubecula*) and Blackcap (*Sylvia atricapilla*) in two polluted areas (MU and EST) and in a reference site (MQ) located in a rural area with no industrialization.

The main objective of this study was to evaluate the use of different passerine bird species as indicators of heavy metals according to different types of atmospheric pollution. Two sampling strategies were applied one of which consisting on the use of nest boxes and nestling Great Tits in forest habitats  and the other consisting on the use of mist nets to capture passerine birds in fragmented non-forested habitats where the use of nest boxes is not possible.

## MATERIAL AND METHODS

### *STUDY AREA*

The study was carried out in two industrial areas, MU and EST, and in a reference site, MQ.

The industrial area EST is a chemistry compound located in the north of Estarreja city, 60Km to the north of the reference site. Agriculture fields and eucalyptus stands dominate the area surrounding the compound. At this site, a variety of factories manufacture several elements such as resins, fertilisers, chlorine ($Cl_2$) and caustic soda (NaOH). It is noteworthy that mercury is used as a raw material in the productive process of chlorine and caustic soda. In 1988, the mercury emission produced by this industry amounted to 8.5mg/l per year, only in liquid effluents (Tavares, 1995). In spite of the decrease of pollution values in the last decade due to the innovation of effluent treatment techniques and productive systems, the present study also focuses on the evaluation of the bioaccumulation of mercury and other heavy metals in the environment.

The industrial area MU is a pulp and paper mill located in the south of Figueira da Foz city, 20Km to the south of the reference site. The area surrounding the factory is a maritime pine forest dominated by *Pinus pinaster* and *Pinus pinea*.

An area located in Mata Nacional de Quiaios (MQ), Figueira da Foz, with presumably very limited pollution, was chosen as a reference site. MQ is a maritime pine forest included in the special protected area of Dunas de Mira (Natura 2000 PTCON055). The area is a 70-80 year old man-made plantation dominated by *Pinus pinaster* and *Pinus pinea.* Agriculture fields are present in the edges of the area.

### *SPECIES CAPTURED, SAMPLING DATA AND STATISTICAL ANALYSIS*

*NEST BOXES*

In MQ and MU nest boxes (n=108) were equally distributed along three quadrangular 4 ha sampling plots. The distance between boxes was 40m. Nest boxes were checked regularly to gather data on several breeding parameters (see Annex II). When the nestlings were 15 (±1) days of age we collected the two outermost tail feathers. Feathers were put in a sterile, metal-free plastic eppendorf tubes and stored at -20ºC until analysis.

Copper, zinc and mercury were compared using a T-test for unequal variances, after checking for normal distribution (Zar 1999). Detailed statistical analysis was not possible in the case of cadmium, nickel and lead due to the high number of samples presenting values below the detection limit.

*MIST NETS*

Birds were caught in mist nets in the 29th of May in EST and in the 9th and the14th of May in MQ. The captured birds were measured, ringed and the two outermost tail feathers were collected. Feathers were put in a sterile, metal-free plastic eppendorf tubes and stored at -20 ºC until analysis.

All the species captured are resident species in both areas. Blackbirds are mainly omnivorous (insects and earthworms) and feed mainly on the ground (Cramp and Perrins, 1988). Robins are mostly insectivorous, eating especially beetles and ants, and also feed mainly on the ground (Cramp and Perrins, 1988). Blackcaps are insectivorous but they also eat berries, feeding mainly on leaves and twigs (Cramp and Perrins, 1992).

Data collected was statistically analysed to detect significant differences between study sites and all results are presented as an average. Due to the small number of samples above detection values, confidence limits were generated for sample estimates by the bootstrap technique - 1000 repetitions (Quinn and Keough, 2002). Cadmium, copper, nickel, lead, zinc and mercury were compared using a T-test for unequal variances, after checking for normal distribution (Zar, 1999).

Analysis of variance (ANOVA) was used to compare metal concentrations between species using species and location as independent variables (Zar, 1999).

***SAMPLE PREPARATION AND METAL ANALYSIS***

In the case of adult birds the analyses were performed at the individual level. However, Tit feathers were pooled from at least 3 nestlings from each nest, according to the recommendations of Dauwe *et al* (2000). This number ensures the collection of a sufficient amount of feathers for heavy metal analysis.

Feathers were washed alternately with deionised water and acetone and were then put in metal-free polypropylene vials. To obtain the feather's dry weight, feathers were put in an oven (60 ºC) till constant weight. About 0.2 g of feathers of each sample were rinsed in tap water and deionised water to eliminate adsorbed external contamination (Burger, 1993). Each sample was treated with nitric acid overnight (15 ml). The wet-ash procedure was continued with heating in a water bath, followed by the addition of hydrogen peroxide (2ml). This treatment was repeated till full digestion. The filtrate was diluted with double distilled water up to a volume of 25ml. Finally, the digested material was analysed for heavy metals using a combination of analytical techniques: ICP-AES (inductively coupled plasma – atomic emission spectrometry) was used to estimate Cu, Zn, Cd, Ni and Pb (EPA procedures: EPA 6010B). Total Hg was estimated using a cold vapor atomic absorption (CVAA) technique (EPA procedures: EPA 7471A). The concentrations are expressed in $mg.kg^{-1}$ based on dry weight values. All samples were analyzed in batches with certified reference material (mussel sample- CRM 278, Community Bureau of Reference) and blanks. Recovered concentrations of the certified samples were within 10 % of the certified values, which is considered an acceptable margin (Gochfeld and Burger, 1998). The limit of detection was 1 $mg.kg^{-1}$ for Cu, Zn, Ni, Pb and 0.1 $mg.kg^{-1}$ for Cd and Hg.

## RESULTS

### *NEST BOXES*

Feathers were collected from 164 nestlings (MQ – 92, MU – 72) from 32 nests (MQ – 19, MU – 13). All elements (Cu, Zn, Hg, Cd, Ni and Pb) were detected in the feathers of Great tit nestlings, except for lead in MU. Cadmium, nickel and lead presented a very low percentage of detection, which did not allow for further statistical analysis. These three elements had a higher percentage of detection in MQ (table 1). Mercury was significantly higher in MU while copper and zinc, although slightly higher in MQ, did not present significant differences (table 2). The confidence limits generated by bootstrap analysis puts in evidence the differences between areas in what concerns mercury and similarity of results in relation to copper and zinc (table 1).

### *MIST NETS*

#### *BLACKBIRD*

Significantly higher cadmium and nickel values were found in EST (table 3). No other element had significant differences, although lead, zinc and mercury were considerable higher in EST, and copper was slightly higher in MQ.

Table 1- Heavy metal levels (mg.kg$^{-1}$) determined in feathers of nestling Great Tits in MQ and MU (mean and CL-confidence limits). ND = not detected (below the limit of detection). Note: mean values do not include levels below limit of detection.

| | MQ | | | MU | | |
|---|---|---|---|---|---|---|
| | **Detection (%)** | **Mean (CL)** | **Range** | **Detection (%)** | **Mean (CL)** | **Range** |
| **Cu** | 100 | 7.15 (6.08-8.24) | 1.85 - 12.43 | 100 | 7.08 (6.31-7.81) | 5.05 - 9.53 |
| **Zn** | 100 | 105.24 (92.82-116.33) | 26.3 - 160.78 | 100 | 98.37(91.11-105.32) | 75.8 - 119.68 |
| **Hg** | 100 | 0.66 (0.37-0.98) | 0.01 - 1.82 | 100 | 1.09 (0.87-1.28) | 0.38 - 1.61 |
| **Cd** | 52.63 | 0.17 (0.12-0.24) | ND - 0.4 | 7.69 | - | ND - 0.2 |
| **Ni** | 63.16 | 2.44 (1.76-6.64) | ND - 7.73 | 15.38 | 1.24 | ND - 1.37 |
| **Pb** | 26.32 | 2.23 (1.38-3.19) | ND - 3.72 | 0 | - | ND |

Table 2- Comparison between heavy metal levels (mg.kg$^{-1}$) determined in feathers of nestling Great Tits in MQ and MU (mean ± standard deviation) using a t-test.

| | **MQ** | **MU** | **t** | **P** |
|---|---|---|---|---|
| **Cu** | 7.15±2.22 | 7.08±1.43 | 0.0998 | 0.9212 |
| **Zn** | 105.24±25.67 | 98.37±13.71 | 0.9618 | 0.3447 |
| **Hg** | 0.66±0.68 | 1.09±0.40 | 2.205 | **0.0365** |

Table 3- Mean metal accumulation (mg.kg$^{-1}$) in the Blackbird and results of bootstrap simulation to generate 95% confidence limits.

| | **Blackbird** | | | |
|---|---|---|---|---|
| | **MQ** | **EST** | **t** | **P** |
| **Cd** | 0.12<br>(0.03 – 0.18) | 0.38<br>(0.16 – 0.5) | 3.051 | **0.0225** |
| **Cu** | 11.00<br>(8.09 – 14.13) | 10.43<br>(8.81 – 12.64) | 0.2735 | 0.7895 |
| **Ni** | 0.78<br>(0.5 – 1.06) | 2.15<br>(1.10 – 2.81) | 2.735 | **0.0410** |
| **Pb** | 2.35<br>(1.52 – 3.15) | 4.89<br>(2.92 – 7.10) | 2.037 | 0.0878 |
| **Zn** | 109.78<br>(102.03 – 121.59) | 140.10<br>(120.46 – 166.88) | 2.109 | 0.0795 |
| **Hg** | 0.50<br>(0.26 – 0.73) | 1.05<br>(0.47 – 1.81) | 1.464 | 0.1935 |

*ROBIN*

Cadmium, lead, zinc and mercury were significantly higher in EST (table 4). The remaining elements were not significantly different. Copper and nickel were slightly higher in MQ.

There was a significant difference in metal accumulation in Robin compared to the other studied species (table 6). Posterior analysis indicate that in MQ Robin presented significantly higher Hg values than those registered for Blackbird (Tukey, $p<0.001$) and those registered for Blackcap (Tukey, $p<0.001$). The same result was obtained in EST (Tukey, Robin vs Blackbird $p<0.05$ and Robin vs Blackcap $p<0.05$).

Table 4- Mean metal accumulation ($mg.kg^{-1}$) in Robin and results of bootstrap simulation to generate 95% confidence limits.

| | Robin | | | |
|---|---|---|---|---|
| | **MQ** | **EST** | **t** | **P** |
| **Cd** | 0.11<br>(0.03 – 0.18) | 0.37<br>(0.06 – 0.65) | 2.356 | **0.0429** |
| **Cu** | 17.06<br>(13.53 – 21.18) | 13.66<br>(8.76 – 18.56) | 1.289 | 0.2170 |
| **Ni** | 1.13<br>(0.60 – 1.73) | 1.02<br>(0.5 – 1.38) | 0.332 | 0.7453 |
| **Pb** | 2.04<br>(1.41 – 2.67) | 4.14<br>(2.48 – 6.60) | 2.587 | **0.0271** |
| **Zn** | 113.57<br>(102.84 – 125.14) | 135.69<br>(108.54 – 150.18) | 2.254 | **0.0407** |
| **Hg** | 2.04<br>(1.52 – 3.75) | 3.44<br>(2.5 – 4.06) | 2.640 | **0.023** |

*BLACKCAP*

Cadmium, zinc and mercury had significantly higher levels in EST (table 5). Nickel was not considered in the analysis because most of the values were below the detection limit. Lead was higher in EST and copper was higher in MQ, although with no significant differences.

There was a significant difference in metal accumulation in Blackcap compared to the other species studied (table 6). Posterior analysis indicate that in MQ Blackcap presented significantly lower Zn values than those registered for Robin (Tukey, $p<0.01$) and those registered for Blackbird (Tukey, $p<0.05$).

Table 5- Mean metal accumulation (mg.kg$^{-1}$) in Blackcap, and results of bootstrap simulation to generate 95% confidence limits.

| | Blackcap | | | |
|---|---|---|---|---|
| | **MQ** | **EST** | **t** | **P** |
| **Cd** | 0.2<br>(0.11 – 0.29) | 0.36<br>(0.24 – 0.51) | 2.144 | **0.0468** |
| **Cu** | 15.04<br>(10.95 – 20.22) | 13.74<br>(11.15 – 16.64) | 0.449 | 0.6610 |
| **Pb** | 3.05<br>(2.30 – 3.84) | 3.57<br>(2.48 – 4.64) | 0.729 | 0.4749 |
| **Zn** | 87.83<br>(79.26 – 96.59) | 121.05<br>(107.09 – 136.44) | 3.671 | **0.0017** |
| **Hg** | 0.32<br>(0.01 – 0.29) | 1.62<br>(0.65 – 2.71) | 2.265 | **0.0412** |

Table 6- Interspecific metal accumulation differences between three bird species studied, Blackbird, Robin and Blackcap, from an ANOVA analysis.

| | MQ | | | EST | | |
|---|---|---|---|---|---|---|
| | **F** | **DF** | **P** | **F** | **DF** | **P** |
| **Cu** | 2.046 | 2.26 | 0.1512 | 1.268 | 2.27 | 0.2988 |
| **Zn** | 7.321 | 2.26 | **0.0033** | 1.222 | 2.27 | 0.3115 |
| **Hg** | 14.03 | 2.22 | **0.0002** | 5.304 | 2.27 | **0.0120** |
| **Cd** | 1.690 | 2.26 | 0.2057 | 0.01227 | 2.26 | 0.9878 |
| **Ni** | 0.6611 | 2.25 | 0.5258 | - | - | - |
| **Pb** | 2.180 | 2.26 | 0.1349 | 0.6759 | 2.27 | 0.5177 |

## DISCUSSION

One objective of our study was to compare metal concentrations in the feathers of nestling Great Tits between two forest habitats exposed to different levels of pollution (an industrial area-MU and a rural area-MQ). The industrial site presented the highest values of Hg. The concentrations of copper and zinc presented no significant differences between areas and they were even slightly higher in the rural site (MQ). In other studies reporting concentrations of heavy metals in nestling birds (Dauwe *et al*, 2000; Janssens *et al*, 2002), the concentrations of copper and zinc were also not significantly different between reference and polluted sites. These authors suggest that Great Tit nestlings' feathers cannot be used as biomonitors for zinc and copper because they may not reflect adequately the nestling body burden. According to Dauwe *et al* (2000) homeostatic mechanisms (e. g., excretion through the excrements) may still adequately control metal levels in tissues at this early stage of maturity.

In the present study, analysis also detected the presence of cadmium, nickel and lead. However, many of the analysed samples produced values below the detection limits, which drastically reduced sample size. For this reason, only the percentage of detection of these heavy metals was compared. Cadmium, nickel and lead presented a higher percentage of presence in the control area. Dauwe *et al* (2000) also found a higher level of cadmium in the reference site. In fact, as previously emphasised (Dauwe *et al*, 2000; Janssens *et al*, 2002) several factors might influence the immobilization of heavy metals in feathers. In what refers to cadmium, this toxic element is generally tightly bound to metallothioneins in the kidney and is therefore not generally available to incorporate into feathers (Elliot and Scheuhammer, 1997).

The presence of agriculture fields, where fertilizers are used, and also the presence of a heavy-traffic road in the surroundings of MQ might account for the higher presence of nickel and lead in this area. Even though nickel and lead were the only heavy metals emitted by the paper and pulp facilities in 2002 (EPER), in MU no traces of lead were detected. In fact, the present results show significant higher levels of mercury in MU when comparing to MQ. Because of its persistence and bioaccumulation potential, this metal is regarded as a global pollutant (EPER). The high presence of mercury in MU could be a result of a past long-term emission, when environmental concerns were not considered a priority and there was a lack of regulations for this kind of emissions. Another possibility refers to current emissions from factories where the production processes still release mercury. Either way, it constitutes a matter of great concern and should be regularly monitored considering its well-known toxicity.

Samples from adult birds collected in the chemical compound area (EST), emphasised that the accumulation of mercury is much higher than that registered in the reference site (MQ). In 1988, the emission to effluents was 8.5mg/l/year (Tavares, 1995) and according to the EPER (2002) emissions amounted to 7.61Kg/year. As expected, EST presented the highest levels of mercury in the three bird species (although not significantly higher in Blackbird).

Cadmium was also significantly higher in EST in all species. Although cadmium is not generally available to incorporate into feathers, in this study cadmium was consistently detected in the three species, possibly implying a very high level of environmental cadmium. The clearly agriculture-dominated landscape and the presence of a fertilizer factory in EST may account for such high cadmium levels considering that cadmium may originate from anthropogenic activities such as the use of phosphate fertilisers (EPER).

Differences in metal levels between species and areas may be produced by different diets, feeding strategies and metabolic rates. Considering that Blackbirds mostly feed on earthworms, this species was expected to accumulate more metals (Llacuna *et al*, 1995). However, results showed that Robins accumulated more metals presenting differences between areas. Robins eat mainly insects and feed on the ground. They are relatively smaller and present a higher metabolic rate (Root, 1990; Eens *et al*, 1999), which might account for the higher accumulation of metals. Blackcaps are also insectivorous and smaller. However, they feed mainly on trees thus reducing their exposure to metals when comparing with species that feed on the ground. The present results highlight that robins may constitute a more efficient species to be used in studies dealing with heavy metal monitorization.

In conclusion, this study suggests that the use of Great Tit nestlings' feathers is a useful method to evaluate the presence of heavy metals, such as mercury, in the forest environment. Furthermore, Great tits are ubiquitous and abundant, and sometimes the only forest passerine species available in suiting densities in polluted areas. They readily nest in manmade nest boxes and so breeding populations can easily be monitored. Great Tit nestlings are also easily monitored because they are relatively insensitive to nest disturbance (Janssens *et al*, 2002). Another advantage of using nestlings is the fact that they are restricted to their nest and will be fed with resources collected in the immediate vicinity of the nest box. Therefore the heavy metal contamination refers to a restricted area around the nest (Furness, 1993). Also, in fragmented habitats where the use of nest boxes is not possible, the use of mist nets and Robin feathers are a reliable method when using birds to evaluate the presence of heavy metals.

**ACKNOWLEDGEMENTS**

Authors are grateful for the support of Eng. António Grácio, Coordinator of Núcleo Regional do Corpo Nacional da Guarda Florestal da Beira Litoral. We thank João Ferreira, Laura Cerqueira, Jorge Vaqueiro and Pedro Rodrigues for their help during the fieldwork. We also thank Catarina Eira for the value comments made during the revision.

**REFERENCES**

Appelquist H, Asbrik S, Drabaek I. 1984. Mercury monitoring: mercury stability in bird feathers. Mar Pollut Bull 15(1): 22-25.

Burger J. 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev Environ Toxicol 5: 203-311.

Burger J. 1996. Heavy metal and selenium levels in feathers of Franklin's gulls in interior North America. Auk 113: 399-407.

Burger J, Gochfeld M. 1993. Lead and cadmium accumulation in eggs and fledgling seabirds in the New York bight. Environ Toxicol Chem 12: 261-267.

Burger J, Gochfeld M. 1997. Age differences in metals in the blood of Herring (*Larus argentus*) and Franklin's (*Larus pipixcan*) gulls. Arch Environ Contam Toxicol 33: 436-440.

Cramp S, Perrins CM. 1988. The birds of Western Paleartic, Vol V. Oxford University Press, Oxford.

Cramp S, Perrins CM. 1992. The birds of Western Paleartic, Vol VI. Oxford University Press, Oxford.

Cramp S, Perrins CM. 1993. The birds of Western Paleartic, Vol VII. Oxford University Press, Oxford.

Dauwe T, Bervoets L, Blust R, Pinxten R, Eens M. 2000. Can excrement and feathers of nestling song birds be used as a biomonitor for heavy metal pollution? Arch Environ Contam Toxicol 39: 541-546.

Denneman WD, Douben PET. 1993. Trace metals in primary feathers of the barn owl (*Tyto alba guttattus*) in the Netherlands. Environmental Pollution 82: 301-310.

Eens M, Pinxten R, Verheyen RF, Blust R and Bervoets L. 1999. Great and Blue Tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety 44: 81-85.

Eeva T, Lehikoinen E. 2000. Recovery of breeding success in wild birds. Nature 403: 851-852.

Elliot JE, Scheuhammer AM. 1997. Heavy metal and metallothionein concentrations in seabirds from the Pacific coast of Canada. Mar Pollut Bull 34: 794-801.

EPER – European Pollutant Emission Register. http://www.eper.cec.eu.int (cited 2/Mar/05).

Esselink H, van der Geld FM, Jager LP, Posthuma-Trumpie GA, Zoun PEF, Baars AJ. 1995. Biomonitoring heavy metals using the barn owl (*Tyto alba guttattus*): sources of variation especially relating to body condition. Arch Environ Contam Toxicol 28: 471-486.

Furness RW. 1993. Birds as monitors of pollutants. In: Furness RW, Greenwood JJD (eds) Birds as monitors of environmental change. Chapman and Hall, London, p 86.

Gochfeld M, Burger J. 1998. Temporal trends in metal levels in eggs of the endangered Roseate Tern (*Sterna dougallii*) in New York. Environmental Research 77: 36-42.

Hahn E, Hahn K, Stoepler M. 1993. Bird feathers as bioindicators in areas of the German Environmental Specimen Bank – bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. The Science of the Total Environment 139/140: 259-270.

Jager LP, Rijnierse FVJ, Esselink H, Baars AJ. 1996. Biomonitoring with the buzzard *Buteo buteo* in The Netherlands: heavy metals and sources of variation. Journal Ornithol 137: 295-318.

Janssens E, Dauwe T, Bervoets L, Eens M. 2002. Inter and intraclutch variability in heavy metals in feathers of Great tit nestlings (*Parus major*) along a pollution gradient. Arch Environ Contam Toxicol 43: 323-329.

Kim EY, Ichihashi H, Saeki K, Atrashkevich G, Tanabe S, Tatsukawa R. 1996. Metal accumulation in tissues of seabirds from Chaun, Northeast Siberia, Russia. Environ Pollut 92: 247-252.

Llacuna S, Gorriz A, Sanpera C, Nadal J. 1995. Metal accumulation in three species of passerine birds (*Emberiza cia*, *Parus major*, and *Turdus merula*) subjected to air pollution from a coal-fired power plant. Arch Environ Contam Toxicol 28: 298-303.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge University Press.

Root M. 1990. Biological monitors of pollution. Bioscience 40: 83-86.

Tavares CMOF. 1995. Contaminação por Hg do solo e plantas dos campos marginais do Esteiro de Estarreja. Dissertação de Mestrado. Universidade de Aveiro.

Zar JH. 1999. Biostatistical analysis. Prentice-Hall.