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Diet and isotopic niche of insectivorous birds in *montado* and their potential role in pest regulation.

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Jaime Albino Ramos (Universidade de Coimbra)

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Table of contents

Abstract

Recent decline or loss of vigour of the *montado*, an agro-silvo-pastoral system characterized by an open tree layer dominated by *Quercus suber* and *Quercus rotundifolia* trees with an understory composed by shrubs and herbs, has threatened it maintenance the last decades, due to biotic and abiotic factors. These factors include insect pest species known to feed on or affect leaves, bark, wood or seeds of the trees. Insectivorous birds, such as the Blue tit (*Cyanistes caeruleus*), Great tit (*Parus major*) and Nuthatch (*Sitta europaea*), are known to prey on insect pest species in many forest habitats worldwide, however its potential role in controlling insect pest species of the *montado* is poorly understood. Recent development in stable isotope analysis (SIA) allows us to compare the diet and isotopic niche of these three species. This enabled us to study in detail, the foraging ecology of these bird species in the *montado* and their role in controlling insect pest species.

We sampled nestlings' diet, during two consecutive years using the ligature-method technique, and identified arthropods present in diet samples to family level, whenever possible. Tertiary feathers of late nestlings and samples of their prey were collected for carbon and nitrogen SIA, to estimate the diet and trophic niche of the three bird species, using SIAR and SIBER packages in R software.

Lepidoptera and Araneae were the most preyed items by all bird species in the two study years. We found significant differences in the annual diet between the three bird species for some arthropod orders such as Coleoptera and Araneae but not for Lepidoptera. Some potential insect pest species, such as Noctuidae, were also predated by all bird species, but the Blue tit was the most likely species to predate on insect pest species, mainly larvae of defoliator Lepidoptera. Individuals of *Lymantria dispar*, a well-known pest species, were identified in the diet of Great tit and Nuthatch, in 2013. Isotopic niche results showed clear differences in size and niche overlaying among the three bird species.

Our results show that diet of nestlings of the three bird species is in accordance with previous studies and that the main prey is Lepidoptera, which should be the most available prey during the breeding season. Because most Lepidoptera larvae are known defoliators, their strong predation by our study bird species indicates their potential role as controllers of insect pest species. Estimates of diet using SIA were a good complement to traditional diet identification, enhancing the importance of some insect families in relation to diet samples analysed. Results obtained for isotopic niche were also expected due to differences in foraging behaviour among the three bird species, explaining differences in predation of some insect orders, such as Coleoptera by Nuthatch, Lepidoptera by Blue tit and Orthoptera by Great tit. The Blue tit feeds mainly on tree foliage branches and twigs, and appears to be the most important predator of defoliator larvae, although the other two species may also be important.

Our study suggest that the studied bird species have a potential role in controlling insect pest species, and SIA can be a helpful method to better understand the diet of terrestrial insectivorous bird species and in discriminating their trophic niche.

Key words: *Montado*, diet analysis, isotopic niche, biological pest control, insectivorous bird species.

Resumo

O recente declínio ou perda de vigor do *montado,* um sistema agro-silvo-pastoril caracterizado por um estrato arbóreo dominado por *Quercus suber* e *Quercus rotundifolia* com um coberto sub-arbustivo composto por arbustos e ervas, tem ameaçado a sua manutenção nas últimas décadas devido a factores bióticos e abióticos. Estes factores incluem espécies de insectos pragas que alimentam de ou afectam folhas, cortiça, madeira ou sementes das árvores. Espécies de aves insectívoras, tais como Chapim-azul (*Cyanistes caeruleus*), Chapim-real (*Parus major*) e Trepadeira-azul (*Sitta europaea*), são conhecidos predadores de pragas em vários habitats florestais a nível mundial, contudo o seu papel como potenciais controladores de pragas do *montado* é pouco estudado. Recentes desenvolvimentos em análise de isótopos estáveis (SIA) permitem estudar e inferir a dieta e nicho trófico de aves. Este método possibilita que estudemos, com maior foco, a ecologia trófica das aves do *montado* e o seu papel como predadores de pragas.

Amostrámos dieta de crias no ninho durante dois anos consecutivos, usando o método-do-colar e identificando os itens presentes nas amostras de dieta até ao nível taxonómico de família, sempre que possível. Penas terciárias das crias e amostras de dieta foram recolhidas para análise de isótopos estáveis de carbono e azoto, para estimar a dieta e nicho trófico das três espécies de aves, usando os pacotes SIAR e SIBER no software R.

Foram obtidas diferenças significativas na dieta anual das três espécies de aves para ordens de artrópodes tais como Coleoptera e Araneae, mas para Lepidoptera não foram obtidas quaisquer diferenças. Lepidoptera e Araneae foram as ordens mais predadas pelas três espécies de aves durante os dois anos. Alguns indivíduos considerados potenciais pragas, tais como os da família Noctuidae, também foram predados pelas três espécies de aves, mas principalmente por Chapim-azul, que apresenta assim o maior potencial como espécie predadora de espécies praga, principalmente larvas desfolhadoras de Lepidoptera. Indivíduos de *Lymantria dispar*, uma conhecida espécie praga, foram identificados na dieta de Chapim-real e Trepadeira-azul, ambos em 2013. Resultados referentes ao SIBER revelaram diferenças no tamanho e sobreposição dos nichos das três espécies de aves, havendo diferenças para carbono e azoto, os dois isótopos estáveis usados neste estudo.

Os resultados obtidos da dieta das três espécies de aves estão de acordo com estudos anteriores, e a principal presa das três espécies são larvas de Lepidoptera. Durante a época reprodutora, este deverá ser o tipo de presa mais disponível. Sendo a maior parte das larvas de Lepidoptera conhecidos desfolhadores, a sua predação pelas aves indica que estas poderão ser potencias controladores de espécies praga. Estimativas do SIAR revelaram ser um bom complemento ao método tradicional de análise de dieta, estando de acordo com os resultados da análise de dieta para algumas famílias e aumentado a importância de outras relativamente à analise das dietas. Os resultados obtidos relativamente ao SIBER eram esperados devido a diferenças nos hábitos de forageamento das três espécies, explicando também a diferenças obtidas na predação de diferentes ordens, tais como Coleoptera por Trepadeira-azul, Lepidoptera por Chapim-azul e Chapim-real e Orthoptera por Chapim-real. O Chapim-azul usa alimenta-se sobretudo nas folhas e ramos finos das arvores, sendo mais provável que prede larvas desfolhadoras de Lepidoptera.

Este estudo sugere que as três espécies de aves estudadas apresentam potencial como controladoras de pragas do *montado* e que SIA é um método útil como complemento de métodos tradicionais para obter uma melhor compreensão da dieta de aves insectívoras terrestres e também para inferir e distinguir entre os seus nichos tróficos.

Palavras-chave: *Montado*, análise de dieta, nicho isotópico, controlo biológico de pragas, aves insectívoras.

1 – Introduction

1.1 – The *montado*

The *montado* is a high biodiverse and sustainable agro-silvo-pastoral system characterized by an open tree layer dominated by a low density of evergreen oaks – cork oak (*Quercus suber*) and holm oak (*Q. rotundifolia*) - , and occupies large and continuous areas in southern Portugal (Belo et al. 2009; Bugalho et al. 2011). The *montado* has large natural, economic and cultural values, of which the most known is perhaps the cork. Portugal produces more than 50% of the cork in the world and it has 33% of the world's cork oak area (Pinto-Correia et al. 2011). However, cork is not the only product of the *montado*, and other important ones are livestock used for food, wood used for fuel or wild game for hunting activities, among many other. The *montado* also provides "invisible" services, as it contributes to carbon sequestration, runoff reduction and groundwater recharge (Belo et al. 2009; Sá-Sousa 2014). These large areas are also important for many animal and plant species, as it act as habitat for a great number of species, some of them with IUCN status, such as Black vulture (*Aegypius monachus*) and Imperial Eagle (*Aquila adaltberti*) (Belo et al. 2009; Tellería 2001). The *montado* is also known as an important habitat for birds, both migratory and resident, and arthropods (Leal et al. 2011; Pereira 2010). The *montado* is considered a High Nature Value Farmland, included in the European Union Habitat Directive, and it is considered one of the world's 25 biodiversity hotspots and a good example of adaptation to the constrains of the Mediterranean climate and balance between socio-economic development and biological conservation (Godinho & Rabaça 2010).

The *montado* occupies an area of 800.000 ha in Portugal (Pinto-Correia et al. 2011) and besides cork oak and holm oak is also composed by other less dominant trees such as *Quercus faginea* and *Quercus pyranaica* (Godinho & Rabaça 2010; Leal, Correia, et al. 2011; Tellería 2001; Belo et al. 2009; Joffre et al. 1999). Along with the tree layer, the *montado* is also characterized by a second layer, the shrub/herbaceous layer, composed by the native vegetation such as *Cistus ladaniferus*, *Cistus salviaefolius*, *Cistus moonspeliensis* and *Erica* spp., *Lavandula* spp., *Rosmarinus* sp., *Ulex* spp. (Pereira & Fonseca 2003; Joffre et al. 1999; Pinto-Correia 1993).

The *montado*, as an agro-silvo-pastoral system, is a result of management practices that optimize the annual fluctuations in productivity, without causing ecological damages to the environment (Pinto-Correia 1993). The recent strong decline of the *montado* is noticeable in the mortality of individual trees and in loss of productivity. This issue has been addressed in different studies, focusing on different causes such as insect pest species, fungus and bacteria, water stress and land management (Olea & Miguel-ayanz 2006; Sousa et al. 2007). Decline can be described as a loss of vigour or weakening of the forest, often without any specific symptoms (Sousa et al. 2007). Decline is a slow process, taking months or even years, usually ending with the death of trees. The decline phenomenon can be described as a positive feedback, in which there is a growth of pathogenic agents or attack by insects species, leading to a decrease in the number of roots, leaf surface and a consequential increase of physiological disorders, which then leads to a decrease of the trees' defences and decrease of vigour, then the trees are susceptible to attack by pathogenic agents and insect attacks again (Sousa et al. 2007). This decline is caused by an interaction between biotic and abiotic factors, such as climatic, edaphic and biological factors (Olea & Miguel-ayanz 2006; Sousa et al. 2007). This situation does not restrain to a single species of trees but it is general to a geographic region (Sousa et al. 2007). The symptoms of the decline are observable in the aerial part of trees: change in leaf colours and progressive death of small branches. Sudden death of trees can also occur, caused by an infection of the radicular system combined with low humidity content. These sudden deaths usually occur in late summer and autumn (Belo et al. 2009).

1.2 – Insect pest species

Insect outbreaks are also one of the known causes of the decline of cork oak and holm oak *montado*. In Portugal, there are 92 species of insects that cause damage to cork oak and holm oak (Ferreira & Ferreira 1991; Sousa et al. 2007) and these can be classified into defoliators, seed-borers and wood-borers, according to the part of the tree they affect. Defoliators attack leafs, reducing the growth of trees but do not kill them. On the other hand, insects that attack the trunk and branches can cause the death of trees, especially if they are already weakened or damaged (Sousa et al. 2007).

Since the beginning of the 20th century, insect populations in *montado* changed in numbers, with the appearance of some new species and the increase of other insect populations, cause by an apparent imbalance in the ecosystem. The first reference to a plague attack in *montado* was from the defoliator *Lymantria dispar* (Sousa et al. 2007). Since then, other insect species that attack trees appeared and their populations are increasing. Among each group of pest species, some species are more important due to their habits and characteristics. Lepidoptera larvae, which include *Catocala nymphagoga*, *Malacosoma neustria* and *Lymantria dispar*, are among the most import defoliator species. In spring, when larvae of Lepidoptera emerge, these species are more abundant and can play a more important role in defoliation of *Quercus* spp. (Toimil 1987; Sanz 2001). Wood-borers are mainly Coleoptera species such as *Cerambyx cerdo*, *Coreobus* spp. and *Platypus cylindrus* (Ferreira & Ferreira 1991). This group affect trees mainly by building galleries inside them or opening holes that may lead to the entrance of pathogenic fungus inside the trees (Martín et al. 2005). Relatively to seed-borers, species such as *Curculio elephas* and *Cydia splendana* are known to affect seed germination and size, due to larvae development of these species inside acorns of *Q. suber* (Soria et al. 1999). The more important insect pest species are listed in appendix III (Ferreira & Ferreira 1991).

1.3 – Birds as pest predators

Insect outbreaks are one of the known causes for the weakening of forest tree species and even decline of some forest species such as the cork oak (*Quercus suber*) in the European Mediterranean region. Bird populations can be very helpful in controlling insect pest species, especially generalist and insectivorous bird species such as Blue tit (*Cyanistes caeruleus*), Great tit (*Parus major*) and European Nuthatch (*Sitta europaea*), which are potential agents of bio-control, resulting in the decline or low population maintenance of insect pest species (Barbaro & Battisti 2011; Greenberg et al. 2000; Mols & Visser 2002; Sanz 2001; Sipura 1999). Despite the importance of birds in insect pest control, few studies have addressed this issue in oak habitats (Marquis & Whelan 1994; Sanz 2001). However, in other habitats this issue has been studied and the positive effect of bird predation on insect pest species is known in habitats such as Pine Forests in south-western Europe and North Africa, coffee plantations, palm oil plantations and apple orchards (Barbaro & Battisti 2011; Greenberg et al. 2000; Johnson et al. 2010; Koh 2008; Mols & Visser 2002; Pimentel & Nilsson 2009; Strong et al. 2000). Several studies have proven that birds can reduce the density of pest species or the damage in trees created by such pest species. Sanz (2001) used nest-boxes to increase the breeding population of *C. caeruleus*, *P. major*, and *Fycedula hipoleuca* on plots with *Quercus pyrenaica*, and showed that the density of caterpillars, the damage caused by caterpillars on the trees and the body mass of caterpillars were significantly lower in the nest-box plots than in the plots without nest-boxes.

1.4 – Insectivorous birds in the *montado*

Tree-foraging insectivorous bird species are of special interest to evaluate if birds can play an important role in controlling cork oak and holm oak pests. Some bird species stand out in this scenario, such as the Blue tit, Great tit and Nuthatch, combining their foraging niche preferences on foliage branches and secondary branches (Leal et al. 2012). Bird community of the *montado* is very rich and diverse, covering species with many different preferred habitats and diet. During the breeding period, most of the bird community in *montados* and oak habitats is composed by insectivorous birds, at least during this period (Diaz & Pulido 1993; Illera & Atienza 1995). Among the insectivorous birds of the *montado*, Blue tit, Great tit and Nuthatch are of great importance in this study because of their foraging and diet preferences, abundance and ease to breed on nest-boxes. These species, mainly insectivorous during breeding season, are very common, easy to manipulate in field experiments and also very well studied in other habitats, being known to prey on forest pest species, fitting the requirements to work with and to better understand their role in pest controlling (Betts 1955; Godinho & Rabaça 2010; Matthysen 1999).

1.5 – Stable Isotopes Analysis to infer diet and trophic niche

A method to assess the potential role of insectivorous birds in pest control is diet sampling. The need to evaluate the diet of insectivorous birds during spring is linked to the breeding season, when almost all of bird community in oak habitats is insectivorous (Illera & Atienza 1995; Pereira 2010). Also, during this season birds are more dependent on arthropods for food as it represents a narrow time-window in which the environment conditions are good enough for the breeding pairs to reproduce and to feed their nestling as well as themselves, because spring is the time of maximum food abundance (Mizutani & Hijii 2002; Mols & Visser 2002; Visser et al. 2006). During spring/summer period, arthropod community may change in numbers and diversity very rapidly, concurring with the period of highest requirements of egg-laying and parental care by breeding birds (Illera & Atienza 1995).

A novel way to study animal ecology, is the use of stable isotopes analysis (SIA) (Post 2002; Caut et al. 2009), of which the stable-nitrogen isotope ratios (^{15}N: ^{14}N, expressed as $\delta^{15}N$) and stable-carbon isotope ratios (^{13}C: ^{12}C, expressed as $\delta^{13}C$) are the most commonly used. This is possible because isotopic ratios of consumers reflect those of their prey (Bakhurin et al. 2008). Stable isotopes are important and useful in tracing diets and location due to a process known as fractioning, in which differences in their mass cause different behaviour during chemical reactions, causing different ration between the light and heavy isotopes of the same element, thus allowing to see differences in isotopic values between different species and individuals (Inger & Bearhop 2008). Different tissues used in SIA indicate diet/habitat during different time-periods, because the different tissues have different turn-over rates and are synthesized at different periods on the life cycle of the individual. A tissue only shows diet or habitat of the individual during the period in which it was synthesized (Inger & Bearhop 2008). This represent an advantage comparing to traditional diet analysis via stomach content or faecal sample because these only give a snapshot of what the individuals ate (Sabat et al. 2013). A trophic leap between prey and predators represent a small increase in the values of isotopic ratio of ^{13}C, about 2‰ (Inger & Bearhop 2008). Isotopic ration of ^{13}C is often used to differentiate between diets based on C_3 or C_4 plants (Post 2002). With nitrogen it is also possible to predict diet of a consumer, due to its distribution and fractioning along the food web. $\delta^{15}N$ is mainly used to infer about trophic level, with a enrichment of about 3-4‰ of isotopic ratio of consumers over those of the diet (Inger & Bearhop 2008; Post 2002). SIA also allows us to infer about isotopic niche. Isotopic niche and trophic niche, are not the same (Jackson et al. 2011). Isotopic niche refers to an area with a set of coordinates given by isotopic values whereas ecological niche is often described as an n-dimensional hypervolume whose axes represent environmental variables (Bearhop et al. 2004; Newsome et al. 2007). Stable isotope analysis gives both quantitative information on resources and habitat, or binomic and scenopoetic, respectively. A set of values of ^{13}C and ^{15}N for several individuals represented in two-axes plot (carbon and nitrogen), a n-dimensional space that contains the niche of a species or individual, gives us the isotopic niche (Newsome et al. 2007).

1.6 - Objectives

The goal of this study is to evaluate the diet and trophic niche of three insectivorous bird species (Blue tit, Great tit and European Nuthatch) and also to evaluate their potential role as bio-controllers of insect pest species. Specifically this study aims to answer three questions: 1) “Does diet and isotopic niche differ among tree-foraging bird species?” 2) “How important are insect pest species in the diet of insectivorous bird species?” and 3) “How efficient are stable isotope techniques to trace the diet of terrestrial insectivorous birds?”.

2 – Methods

2.1 – Study area

Field work was done in Herdade do Freixo do Meio, in Foros de Vale Figueira (38º42'11.54''N, 08º19'31,88''O), located approximately 15 km from Montemor-o-Novo, with approximately 1140 ha of cork-holm oak *montado* in Alentejo, south Portugal.

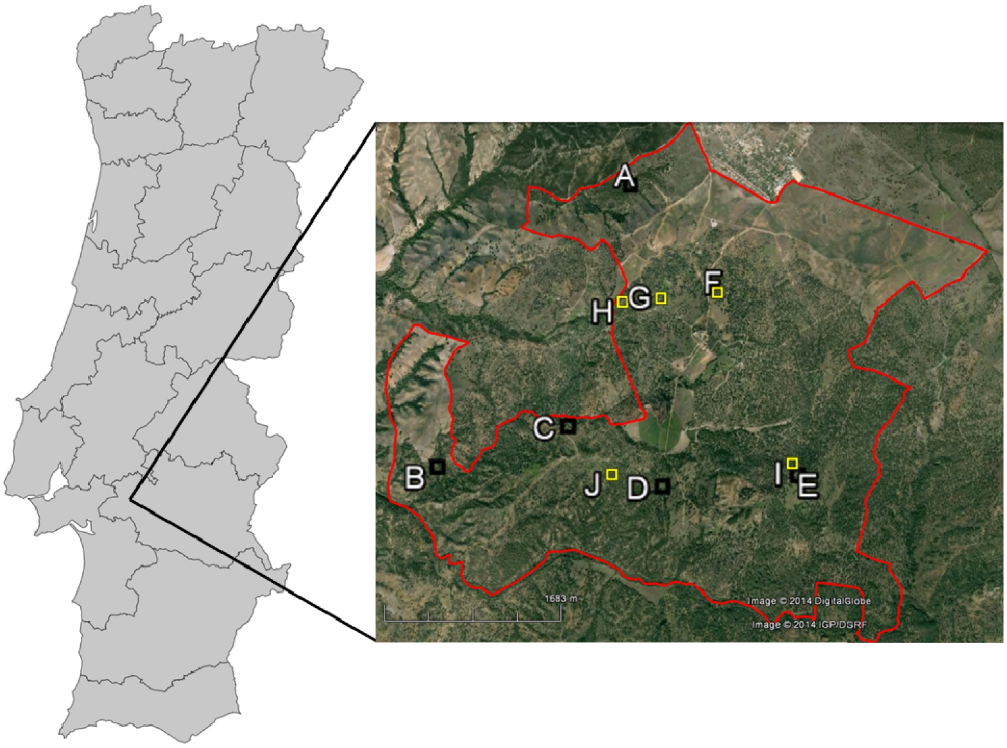

Figure 1 – Location of the study site showing sampling areas (black squares: A to E were sampled in 2013 and 2014 whereas yellow squares: F to J were sample in 2014) and the limits of the Herdade do Freixo do Meio (red line).

This area, mainly composed by cork oak and holm oak *montado*, has typically Mediterranean climate with a mean annual temperature being 16.6º C (Gouveia & Freitas 2008) and 22º C in summer months (Belo et al. 2009). Rainfall in typical *montado* mainly occurs during the winter and summer months are usually dry. Mean rainfall values varies between 300 mm and over 600 mm (Belo et al. 2009; Gouveia & Freitas 2008). These rainfall values influence tree cover in the *montado*, with lower rainfall favouring holm oak and higher rainfall values favouring cork oak (Belo et al. 2009).

2.2 – Study design

Nest-boxes were used to access nestlings of the three study species (Blue tit, Great tit and Nuthatch). Nest-boxes were made of wood with 10 x 7 x 6 cm, and entrance hole in the front with 25mm diameter approximately. During the autumn of 2012, 100 nest-boxes were placed in 5 different areas (A to E) separated approximately by 1 - 3 Km, to collect diet samples in the spring of 2013. In each area there were 20 nest-boxes placed at every 50 m, in 2 lines separated 50 m from each other. Therefore, we had 2 lines of 10 nest-boxes in each area, each nest-box separated from the next by 50 meters. In the autumn of 2013, another 100 nest-boxes were similarly placed in other 5 areas (F to J) in order to collect diet samples in spring of 2014.

Figure 2 - Nest-box placed on a Holm oak tree (*Q. rotundifolia*).

2.3 – Nestlings' Diet

Between April and June of 2013 and 2014, every nest-box was visited regularly to register developments in nest-building, egg-laying and hatching. Nestlings were ringed and the ligature method was used to collect diet samples in nestlings older than 8-9 days. The ligature method consists in positioning a cotton coated wire in the throat of nestlings, tight so birds can breathe but food cannot be swallowed (Almeida 1996;

Johnson et al. 1980; Marques et al. 2003; Mellot & Woods 1980; Robinson et al. 2010). The ligature was left in the nestling for approximately one hour before it was removed and diet samples collected when present. Diet samples are referred to as a bolus, or a single "meal" for a nestling. Nest-boxes were visited only twice a day to avoid unnecessary disturbance, first to put the ligature and later to remove it and collect samples. Diet samples were preserved in ethanol 70% until posterior identification (Marques et al. 2003).

Figure 3 - Diet sampling with ligature-method technique used on a nestling of Great tit.

Diet samples were identified in the laboratory, using a binocular loupe with 60x magnifying glass, to family level whenever possible using insect identifying guides (Borror & DM 1964; Chu 1949; Thyssen 2010; Viejo & Romera 2004), both for mature and immature forms. Items were measured using graph paper and total biomass of each arthropod group in the diet was calculated using the length (mm) of each item. Mathematical formulas were used as described in Ganihar (1997), for Araneae and Dermaptera, and Sample et al. (1993), for the other arthropods [i.e., Insecta (adults), Hemiptera (adults), Coleoptera (adults), Carabidae (adults), Lepidoptera (adults and larvae), Noctuidae (larvae), Geometridae (larvae), Diptera (adults), Nematocera (adults), Cyclorrapha (adults) and Hymenoptera (adults); Note that Nematocera and Cyclorrapha include the families Tipulidae and Calliphoridae, respectively]. All equations are presented in appendix (IV). For some groups present in diet, for which the use of a length-weight regression equation was not applicable, such as Annelida, Gastropoda and Diplopoda, biomass was estimated by calculating the mean biomass of every item in the diet of the three species, for each year. In the case of items of Vegetable matter and Eggs, six items of each were weighted and the mean value for these was applied for every item of these groups, for both years. Non-identified items were measured whenever possible and the equation for Insecta was used whenever applicable.

2.4 – Stable Isotopes Analysis

After identification, diet samples were maintained in 70% ethanol for stable isotope analysis of prey. Simultaneously, nestlings' feathers were used to measure stable isotopic values. Note that SIA was only performed with diet and feathers collected in 2013. In each nest-box, two tertiary feathers were collected from three nestlings a few days before fledgling. Feathers were stored in zipped plastic bags until further treatment.

Figure 4 - Feather collection on a nesting of Nuthatch for further SIA (Stable Isotope Analysis).

After the evaporation of ethanol, diet samples were placed in eppendorfs, separated by families, to initiate their preparation. All eppendorfs were filled with the 2:1 chloroform:methanol solution to clean and dilapidate the samples. The samples were stirred, centrifuged and bathed in ultra-sounds and afterwards the remaining solution in the eppendorfs was removed. This procedure was repeated until the solution removed was clean, meaning all the lipids were removed from the samples. After cleaning and dilapidation, diet samples were dried inside an aspirating hood for 24 hours at 50ºC and then grounded to dust and small pieces and stored in eppendorfs. Note that the whole arthropod was used in this step. The feathers were washed in successive bathes in a 2:1 chloroform:methanol solution to remove all impurities and dirt, then samples were wrapped in tin foil and placed inside an aspirating hood for 24 hours at 50 ºC. The dry samples were afterwards cut in small pieces and stored in eppendorfs.

Dusted diet samples and cut feathers were weighted to 0.25-0.40 mg and encapsulated in small tin cups. All instruments and working bench were cleaned with ethanol after every encapsulated sample to prevent contaminations. Isotope ratios of carbon and nitrogen were determined using a standard procedure by continuous-flow isotope ratio mass spectrometry, utilizing an EA-IRMS (Isoprime, Micromass, UK) at IMAR – Institute of Marine Research, Coimbra.

2.5 – Data analysis

Our sampling unit was the biomass of each order and family per bollus of chick (logarithmic transformed for statistical analysis). We compared the mean bolus biomass per chick between years, species and interaction only for the most important families of the diet in both years. Regarding the presence of families in the diets, only the most relevant families in the diet of the three bird species were used. The following families were selected: 2013 – Carabidae (Coleoptera), Crambidae, Geometridae and Noctuidae (Lepidoptera) and Forficulidae (Dermaptera); 2014 – Andreniidae and

Formicidae (Hymenoptera), Carabidae (Coleoptera), Crambidae, Lasiocampidae, Noctuidae, Notodontidae (Lepidoptera), Forficulidae (Dermaptera) and Tettigoniidae (Orthoptera). These families were selected because in each year, the total biomass of each in the diets was superior than the total biomass of other families. It was also counted the total biomass of non-identified Coleoptera and Lepidoptera. With the data of families present in the diets we have done a graphic representation of the percentage of biomass of each order over the total biomass collected for each bird species.

We performed Generalized Linear Models (GLM) with a Type III sum of squares and Poisson distribution for Araneae, Coleoptera, Diptera, Gastropoda and Lepidoptera, using Species and Year as fixed factors, in order to assess differences in the consumption of these prey types among the three bird species and between years. The analysis was performed with the Statistica 7 software (Statsoft, 2004).

A Principal Component Analysis (PCA) was performed on the diet of the three bird species, using the total biomass of the following groups for each bird species in each year: Araneae, Coleoptera, Hemiptera, Hymenoptera, Orthoptera, Lepidoptera and “Others”. The category “Others” grouped all the other items present in small quantities in the diet of each species. We assessed the importance of each prey group to explain the PCA axes and used the scores in the first, second and third PCA components in a factorial ANOVA, followed by post hoc Tukey test, to evaluate the effects of Species, Year and their interaction on the PCA scores (which represent a composite index of the diet). The analysis was performed with the Statistica 7 software (Statsoft, 2004).

A MANOVA was used to evaluate whether both isotope values differed among the three species, using Species as a dependent variable and $\delta^{13}C$ and $\delta^{15}N$ as independent variables, followed by a factorial ANOVA to evaluate whether each isotope value, $\delta^{13}C$ and $\delta^{15}N$ differed among species. The analysis was performed with the Statistica 7 software (Statsoft, 2004).

To estimate contribution of each insect group to the diet of each bird species during the breeding period of 2013 we used a Bayesian multi-source stable isotope mixing model (Stable Isotope Analyses in R, SIAR; Parnell et al., 2010). This model allows to estimate the distributions probability of multiple sources of prey to a mixture of predator's diet taking into account the variability in source and mixture isotopic signatures, dietary isotopic fractionation and elemental concentration (Polito et al. 2011). In this study, SIAR was used to estimate diet of nestlings using both isotope values ($\delta^{13}C$ and $\delta^{15}N$). In the model we used the isotopic values of insect families, of two orders (Araneae and Lepidoptera) and one insect pest species (*Lymantria dispar*) present in the diet. The isotopic values used for Lepidoptera were the mean $\delta^{13}C$ and $\delta^{15}N$ values obtained for all Lepidoptera families, and the values used for Araneae were the mean $\delta^{13}C$ and $\delta^{15}N$ values of 10 individuals. Thus, the following families were analyzed in SIA: Geometridae, Noctuidae, Tettigoniidae and Lymantriidae for Great tit; Crambidae, Noctuidae, Tischeriidae and Lymantriidae for Blue tit; and Carabidae, Forficulidae and Lymantriidae for Nuthatch. These items used in the model comprised more than 5% of the diet. We used a trophic enrichment from arthropods to birds of 0.75 and 2.75 ‰, and a standard deviation of 0.10 and 0.11‰, respectively for carbon and nitrogen (Caut et al. 2009).

To analyse stable isotope data in the context of isotopic niche between species, we used the recent metrics based in a Bayesian framework (Stable Isotope Bayesian Ellipses in R: SIBER; Jackson et al. 2011). The standard ellipse area corrected for small sample sizes (SEAc, an ellipse that has 40% probability of containing a subsequently sampled datum) was used to infer about isotopic niche of the three species and compare them among the three bird species (Ceia et al. 2014; Mancini et al. 2014). The SIA was also used to establish the isotopic niche of the three bird species by applying a metric called SIBER (Stable Isotope Bayesian Ellipses in R), which allows for a robust statistical analysis (Jackson et al. 2011). A SEACc (Standard Ellipse Area) adjusted for small samples. Also, a Bayesian estimate of standard ellipse and its area (SEA_B) was used to compare niche size among species, i.e., p, the proportion of ellipses of species 1 that is smaller than species 2 using 10^4 replicates (Jackson et al. 2011).

Both SIAR and SIBER results were analyzed in R software version 3.0.3, using respectively the SIAR model package (Parnell & Jackson 2013) and the SIBER package (Jackson et al. 2011).

3 – Results

3.1 – Nestlings' Diet

A total of 346 bolus corresponding to nestlings' diet of the three bird species were analysed (Table I). Information on the number of items and total biomass per order and family for the three bird species, both in 2013 and 2014 is presented in Appendix I and Appendix II.

Table I – Summary of diet sampling data.

Species	No. of nest-boxes sampled	No. of nestlings sampled	No. of bolus collected
Blue tit			
2013	14	19	39
2014	15	17	27
Great tit			
2013	14	36	65
2014	17	39	57
Nuthatch			
2013	4	14	83
2014	20	44	75

We compared the number of items and biomass (mg) per bolus x chick^{-1} among the three species and between 2013 and 2014. In both years, Nuthatch fed more bolus to the nestlings than the other two species, and the mean number of items per bolus was also higher for Nuthatch in both years (Table II). However, the biomass (mg) per bolus chick^{-1} did not differ among species ($p=0.54$) and between years ($p=0.93$).

Table II – Total number of items present in the diet of Blue tit, Great tit and Nuthatch, and estimated biomass per bolus sampled in the breeding seasons of 2013 and 2014.

Species	Total No. items	Mean No. items / bolus	Mean biomass / bolus (mg)	Total biomass (mg)
Blue tit				
2013	69	1.8	48.1	1876.1
2014	90	3.3	121.7	3285.3
Great tit				
2013	95	1.5	162.8	10583.7
2014	88	1.5	99.9	5694.9
Nuthatch				
2013	321	3.9	139.4	11568.9
2014	295	3.9	188.1	14107,0

In 2013 (Figure 5), the order Araneae clearly dominated the diet of all species in terms of biomass (Blue tit - 43.70%; Great tit - 75.74%; Nuthatch - 53.93%), followed by Lepidoptera (Blue tit - 39.18%; Great tit - 13.65%; Nuthatch - 23.99%) and Diptera for Blue tit (3.08%), Orthoptera for Great tit (6.96%) and Coleoptera for Nuthatch (8.42%).

Similarly to 2013, in 2014 (Figure 6) the most representative order in the diet of the three species was Araneae (Blue tit - 63.24%; Great tit - 46.53%; Nuthatch - 71.41%), followed by Lepidoptera (Blue tit - 24.83%; Great tit - 29.30%; Nuthatch - 12.60%) and Dermaptera for Blue tit (3.73%), Orthoptera for Great tit (9.34%) and Coleoptera for Nuthatch (7.75%).

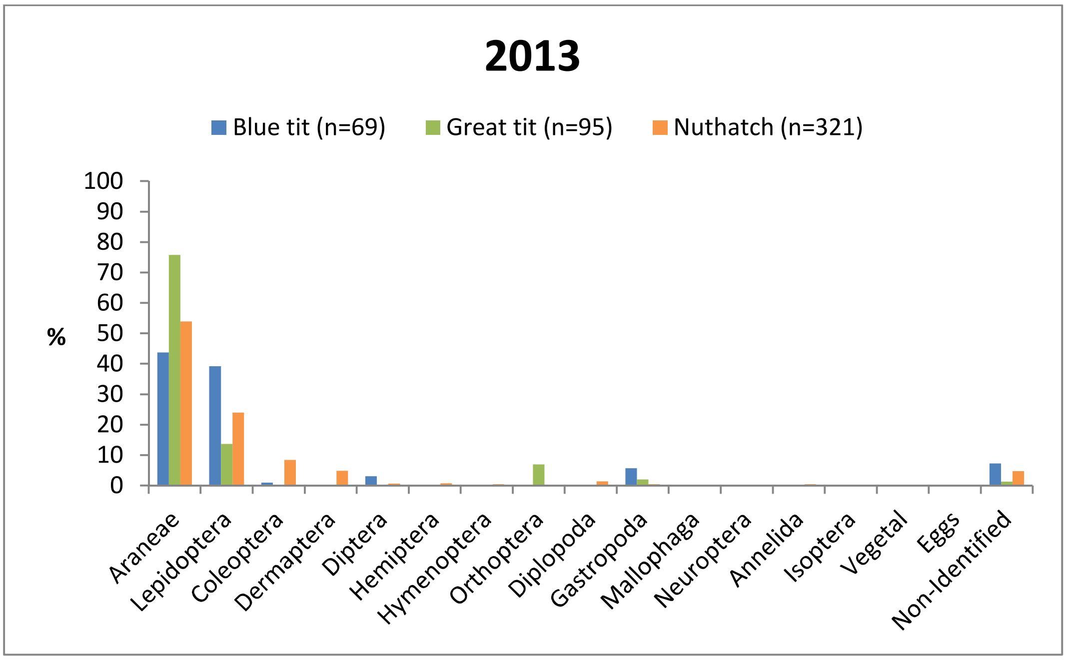

Figure 5 – Comparison of the diet of the three bird species in 2013 in terms of % of biomass of each order in relation to total biomass ingested by each bird species. Numbers in brackets represent the number of bolus collected for each bird species.

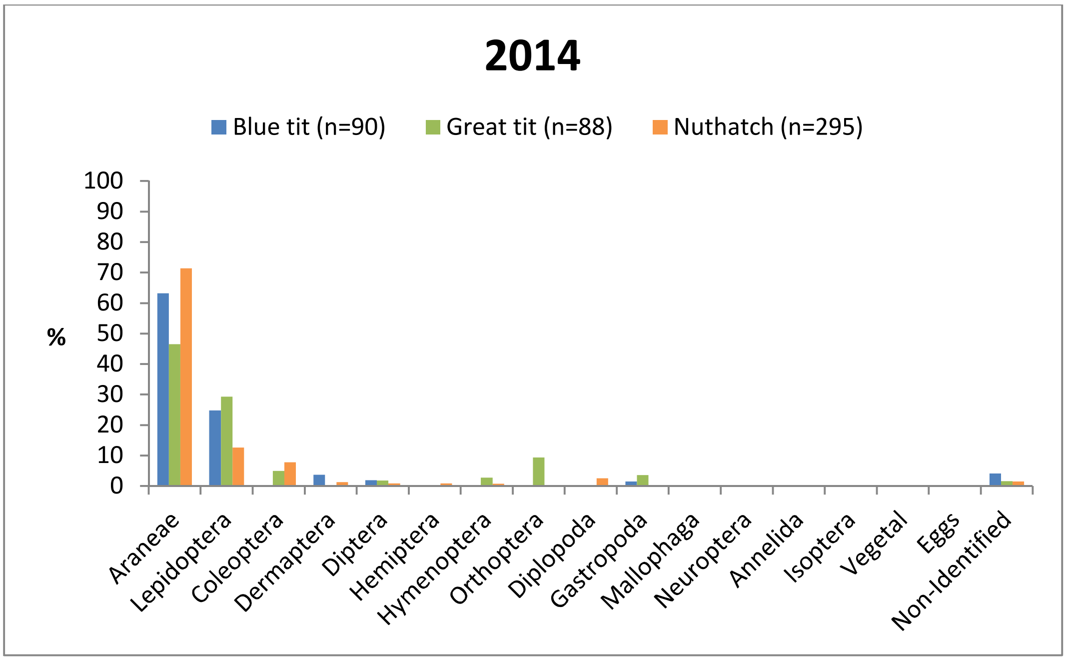

Figure 6 – Comparison of the diet of the three bird species in 2014 in terms of % of biomass of each order in relation to total biomass ingested by each bird species. Numbers in brackets represent the number of bolus collected for each bird species.

Comparing both years, Araneae was more important in the diets of Blue tit and Nuthatch in 2014 and less important in Great tit, whereas Lepidoptera and Coleoptera were more important in 2014 for Great tit and less important in Blue tit and Nuthatch. Some orders such as Dermaptera, Diptera and Hemiptera were more important for Blue tit, Great tit and Nuthatch, respectively. Dermaptera, which in 2013 was only identified in the diet of Nuthatch (4.80%), in 2014 was also present in the diet of Blue tit (3.73%) as well as in the diet of Nuthatch (1.28%). Diptera identified in the diet of the three species in 2013 (Blue tit - 3.08%; Great tit – 0.12%; Nuthatch – 0.59%) were less important in the diet of Blue tit in 2014 (1.97%) and more important in the diets of Great tit (1.78%) and Nuthatch (0.89%) in 2014. As for Hemiptera, it was more important for all species in 2014, increasing its presence in 2014 (Blue tit – 0.31%, Great tit – 0.08%, Nuthatch – 0.87%) when comparing with 2013 (Blue tit and Great tit – 0%, Nuthatch – 0.72%). Non-identified individuals maintained a similar presence in the diet of 2013 (Blue tit – 7.29%; Great tit – 1.29%; Nuthatch – 4.73%) and 2014 (Blue tit – 4.08%, Great tit – 1.63%; Nuthatch – 1.52%).

In 2013, the family Noctuidae was the most predated by all species (Figure 7). Nuthatch shows a more diverse diet compared to Blue tit and Great tit, as this species preyed mainly on Noctuidae (0.078%), Forficulidae (0.048%) and Carabidae (0.043%) while Blue tit preyed more on Noctuidae (0.233%), Crambidae (0.026%) and Geometridae (0.007%), which are all Lepidoptera families. Great tit fed mainly on Noctuidae (0.043%), Crambidae (0.007%) and Geometridae (0.009%), also all Lepidoptera families. Non-identified Coleoptera represented 0.01% and 0.04% of the diet of Blue tit and Nuthatch, respectively. Non-identified Lepidoptera represented 0.10%, 0.06% and 0.13% of the diet of Blue tit, Great tit and Nuthatch, respectively,

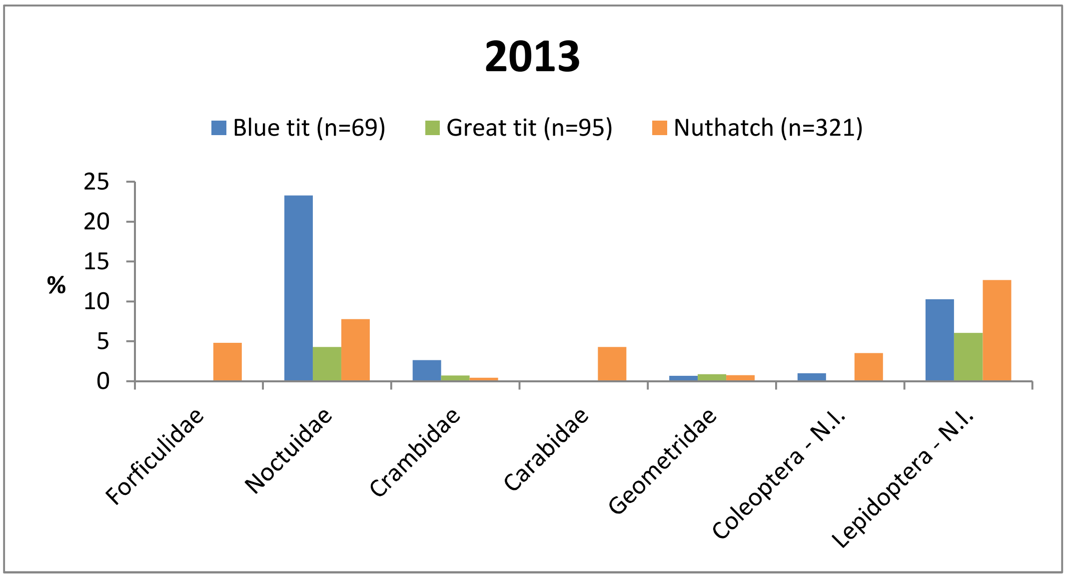

Figure 7 – Comparison of the diet of the three bird species in 2013 in terms of % of biomass of each family in relation to total biomass ingested by each bird species. Numbers in brackets represent the number of bolus collected for each bird species.

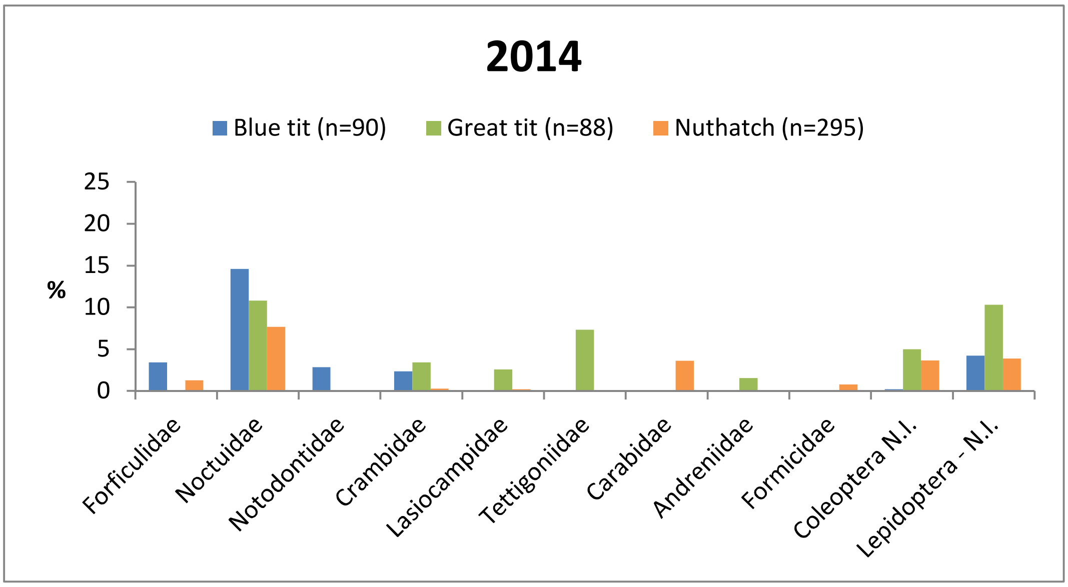

Figure 8 - Comparison of the diet of the three bird species in 2014 in terms of % of biomass of each family in relation to total biomass ingested by each bird species. Numbers in brackets represent the number of bolus collected for each bird species.

In 2014 (Figure 8), the family Noctuidae was the most important in the diet of all three bird species (Blue tit – 14.6%; Great tit – 10.8%; Nuthatch – 7.7%). Blue tits, apart from Noctuidae, preyed more on Forficulidae (3.4%) and Notodontidae (2.8%). Great tit, besides Noctuidae, have a higher presence of Crambidae (3.4%) and Tettigoniidae (7.3%). As for the Nuthatch, after Noctuidae, the most important families in its diet were Carabidae (3.6%) and Forficulidae (1.3%). Non-identified items of Coleoptera comprised 1.92%, 4.9% and 3.6% of Blue tit, Great tit and Nuthatch diet while non-identified Lepidoptera represented 4.2%, 10.3% and 3.9% of their diet, respectively.

Regarding insect pest species, in 2013 there were 7 individuals of *Lymantria dispar* (178.32 mg) in the diet of Nuthatch and 1 individual (31.51 mg) in the diet of Great tit. Other insect pest species were not identified in the diet samples in 2013 or in 2014. However, several items of families containing potential pest species were identified in 2013 and in 2014. Individuals of the families Curculionidae, Formicidae, Lymantriidae, Noctuidae, and Notodontidae were identified in 2013 diets while individuals of the families Cerambycidae, Curculionidae, Formicidae, Lasiocampidae, Noctuidae, Noctuidae and Tenthredinidae were identified in 2014 diets. These data was used to see the percentage of potential pest species individuals in the diet of each bird species (Figure 9).

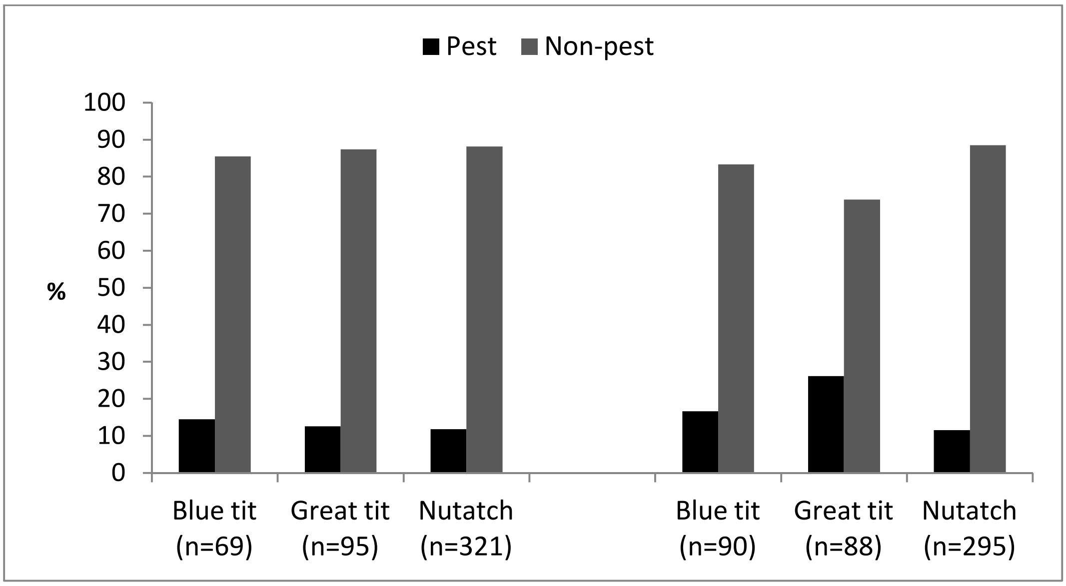

Figure 9 – Comparison of insect pest species and Non-pest species contribution to the diet of each bird species on 2013 (Left) and 2014 (Right), represented in % of biomass of potential pest species and non-pest species in relation to total biomass ingested by each bird species. Numbers in brackets represent the number of bolus collected for each bird species.

In 2013, the predation on individuals of potential pest species was, for Blue tit and Great tit, lower than in 2014, (2013: Blue tit – 14.49%, Great tit – 12.63%, Nuthatch – 11.84%; 2014: Blue tit – 16.67%, Great tit – 26.14%, Nuthatch – 11.53%). Blue tit and Great tit, which fed mainly on Lepidoptera (Figures 5 and 6), are the bird species with more potential insect pest individuals on their diet. This is a result of feeding on Lepidoptera larvae, an abundant resource during the breeding season. All items identified to family level were then classified in “Potential pest species” or “Non-pest species” according to their life cycle and foraging preferences (Ferreira & Ferreira 1991).

A PCA reduced the original 211x8 matrix to three independent principal components that explained 24.1, 15.1 and 14.0 % of the variance, with eigenvalues of 1.93, 1.21 and 1.12, respectively (Figure 10). The first component was negatively correlated with Hemiptera (-0.693), Dermaptera (-0.690) and Hymenoptera (-0.547), the second component was positively correlated with Lepidoptera (0.666) and negatively correlated with Orthoptera (-0.697), and the third component was positively correlated with Araneae (0.718) (Table III).

Table III – PCA statistics and factor coordinates of each order present in the diet in the diet of Blue tit, Great tit and Nuthatch.

	PC1	PC2	PC3
Eigenvalues	1.93	1.21	1.12
% Variance explained	24.1	15.1	14.0
Cumulative %	24.1	39.2	53.3
Araneae	-0.301	0.174	0.718
Coleoptera	-0.443	0.310	0.316
Dermaptera	-0.690	0.163	-0.127
Hemiptera	-0.693	-0.169	-0.229
Hymenoptera	-0.547	-0.309	-0.443
Orthoptera	0.171	-0.697	0.090
Lepidoptera	0.200	0.666	-0.473
“Others”	-0.561	0.003	0.090

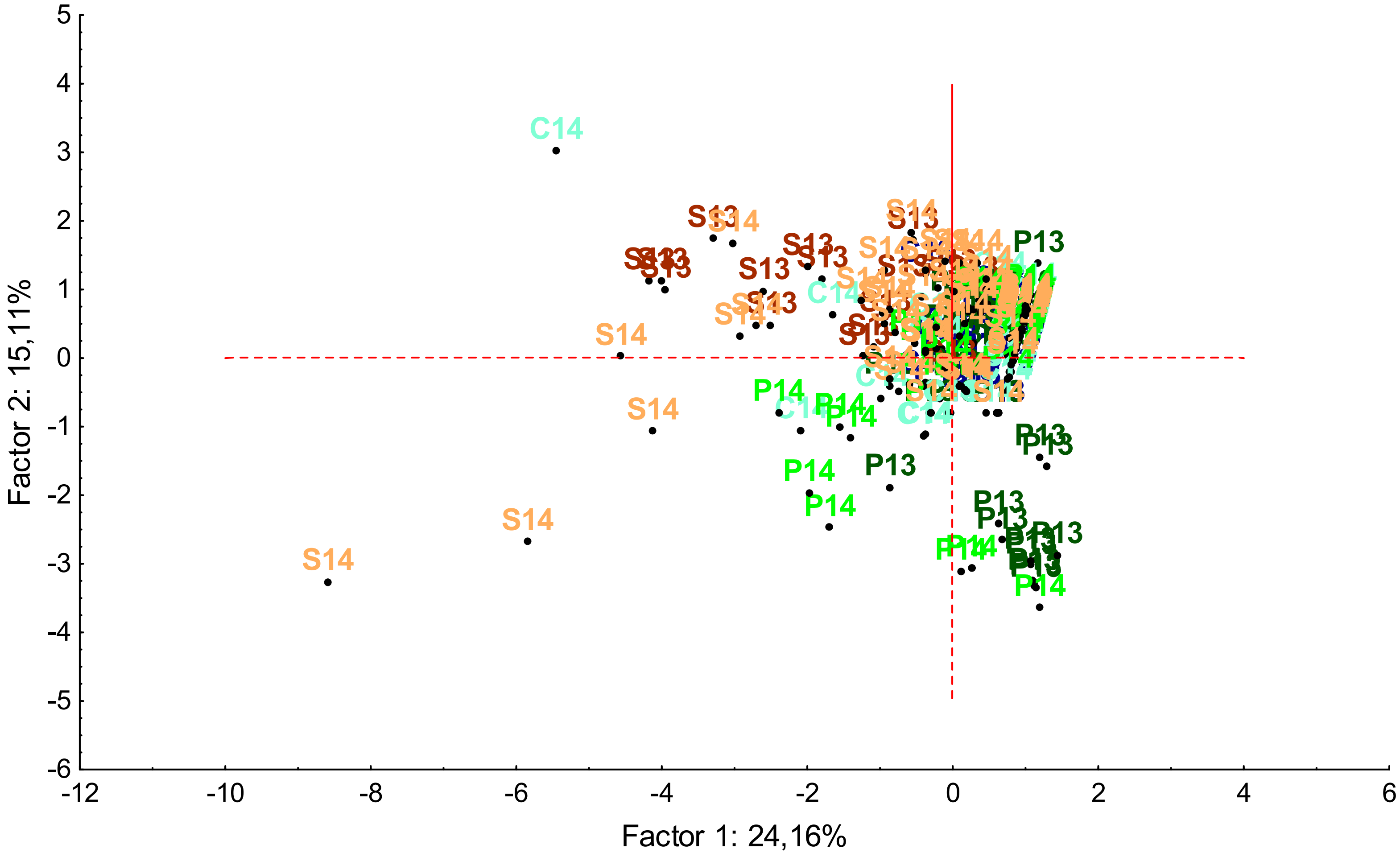

Figure 10 – Factor scores for nestlings sampled in 2013 and 2014, separated by bird species (C: Blue tit, P: Great tit, S: Nuthatch), in the two first factors extracted by a PCA using the log+1 – transformed biomass collected per nestling (i.e. total biomass divided by the number of bolus collected for each nestling).

The first factor separated Nuthatch from Blue tit and Great tit, while the second factor separated Nuthatch from Great tit.

The factorial ANOVA on the scores of the three principal components differed significantly among the three species and the interaction species*year for the first and second component (Table IV). No difference in the diet among years was revealed by the Factorial ANOVA.

Table IV – Factorial ANOVA results for comparison between Species, Year and interaction Species*Year for factor coordinates of each nestling in Components 1 to 3.

	PC1	PC2	PC3
Species	$F_{2,205}$=27.29, p<0.0001	$F_{2,205}$=13.69, p<0.0001	$F_{2,205}$=3.26, p=0.04
Year	$F_{1,205}$=0.59, p=0.442	$F_{1,205}$=0.14, p=0.70	$F_{1,205}$=2.07, p=0.15
Species*Year	$F_{2,205}$=7.21, p<0.0001	$F_{2,205}$=4.19, p=0.01	$F_{2,205}$=0.46, p=0.62

Post hoc Tukey tests showed that for the first component, Nuthatch differed significantly from Blue tit (p<0.001) and Great tit (p<0.001). According to post hoc Tukey test results, Nuthatch in 2014 differed from Great tit in 2013 (p<0.001) and 2014 (p=0.01), from Blue tit in 2013 (p<0.01) and Nuthatch in 2013 (p<0.01) but did no differed from Blue tit in 2014 (p=0.61). The second component, according to post hoc Tukey test, explained differences between Nuthatch and Great tit (p<0.0001) but not between Nuthatch and Blue tit (p>0.05), and also shows differences among species*year interaction: Nuthatch in 2013 differed significantly from Great tit in 2013 (p<0.001) and Great tit in 2014 (p<0.01) while Nuthatch in 2014 differed from Great tit in 2013 (p<0.001) The third component explains differences observed between Nuthatch and Great tit (p=0.04).

A Generalized Linear Model (GLM) was used to compare the importance of the following orders among species and between years: Araneae, Coleoptera, Diptera, Gastropoda and Lepidoptera. There was a clear effect of species for Araneae and Coleoptera and an almost significant difference for Diptera. The interaction species*year only had a significant effect for Coleoptera (Table V). The parameter estimates (β=-1.06 ± 0.514, p = 0.04) show that the Coleoptera consumption of Blue tit differed significantly from that of Nuthatch (the reference category in the model), meaning that Blue tit consumed less Coleoptera than Nuthatch. As for Diptera, the parameter estimates indicates a significant difference between Great tit and Nuthatch (β=-0.871 ± 0.436, p = 0.04), thus meaning that Great tit consumed less Diptera than Nuthatch. For Gastropoda and Lepidoptera, no significant differences were detected.

Table V - Comparison of contribution of each order to the diet of Blue tit, Great tit and Nuthatch and differences between species, year and interaction species*year.

		Df	X^2	P
Araneae	Species	2	12.247	0.002
	Year	1	0.850	0.356
	Species*Year	2	1.941	0.379
Coleoptera	Species	2	67.589	<0.001
	Year	1	0.714	0.398
	Species*Year	2	8.260	0.016
Diptera	Species	2	5.461	0.065
	Year	1	0.303	0.582
	Species*Year	2	1.020	0.600
Gastropoda	Species	2	2.602	0.272
	Year	1	0.290	0.590
	Species*Year	2	0.229	0.892
Lepidoptera	Species	2	1.149	0.563
	Year	1	0.023	0.879
	Species*Year	2	1.926	0.382

3.2 – Stable Isotope Analysis

The feather isotopic signature of nestlings during the breeding season of 2013 differed between bird species (MANOVA, $F_{4,128}$=18.17, p<0.0001). An ANOVA for each stable isotope showed a significant difference among bird species for $\delta^{15}N$ ($F_{2,4.78}$=4.19, p=0.02) and also for $\delta^{13}C$ ($F_{2,15.44}$=41.28, p<0.0001). The post hoc Tukey test indicates differences in $\delta^{15}N$ between Nuthatch and Great tit (p=0.01) and also differences in $\delta^{13}C$ between Nuthatch and Great tit (p<0.001) and Blue tit and Great tit (p<0.001).

Results from the SIAR model suggest that for Blue tit, the family Noctuidae was not the main prey ingested, contrary to the results obtain in diet analysis (Figure 7), in which the main prey were Noctuidae, Crambidae and Geometridae. According to the SIAR model, the main prey of Blue tit in 2013, in terms of families, were Tischeriidae (mean=0.243), Crambidae (mean=0.134) and Noctuidae (mean=0.119). However, Araneae was the main prey of Blue tit in 2013 (mean=0.249). Although it was not identified in the diet, in 2013, *Lymantria dispar* was also included in the SIAR model and results show that this species does not contribute much to the diet of Blue tit (0.113) (Figure 11).

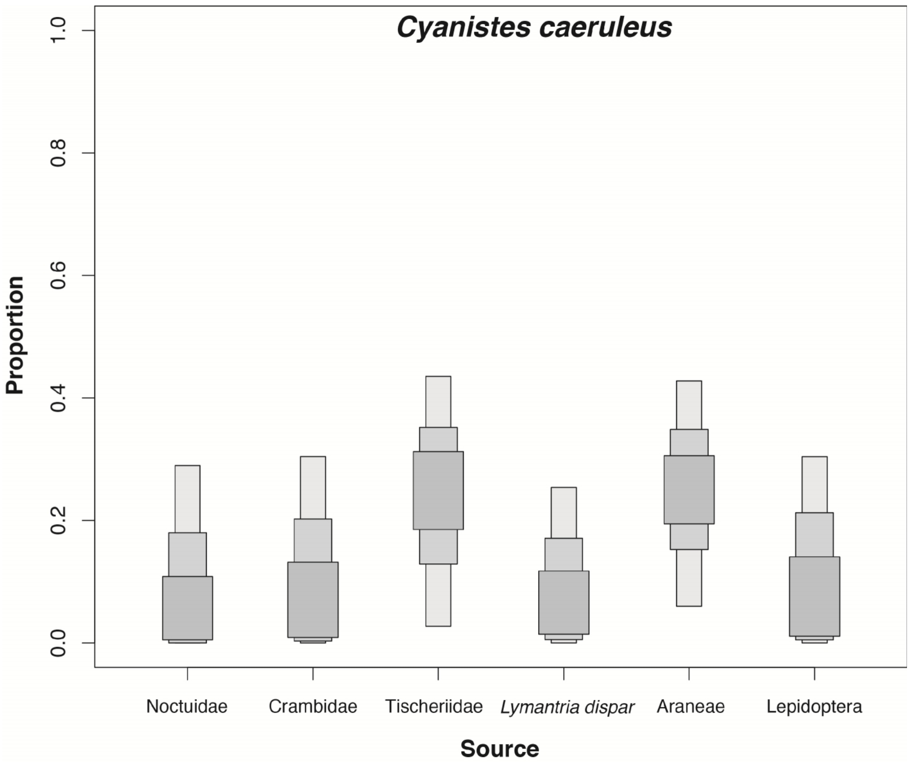

Figure 11 – Estimated proportion of the main families, Araneae and Lepidoptera and *L. dispar* in the diet of Blue tit, in 2013. The bars represent the 50, 75 and 95% confidence intervals given by SIAR.

Regarding Great tit, SIAR model, highlights the importance of the family Geometridae (mean=0.106) and *Lymantria dispar* (mean=0.116), over Noctuidae (mean=0.036) and Tettigoniidae (mean=0.089). However, Araneae dominated the diet of this species (mean=0.439), with Lepidoptera also having an important contribution for the diet (mean=0.212) (Figure 12). *L. dispar* gained importance with SIA, given that in diet analysis only one individual was identified, while in SIAR model this species contributes more to the diet than Noctuidae or Tettigoniidae (which were more important in diet).

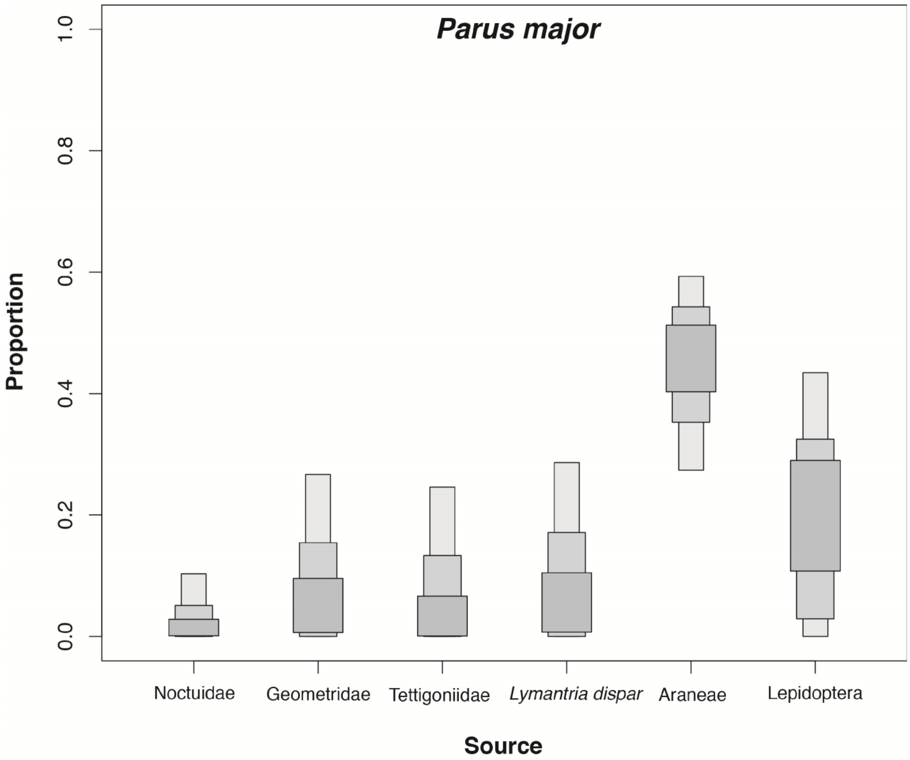

Figure 12 - Estimated proportion of the main families, Araneae and Lepidoptera and *L. dispar* in the diet of Great tit, in 2013. The bars represent the 50, 75 and 95% confidence intervals given by SIAR.

For Nuthatch, SIAR results clearly highlight three groups: Araneae (mean=0.264), *Lymantria dispar* (mean=0.260) and Forficulidae (mean=0.202). Carabidae, although identified in greater number in diet samples than *L. dispar*, according to SIAR, contributes less to the diet (mean=0.116) (Figure 13).

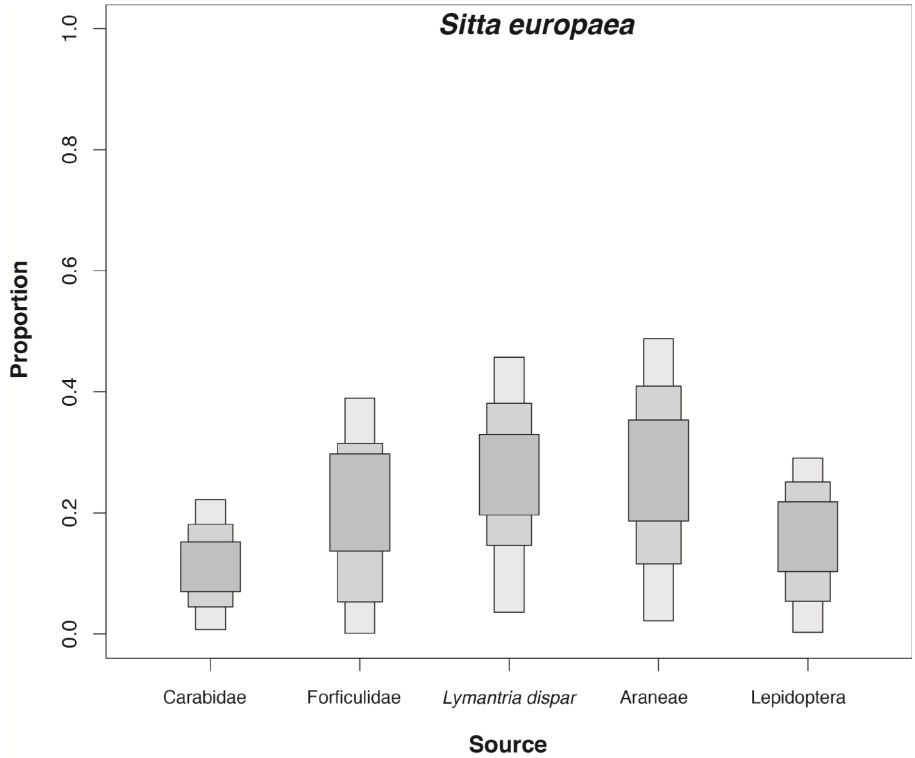

Figure 13 - Estimated proportion of the main families, Araneae and Lepidoptera and *L. dispar* in the diet of Nuthatch, in 2013. The bars represent the 50, 75 and 95% confidence intervals given by SIAR.

Results from Stable Isotopes Bayesian Ellipses (SIBER model) (Figure 14), show differences in the isotopic niche among the three bird species. Nuthatch was the species with the smallest isotopic niche (SEAc=0.553) compared to Blue tit (SEAc=1.637) and Great tit (SEAc=3.110). Nuthatch is the species with the smaller isotopic niche, with was expected by results of previous studies. Nuthatch has a significantly smaller isotopic niche than Great tit (SEA_B, p=0.001) and is also smaller, although not significantly, than Blue tit niche (SEA_B, p=0.07).

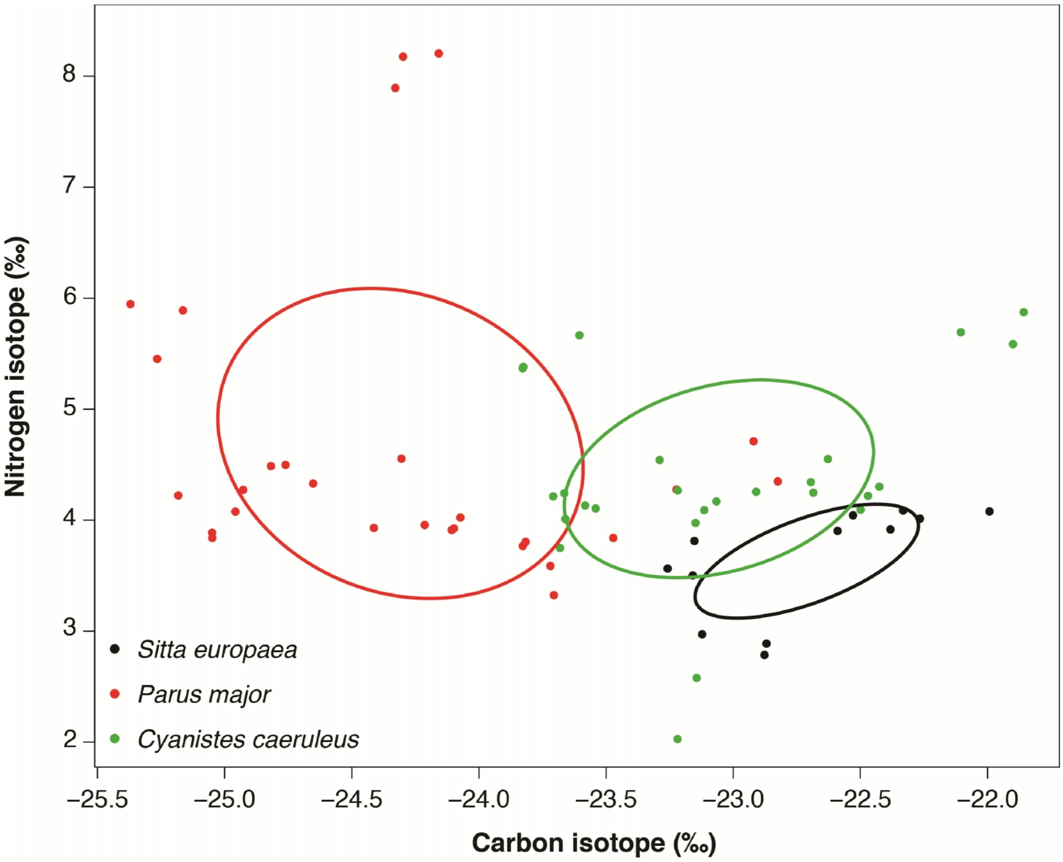

Figure 14 - Isotopic niche area of each bird species on stable isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) of nestling tertiary feathers.

4 – Discussion

4.1 – Diet and isotopic niche

The results obtained from both diet analysis and SIAR revealed distinct diets among the three insectivorous bird species, during the 2013 and 2014 breeding seasons. Both methods clearly showed a dietary segregation among Blue tit, Great tit and Nuthatch, particularly between Nuthatch and the other two species. This result was expected from previous studies on the diet of these three bird species and their foraging niche, which shows a clear segregation between species (Betts 1955; Cowie & Hinsley 1988; Matthysen 1999; Leal et al. 2012). Nuthatch forages mainly in bark while Blue tit and Great tit forage mainly on foliage and twigs, and also on the ground, in the case of Great tit (Diaz et al. 1998; Illera & Atienza 1995; Herrera 1978). More information about foraging niche showed differences in tree section used between bird species, in spring: Nuthatch mainly uses principal branches with diameter superior to 5 cm, Great tit mainly uses inner branches between 0.5 and 5 cm diameter while Blue tit mainly forages on leaves, twigs, flowers and acorns (Almeida & Granadeiro 2000). Generally, all species had a high presence of Araneae in their diet, in accordance to SIAR results, although its biomass ingested differed among species. Number of items per bollus was higher for Nuthatch, both in 2013 and 2014, relatively to Blue tit and Great tit, possibly by the fact that Great tits usually prey on only one relatively large individual at a time (Naef-Danzer et al. 2000). This result in conformity with a study realized by Neaf-Danzer et al. (2000), which showed that up to 75% of the biomass fed to nestling by Great tits, in a mixed deciduous forest, was Araneae. A study by Obeso (1985) showed that the main prey, in terms of percentage of items, in the diet of Nuthatch were Coleoptera (79.2%) followed by Araneae (21,9%). Despite the opposite result obtained in our study, the measure used in analysis was biomass per bolus chick^{-1}, possibly explaining differences in the percentage of each order in the diet of the bird species. Again, for Blue tit, this result was in concordance with a previous study by Pulido & Diaz (1990), which showed Araneae as the major prey for both young and adult birds. Also for Coleoptera there were differences in their consumption by Blue tit and Nuthatch, with Nuthatch consuming more Coleoptera than Blue tit. Regarding Lepidoptera there were no differences in its consumption among bird species. Lepidoptera families were important in both the diet samples and the SIAR model. Lepidoptera are an important source of prey for all bird species during the breeding season, when energetic requirements are higher and immature insects are more abundant and less mobile, and several studies showed that they are main prey for many insectivorous bird species (Barbaro & Battisti 2011; Mols & Visser 2002; Illera & Atienza 1995). The Blue tit was, among the three bird species, the main predator of Lepidoptera, mainly of larval stages, followed by Great tit. Again, former studies on feeding ecology of these two species showed that during the breeding season, larval stages of Lepidoptera are the main prey of Great tit and Blue tit (Betts 1955; Cowie & Hinsley 1988). The fact that Orthoptera were mainly identified in diet of Great tit is explained by the foraging habits of this species, which forages more on the ground (where Orthoptera are more abundant) (Leal et al. 2012). Regarding Nuthatch, SIAR results are, partially, in accordance with results from diet samples collected with the ligature-method due to the importance of Araneae and Lepidoptera, including *L. dispar*, shown by both methods. Forficulidae, which were present in some of the nest-boxes in 2013, occupying the lid, is present in diet samples of Nuthatch only, but less than other families, such as Carabidae, and the SIAR results showed a considerable importance of Forficulidae in Nuthatches diet when compared with Carabidae.

Orders such as Diptera and Hymenoptera, also referred as prey of all the three bird species, were also identified in diet samples all bird species, although Hymenoptera were only identified in diet samples of Great tit and Nuthatch in 2013 but in the diet of all bird species diet in 2014. As for Hemiptera, which was only present in the diet of Nuthatch in 2013, was identified in the diet of all bird species in 2014. Reported main prey orders for nestlings of Nuthatch are, according to literature on *Quercus* spp. habitats, Lepidoptera, Coleoptera and Hemiptera (Cholewa & Wesołowski 2011). The fact that several orders were found only in one of the studied years, in the diet of some of the bird species, could be explained by seasonal variation in nestlings diet (Cholewa & Wesołowski 2011). Diet studies nestlings raised in nest-boxes, could influence the diet of nestlings due to the modification of habitat and potential increase in bird densities and inter- and intra-specific competition, although almost every study on this subject was carried out using nest-boxes (Cholewa & Wesołowski 2011). Nest-boxes are a reliable option to study diet of nestlings, allowing more control and consistency in sampling, which would not be possible using other method such as the use of mist-nets, which would lead to uncertainties in sampling of each bird species.

While diet samples only provide a snapshot of a birds diet in a small period of time, SIA allows us to infer about diet over larger periods of time. Animal tissues have different synthesis rates the isotopic composition of this tissues reflects the diet or habitat of animal during the time at which tissues are synthesized. Feathers, as other inert tissues, provide isotopic information about the time they are formed, during weeks or months (Inger & Bearhop 2008). The comparison between SIAR and diet results show differences of proportion of some families in the diet of the three insectivorous bird species, meaning that during feather growing period, diet samples provide a narrow idea of the general diet. Regarding family Noctuidae, with great presence in diet samples of every bird species, its proportion in SIAR are relatively low when comparing to other families which were less present in diet samples, such as Crambidae and Tischeriidae (in Blue tit), Geometridae or even *L. dispar* (in Great tit). As for Nuthatch, SIA clearly highlights *L. dispar* as a main prey, over families more present in the diet, such as Carabidae or group Lepidoptera. This highlights the importance of SIA as complementary method to study diet of terrestrial insectivorous birds.

Isotopic niche, according to SIBER results differed also among the three bird species as expected from previous studies on trophic niche of these species (Cramp et al. 1993; Diaz et al. 1998; Illera & Atienza 1995; Herrera 1978; Leal, Correia, et al. 2011). The Great tit is a more generalist insectivorous bird species than the Blue tit and the Nuthatch (Cowie & Hinsley 1988; Matthysen 1999; Stauss et al. 2005) explaining the results from SIBER which show a larger isotopic niche for Great tit than for the other two species. Of the three studied species, Nuthatch had a smaller isotopic niche, possibly explained by its higher specialization in trunk- and bark-foraging. A study on cork oak *montado* by Leal et al. (2011) showed that Nuthatch spent 91.2% of time foraging in bark and only 4.7% on foliage-foraging, much lesser than the time spent by Great tit and especially Blue tit on foliage-foraging (54.6% and 70.2%, respectively). Other study by Leal et al. (2012), conducted in a cork oak *montado* revealed that Nuthatch spent only 11% of time foraging in foliage branches while Great tit and Blue tit spent 66% and 88.5%, respectively. The fact that isotopic niche differ between all bird species may explain the different diet composition of each species, due to different foraging strategies and foraging areas of the tree.

Significant differences were found for both $\delta^{13}C$ and $\delta^{15}N$ in all of the three insectivorous bird species. Relatively to $\delta^{15}N$, differences were found between Nuthatch and Great tit, with the mean value of $\delta^{15}N$ superior for Great tit. $\delta^{15}N$ usually is an indicator of trophic level, appearing to be biomagnified across trophic levels (Bodey et al. 2013; Sabat et al. 2013). This could indicate that Nuthatch and Great tit are in different trophic levels. According to SIAR, Great tit preyed more on Araneae than Nuthatch, and as Araneae are predators of other arthropods, it is expected that their $\delta^{15}N$ value is superior than that of phytophagous arthropods, which should contribute to explain the higher trophic level of the Great tit. However, this result should be taken cautiously as the wide range of $\delta^{15}N$ values in Nuthatch and Great tit could arise from a great variation in $\delta^{15}N$ values in primary producers (Sabat et al. 2013). In fact, according to Sabat et al. (2013), birds that feed exclusively on insects have a wider range of $\delta^{15}N$ values than birds that feed almost exclusively on vegetal material. Also, arthropods found in the diet were likely to feed in a great variety of plants or to predate on other arthropods, certainly with a wider variety of $\delta^{15}N$ values than those of the prey of insectivorous birds, causing great variation in $\delta^{15}N$ values among birds, hence leading to differences between species. The variation found relatively to $\delta^{13}C$, can be explained due to differences in the basis of the food web, specifically plants. Plants can be divided into three different groups according to their photosynthetic pathway, or way of fixating atmospheric CO_2: C_3, C_4 and Crassulacean Acid Metabolism (CAM) (Fry et al. 1978; Hobson 1999; Kelly 2000). These different metabolism in plants, leads to different isotopic signatures of $\delta^{13}C$, also caused by different water-use efficiency in C_3 plants (Michener & Lajtha 2007). We found a significant difference in $\delta^{13}C$ between the three insectivorous bird species, possibly meaning that the diet of the three species have different plants as food sources and also phytophagous insects which feed on plants with different photosynthetic metabolism, or with different water-use efficiency. Fry et al. (1978) showed, with grasshoppers collected in the same area, different $\delta^{13}C$ values due to different feeding habits, on C_3 or C_4 plants. Thus, differences found on $\delta^{13}C$ values may be explained by different foraging behaviour of the prey present in the diet, such as Hemiptera or Orthoptera, which were found mainly in Nuthatch and Great tit diet, respectively. These data was supported by the PCA results, which showed a clear segregation by Nuthatch and Great tit by the first component and also a clear relationship between Hemiptera and the first component. As shown by Fry et al. (1978), the different species of these two orders, found on different proportions on the diets of Nuthatch and Great tit can explain the variability of $\delta^{13}C$ values. Woody plants, such as *Quercus* spp. present a C_3 photosynthetic pathway (Nelson et al. 2004). The most likely hypothesis for the variation found in $\delta^{13}C$ isotopic ratios between the studied bird species is the existence of a mixed C3/C4 grassland that is the basis of the food web in this area, explaining the variation of $\delta^{13}C$ in upper trophic levels (Aires et al. 2008; Hobson 1999). Herbaceous plants with C4 photosynthetic pathways are known to be well adapted to warm and dry climates, such as the Mediterranean area (Rao et al. 2012).

In conclusion, all bird species have their diet dominated by Lepidoptera and Araneae, enhancing the importance of this type of prey during the breeding period, when energetic needs are higher and prey items are more available. As for isotopic niche, all species differ in isotopic niche, in accordance to their foraging habits, also reflected in their diet.

4.2 – Insect pest species in the diet of insectivorous bird species

Comparing both years, only in 2013 it was possible to identify one insect pest species in the diet of Great tit and Nuthatch. Other individuals in the diet were identified to family level only and yet some of those individuals are potential pest species. In 2013, we found 7 individuals of *L. dispar* on the diet of Nuthatch and 1 in the diet of Great tit. The SIAR results suggest that *L. dispar* is more important in the diet of Nuthatch and Great tit than expected from the diet samples. These two bird species, which have different isotopic niches according to SIBER results, were the main predators of *L. dispar*. All of our three studied bird species are known to prey on insect pest species, including *L. dispar* (Higashiura 1980; Higashiura 1991; Mols & Visser 2002; Sanz 2001). *L. dispar* is a pest species, not only in Mediterranean habitats but also on other habitats, such as Japanese broad leaved natural forests and oak forests in southern United States (Furuta 1982; Schultz & Baldwin 1982). In Japanese broad leaved natural forest Nuthatches are known predators of *L. dispar*, causing high-mortality on this species, and maintaining them at low population levels (Furuta 1982). As for Blue tit and Great tit, they also preyed on insect pest species in both orchards and forests. In the breeding season, when insect pest species such as larvae of tortricid and winter moths, are more abundant, the number of these prey caught by birds was also higher. Great tits and also Blue tit can reduce damage caused by defoliator larvae in orchards and reduce insect abundance, between a range of densities, being ineffective when insect densities are either low or above a threshold (Mols & Visser 2002). These two species, also reduced caterpillar damage and densities in plot with nest-boxes within Pyrenean Oak forests, thus increasing bird density and reducing damage (Sanz 2001). The Great tit is also known to prey on other pest species, such as Processionary moth (*Thaumetopea pityocampa*), in Pine Forest in central Portugal, increasing in abundance in infested areas (Pimentel & Nilsson 2009). Other bird species, also present potential as predators of insect pest species, such as Pied Flycatcher (*Fycedula hipoleuca*) (Sanz 2001), Great Spotted Cuckoo (*Clamator glandarius*), Common Cuckoo (*Cuculus canorus*), European Nightjar (*Caprimulgus europaeus*), Crested tit (*Lophophanes cristatus*), Coal tit (*Periparus ater*) and European hoopoe (*Upupa epops*), this last species is a known predator of the Processionary moth (Barbaro & Battisti 2011).

Some items in the diet were not identified to family level (2013: Blue tit – 43.48%, Great tit – 38.95%, Nuthatch – 38.01%; 2014: Blue tit – 34.44%, Great tit – 39.77%, Nuthatch – 31.19%), excluding from this the orders Araneae and Annelida and vegetal and egg items. Most items were not identified to lower than order taxonomic levels due to damages suffered by bird predation. Relatively to Lepidoptera larvae, some bird species, have developed strategies to cope with hairy larvae with urticating setae, which consists in rupture head and thoracic segments of larvae to feed only of the viscera, removing hairs and setae (Barbaro & Battisti 2011). Some insect pest species of the *montado* are hairy with urticating setae, such as *L. dispar* and *Malacosoma neustria*, and this feeding strategy of birds undermines the hypothesis of identify larvae to lower taxonomic levels. Therefore, diet analyses probably underestimate the presence of Lepidoptera pest species in the diet of the birds. In fact, SIAR results suggest a strong presence of *L. dispar* in the diet of Nuthatch and Great tit.

The classification of insect families as Potential pests and Non-pests (Figure 9) indicate that Blue tit and Great tit are major predators of potential pest species. This is explained by feeding habits of individuals identified in the diets of the three bird species. According to our diet data, Blue tit is the major predator of Lepidoptera individuals, most of them in larval stage, in both years. In this classification, among other families, there is the family Noctuidae which was well represented in the diet of the three bird species, both in 2013 and 2014; and the families Lasiocampidae, Notodontidae (Lepidoptera) and Curculionidae and Cerambycidae (Coleoptera), identified in 2013 and 2014, but not in the diet of all the bird species, all listed by Ferreira & Ferreira as pest species of cork and holm oak (1991). These families are described by Moran & Southwood (1982) as phytophagous, with feeding habits such as defoliators, miners or gall-formers. The high level of predation of Noctuidae by all bird species, along with other defoliators such as Geometridae, Lasiocampidae and Notodontidae, reveals a potential of these bird species to control insect pest species, especially Blue tit and Great tit, which fed mainly in Lepidoptera larvae.

The results presented in this study suggest an important predation of insect pest species, mainly Lepidoptera, by Nuthatch, Blue tit and Great tit. These three bird species, in situation where numbers of larvae are in moderate densities, are likely to maintain population of insect pest species at low levels (Mols & Visser 2002).

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Appendix I: Diet composition, in biomass (mg), of each bird species by order and family, in 2013

Diet items	Blue tit (n=69)		Great tit (n=95)		Nuthatch (n=321)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Annelida	0	0.000	0	0.000	1	53.616
Araneae	8	819.956	10	8016.571	34	6239.049
Coleoptera	1	18.626	2	11.674	60	974.158
Carabidae	0	0.000	0	0.000	30	497.003
Coccinellidae	0	0.000	0	0.000	1	0.651
Curculionidae	0	0.000	0	0.000	8	46.835
Histeridae	0	0.000	2	11.674	3	11.728
Lathridiidae	0	0.000	0	0.000	2	10.051
Mycetophagidae	0	0.000	0	0.000	1	1.083
N.I.	1	18.626	0	0.000	15	406.807
Dermaptera	0	0.000	0	0.000	27	555.057
Forficulidae	0	0.000	0	0.000	27	555.057
Diptera	4	57.751	3	12.223	9	67.705
Asilidae	0	0.000	0	0.000	1	4.928
Calliphoridae	0	0.000	0	0.000	2	15.397
Phoridae	0	0.000	1	11.280	2	1.544
Tabanidae	0	0.000	0	0.000	1	10.503
Tipulidae	0	0.000	0	0.000	1	9.831
N.I.	4	57.751	2	0.942	2	25.501
Gastropoda	2	107.233	4	214.466	1	53.616
Hemiptera	0	0.000	0	0.000	32	83.227
Ciccadellidae	0	0.000	0	0.000	6	21.500
Miridae	0	0.000	0	0.000	5	10.678
Nabidae	0	0.000	0	0.000	2	8.317
Tingidae	0	0.000	0	0.000	6	6.903
N.I.	0	0.000	0	0.000	13	35.830

Appendix I (cont.): Diet composition, in biomass (mg), of each bird species by order and family, in 2013

Diet items	Blue tit (n=69)		Great tit (n=95)		Nuthatch (n=321)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Hymenoptera	0	0.000	1	10.216	16	51.594
Dryinidae	0	0.000	0	0.000	1	0.436
Formicidae	0	0.000	0	0.000	10	39.630
N.I.	0	0.000	1	10.216	5	11.528
Isoptera	0	0.000	0	0.000	1	1.471
Kalotermitidae	0	0.000	0	0.000	1	1.471
Diplopoda	0	0.000	0	0.000	3	160.849
Lepidoptera	35	735.002	41	1444.793	74	2775.309
Arctiidae	0	0.000	0	0.000	1	76.737
Carposinidae	2	7.198	0	0.000	0	0.000
Coleophoridae	1	1.140	0	0.000	0	0.000
Crambidae	7	49.362	4	77.795	5	51.410
Geometridae	2	13.013	8	93.931	5	86.948
Lasiocampidae	0	0.000	1	30.494	1	14.531
Lymantriidae	0	0.000	1	38.511	7	178.318
Lymantria dispar	0	0.000	1	38.511	7	178.318
Lyonetiidae	1	18.538	1	30.494	0	0.000
Noctuidae	10	437.097	9	454.105	12	899.403
Notodontidae	0	0.000	1	42.679	0	0.000
Pyralidae	0	0.000	1	11.670	0	0.000
Sphingidae	0	0.000	1	21.968	0	0.000
Tischeriidae	4	15.858	0	0.000	0	0.000
N.I.	8	192.796	14	643.145	43	1467.962

Appendix I (cont.): Diet composition, in biomass (mg), of each bird species by order and family, in 2013

Diet items	Blue tit (n=69)		Great tit (n=95)		Nuthatch (n=321)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Mallophaga	0	0.000	1	0.346	0	0.000
Neuroptera	0	0.000	0	0.000	1	2.618
Orthoptera	0	0.000	19	736.230	3	2.470
Acrididae	0	0.000	2	71.988	0	0.000
Tettigoniidae	0	0.000	11	519.917	0	0.000
Gryllidae	0	0.000	0	0.000	1	1.195
N.I.	0	0.000	6	144.325	2	1.275
Vegetal matter	4	0.258	0	0.000	21	1.355
Ootecs	6	0.613	5	0.511	1	0.102
N.I.	9	136.678	9	136.678	37	546.739
Total	69	1876.117	95	10583.708	321	11568.936

Appendix II: Diet composition, in biomass (mg), of each bird species by order and family, in 2014

Diet items	Blue tit (n=90)		Great tit (n=88)		Nuthatch (n=295)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Araneae	10	2077.749	8	2649.750	33	10074.453
Coleoptera	1	6.295	4	283.931	46	1093.231
Carabidae	0	0.000	0	0.000	14	509.498
Cerambycidae	0	0.000	0	0.000	2	38.263
Coccinellidae	0	0.000	0	0.000	1	2.041
Cucujidae	0	0.000	0	0.000	5	27.601
Curculionidae	0	0.000	0	0.000	1	0.601
Mordellidae	0	0.000	0	0.000	1	1.231
N.I.	1	6.295	4	283.931	22	513.996
Dermaptera	9	122.404	0	0.000	19	180.258
Forficulidae	9	122.404	0	0.000	19	180.258
Diptera	2	64.706	2	101.340	11	125.114
Asilidae	0	0.000	0	0.000	1	20.367
Phoridae	0	0.000	0	0.000	3	12.287
Tabanidae	0	0.000	0	0.000	1	3.685
Tachinidae	0	0.000	0	0.000	1	7.491
Tipulidae	1	14.345	0	0.000	4	69.671
N.I.	1	49.362	2	101.340	1	11.613
Gastropoda	1	50.850	4	203.399	1	50.850
Hemiptera	14	10.311	1	4.451	71	122.739
Aphididae	10	5.564	0	0.000	0	0.000
Cercopidae	0	0.000	1	4.451	0	0.000
Ciccadellidae	0	0.000	0	0.000	13	42.595
Miridae	1	1.784	0	0.000	52	70.332
N.I.	3	2.963	0	0.000	6	9.813

Appendix II (cont.): Diet composition, in biomass (mg), of each bird species by order and family, in 2014

Diet items	Blue tit (n=90)		Great tit (n=88)		Nuthatch (n=295)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Hymenoptera	1	3.152	7	158.700	6	108.740
Andrenidae	0	0.000	5	87.458	0	0.000
Formicidae	0	0.000	0	0.000	5	107.945
Tenthedinidae	0	0.000	1	41.698	0	0.000
N.I.	1	3.152	1	29.544	1	0.795
Diplopoda	0	0.000	0	0.000	7	355.948
Lepidoptera	37	815.584	44	1668.439	58	1776.890
Arctiidae	1	6.828	0	0.000	0	0.000
Crambidae	8	77.005	6	194.046	5	42.081
Geometridae	0	0.000	2	32.023	3	35.438
Incurvariidae	0	0.000	1	32.140	0	0.000
Lasiocampidae	0	0.000	3	147.436	1	26.159
Noctuidae	14	479.187	14	615.987	25	1081.277
Notodontidae	1	93.420	1	5.211	0	0.000
Pyralidae	0	0.000	2	54.749	1	43.192
Thyatiridae	1	16.762	0	0.000	0	0.000
Tischeriidae	1	3.455	0	0.000	0	0.000
N.I.	11	138.927	15	586.846	23	548.744

Appendix II (cont.): Diet composition, in biomass (mg), of each bird species by order and family, in 2014

Diet items	Blue tit (n=90)		Great tit (n=88)		Nuthatch (n=295)	
	No. items	Biomass (mg)	No. items	Biomass (mg)	No. items	Biomass (mg)
Neuroptera	0	0.000	0	0.000	1	3.167
Orthoptera	0	0.000	6	531.692	0	0.000
Tettigoniidae	0	0.000	4	418.036	0	0.000
N.I.	0	0.000	2	113.656	0	0.000
Vegetal matter	0	0.000	1	0.065	12	0.774
Ootecs	2	0.204	4	0.409	0	0.000
N.I.	13	134.028	7	92.772	30	214.849
Total	90	3285.284	88	5694.946	295	14107.013

Appendix III: Insect pest species of the *montado*

Target species: *Quercus* spp.		
Species	Order	Family
Attelabus nitens	Coleoptera	Attelabidae
Catacola nymphagoga	Lepidoptera	Noctuidae
Cerambyx cerdo	Coleoptera	Cerambycidae
Coeliodes ruber	Coleoptera	Curculionidae
Coroebus florentinus	Coleoptera	Buprestidae
Coroebus undatus	Coleoptera	Buprestidae
Curculio elephas	Coleoptera	Curculionidae
Euproctis chrysorrhoea	Lepidoptera	Lymantriidae
Gracilia minuta	Coleoptera	Cerambycidae
Lymantria dispar	Lepidoptera	Lymantriidae
Phalera bucephala	Lepidoptera	Notodontidae
Phymotofdes testaceus	Coleoptera	Cerambycidae
Rhynchaenus erythropus	Coleoptera	Curculionidae
Rhynchaenus irroratus	Coleoptera	Curculionidae
Tortrix viridana	Lepidoptera	Tortricidae
Xylerobus saxeseni	Coleoptera	Scolytidae
Zeuzera pyrina	Lepidoptera	Cossidae

Target species: *Quercus rotundifolia*		
Archips xylosteana	Lepidoptera	Tortricidae
Attelabus nitens	Coleoptera	Attelabidae
Coeliodes ruber	Coleoptera	Curcuionidae
Curculio elephas	Coleoptera	Curcuionidae
Euproctis chrysorrhoea	Lepidoptera	Lymantriidae
Lymantria dispar	Lepidoptera	Lymantriidae
Malacosoma neustria	Lepidoptera	Lasiocampidae
Periclista andrei	Hymenoptera	Tenthredinidae
Periclista dusmeti	Hymenoptera	Tenthredinidae
Phalera bucephala	Lepidoptera	Notodontidae
Platypus cylindrus	Coleoptera	Platypodidae
Polysidrus setifrons	Coleoptera	Curcuionidae

Appendix III (cont.): Insect pest species of the *montado*

Target species: *Quercus suber*		
Archips xylosteana	Lepidoptera	Tortricidae
Attelabus nitens	Coleoptera	Attelabidae
Catacola nymphagoga	Lepidoptera	Noctuidae
Cerambyx cerdo	Coleoptera	Cerambycidae
Coeliodes ruber	Coleoptera	Curculionidae
Coroebus florentinus	Coleoptera	Buprestidae
Coroebus undatus	Coleoptera	Buprestidae
Crematogaster scutellaris	Hymenoptera	Formicidae
Curculio elephas	Coleoptera	Curculionidae
Euproctis chrysorrhoea	Lepidoptera	Lymantriidae
Haltica ampelophaga	Coleoptera	Chrysomelidae
Lymantria dispar	Lepidoptera	Lymantriidae
Malacosoma neustria	Lepidoptera	Lasiocampidae
Periclista andrei	Hymenoptera	Tenthredinidae
Periclista dusmeti	Hymenoptera	Tenthredinidae
Phalera bucephala	Lepidoptera	Notodontidae
Platypus cylindrus	Coleoptera	Platypodidae
Polydrosus setifrons	Coleoptera	Curculionidae
Prinibius scutellaris	Coleoptera	Cerambycidae
Rhynchaenus erythropus	Coleoptera	Curculionidae
Rhynchaenus irroratus	Coleoptera	Curculionidae
Tortrix viridana	Lepidoptera	Tortricidae

Appendix IV: Length-regression equations used to estimate biomass of arthropods

Group	Equation	a/b_0	b/b_1	Reference
Araneae	weight = b_0+ $(\text{length})^{b1}$	-3.2105	2.4681	Ganihar 1997
Dermaptera	weight = b0+ $(e)^{b1.(\text{length})}$	-0.4524	0.2037	Ganihar 1997
Insecta (Adult)	In(weight)=In(a) + b In(length)	2.494	-3.628	Sample *et al*. 1993
Hemiptera (Adult)	In(weight)=In(a) + b In(length)	3.075	-4.784	Sample *et al*. 1993
Ciccadellidae	In(weight)=In(a) + b In(length)	2.561	-3.735	Sample *et al*. 1993
Cercopidae	In(weight)=In(a) + b In(length)	1.583	-1.470	Sample *et al*. 1993
Miridae	In(weight)=In(a) + b In(length)	1.491	-2.257	Sample *et al*. 1993
Neuroptera	In(weight)=In(a) + b In(length)	2.570	-4.483	Sample *et al*. 1993
Coleoptera (Adult)	In(weight)=In(a) + b In(length)	2.492	-3.247	Sample *et al*. 1993
Carabidae	In(weight)=In(a) + b In(length)	2.755	-3.724	Sample *et al*. 1993
Lepidoptera (Adult)	In(weight)=In(a) + b In(length)	3.122	-5.036	Sample *et al*. 1993
Lepidoptera (Larvae)	In(weight)=In(a) + b In(length)	2.959	-5.909	Sample *et al*. 1993
Noctuidae (Larvae)	In(weight)=In(a) + b In(length)	2.845	-5.424	Sample *et al*. 1993
Geometridae (Larvae)	In(weight)=In(a) + b In(length)	2.625	-5.493	Sample *et al*. 1993
Arctiidae (Adult)	In(weight)=In(a) + b In(length)	2.658	-3.755	Sample *et al*. 1993
Notodontidae (Larvae)	In(weight)=In(a) + b In(length)	3.263	-6.688	Sample *et al*. 1993
Diptera (Adult)	In(weight)=In(a) + b In(length)	2.213	-3.184	Sample *et al*. 1993
Nematocera	In(weight)=In(a) + b In(length)	2.212	-3.675	Sample *et al*. 1993
Cyclorrapha	In(weight)=In(a) + b In(length)	2.632	-3.619	Sample *et al*. 1993
Hymenoptera (Adult)	In(weight)=In(a) + b In(length)	2.696	-4.284	Sample *et al*. 1993
Formicidae	In(weight)=In(a) + b In(length)	2.919	-4.727	Sample *et al*. 1993