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AN EVALUATION OF THE USE OF A SPOONED SPATULA TO ASSESS THE DIET OF CARDUELINE NESTLINGS

José. A. GIL-DELGADO* [1] and Daniel GUIJARRO*

SUMMARY.—*An evaluation of the use of a spooned spatula to asses the diet of cardueline nestlings.*

Aims: Sampling diet of passerine nestlings can be associated with bias and danger to the birds, according the methods adopted, but an understanding of diet is essential to development of conservation measures. This paper describes a new technique for examining the diet of cardueline finches that store food in gullet pouches prior to digestion. We applied this method to nestlings of goldfinches *Carduelis carduelis* of 31 nests. Goldfinch nestlings were selected because the diet of their chicks is poorly known.

Location: Our study was conducted near Sagunto in eastern Spain (39º 42' N, 0º 15' W). The area was located within extensive orange monocultures that are widely spraying in the east of Spain.

Methods: A small spoon was used to obtain the food directly from the gullets. Samples were obtained from 3 - 10 day old nestlings and samples from nestlings in the same nest were combined. Animal prey seeds were separated. Seeds of the same species were put together. We dried each sample at 103 ºC for two hours to assess dry weight. After drying, each food type from a nest was weighed. In order to test the method effect on the nestlings we compared the breeding success between the sampled nests and another 31 nests that were not manipulated and hatching close to the sampled nests.

Results: Thistles of two species, *Sonchus oleraceus* and *Carduus pycnocephalus* were the main food and the contributions to the diet varied across the breeding season. The two species of thistles showed opposite trends. The contribution of animal foods in the diet decreases as the season progresses. Daily survival rates and the average number of fledglings per nest were similar in both groups of nests suggesting that the use of a spoon to analyse the diet have no impact on the nestlings.

Conclusions: The main value of this method is that food samples are obtained before swallowed and thus the food is easy to recognize. This is an inexpensive method because the spoon tool can be used repeatedly.

Key words: diet analysis, goldfinch, gullet, nestling, spoon-spatule, food stored.

RESUMEN.—*Una evaluación de la utilización de una cucharilla para determinar la dieta en los carduelinos.*

Objetivos: El muestreo de la dieta en los pollos de los paseriformes esta frecuentemente sesgado al tiempo que puede representar un peligro para los jóvenes. No obstante, conocer la dieta de los mismos es necesario para desarrollar medidas de conservación. En este estudio describimos un nuevo método que nos permite examinar la dieta de los pollos de los carduelinos, aprovechando la capacidad que estas especies tienen para almacenar el alimento en sus buches antes de tragárselo. El método lo probamos con pollos de 31 nidos de jilgueros *Carduelis carduelis*. El Jilguero fue elegido por estar poco conocida la dieta de los pollos en esta especie.

* Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad deValencia, Apartado Oficial 2085, Valencia, E-46071 Spain.

[1] Corresponding author: gild@uv.es

Localidad: El estudio se realizó en las inmediaciones de Sagunto en el Este de España (39º 42' N, 0º 15' W). El área es un naranjal que se inserta en las grandes superficies de estos cultivos del Este de España.

Métodos: Utilizamos una cucharilla para extraer el alimento directamente de sus buches, procedimiento que facilita el reconocimiento de los diferentes tipos de alimento al recolectarse antes de ser tragado. Para conocer el efecto sobre el desarrollo de los pollos a los que les extrajimos alimento comparamos la supervivencia de estos pollos con los correspondientes a otra serie de 31 nidos que se desarrollaron en períodos muy próximos a los nidos manipulados.

Resultados: El principal alimento son dos especies de cardos, *Sonchus oleraceus* y *Carduus pycnocephalus*, y cuya contribución varia en el curso de la estación de nidificación. Las dos especies de cardos muestran tendencias opuestas. La contribución del alimento de origen animal decrece según avanza la estación de nidificación. Las tasas diarias de supervivencia y el número medio de volantones fueron similares en los dos grupos de nidos.

Conclusiones: La similitud en la supervivencia entre los pollos a los que se les extrajo el alimento de sus buches y aquellos que no fueron tratados sugiere que el uso de la cucharilla no afecta negativamente al desarrollo de los pollos en los carduelinos. El método utilizado es barato pues la herramienta aplicada puede utilizarse repetidamente. El método utilizado analiza el alimento antes de ser tragado lo que facilita su identificación. Además, permite tratar la alimentación usando directamente los pesos de cada tipo de alimento.

Palabras clave: análisis de la dieta, jilguero, pollos, cucharilla-espátula, extracción de alimento, almacenamiento, buche, semillas.

Introduction

Information about food habits is an important introduction to the natural history of any species. Litvaitis (2000) evaluated the different techniques used to investigate food habits in vertebrates and he pointed out their advantages and disadvantages, including its expensive or inexpensive cost. Techniques that allow the diet studies on birds have also been described (Sutherland, 2004), including those applied on birds which stored the food in their gullets (Newton, 1967; Poulin *et al.*, 1994; Poulin and Lefevre, 1996; Valera *et al.*, 1999).

The use of post-ingestion samples such as pellets or feces presents some disadvantages (Litvaitis, 2000). For birds, determining diet entails many problems. For example, differences in digestibility may create bias because different food types can be over or under represented depending on the method used (Johnson *et al.,* 1980; Jenni *et al.,* 1990; Moreby and Stoate, 2000). Obtaining food samples before the items enter the digestive tract could eliminate such bias. The ligature method collects the sample before the swallowing of food although does not evict in disturbing to nestlings because must be applied during a one or more hours (Johnson *et al*., 1980; Barba and Gil-Delgado, 1990).

Here, we present a method for studying the diet of nestling goldfinches *Carduelis carduelis* that should also be suitable for use with other species when food is stored in the crop. We chose to apply the method in goldfinches instead of other species that also stored the food in the crops, because the nestling diet in this species has been little studied (Cramp and Perrins, 1994). Recently, it has been widely reported throughout Western Europe that bird populations of some species which inhabit in farmland present a decline associated to agricultural intensification (Fuller *et al*., 1995; Wilson *et al*., 1997; Siriwardena *et al*., 1998, 2001; Chamberlain *et al*., 1999; Gil-Delgado *et al*., 2002). The Goldfinch is a cardueline species that shows preference for orchards, parks, gardens and farmlands (Newton, 1972; Cramp and Perrins, 1994). In our study area, an orange plantation, this species was rare until 1985. Then, the breeding population increased (Gil-Delgado *et al*., 1991).

Material and methods

Study area

Our study was conducted near Sagunto in eastern Spain (39º 42' N, 0º 15' W). The area was located within extensive orange monocultures that are widely spraying in the east of Spain (Zaragoza, 1988). More information about the study area can be found in previous reports (*e.g.*, Gil-Delgado and Lacort, 1996; Ponz *et al*., 1996; Gil-Delgado *et al.*, 2002, 2005). Since 1975, mapping methods and nest searching techniques have been applied. Every year, nest searching started in the first week of March and went on until early August (Gil-Delgado and Lacort, 1996, Gil-Delgado *et al.*, 2002, 2005). Trees were revised every 7 - 12 days.

Food sampling and nestling manipulation

In order to obtain each sample five steps were followed, i) nestlings were removed and then put in a small cloth bag; ii) each nestling was handled with care according to the standard method, known as the ringer's grip (Gosler, 2004); iii) the food from the crop was obtained using a small spoon (Fig. 1); iv) the nestling was returned to its nest and v) the food extracted was kept in blotting paper. This protocol was done by two observers and it was repeated for each sibling.

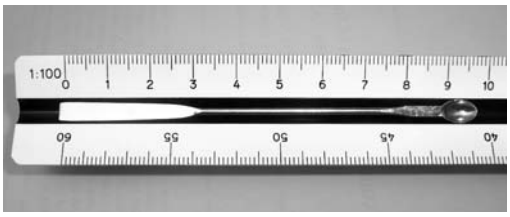

Fig. 1.—Spooned spatula tool used in the food extraction. Length: 10 cm.
[Imagen de la cucharilla de 10 cm de longitud empleada para extraer el alimento de los buches.]

During the breeding season of 1998 and 1999, we obtained 126 food samples from 108 nestlings (31 nests). All nests were located in a widely described plot of almost 17 ha (Gil-Delgado *et al*., 2002, 2005). Samples were obtained from 3 - 10 day old nestlings and samples from nestlings in the same nest were combined. Food samples were obtained directly from crops using a small spooned spatula: the dimensions of the spoon are 8.2 (length) x 6.5 (width) x 2 (depth) mm. It is a metallic tool with a spoon on one end and a spatula on the other. This tool is commercially available (Fig. 1), and more detailed information can be found on the following web page: http//www.pobel.com (reference ESCP 0150).

We extracted a spoonful of food from the crop of each nestling, and the contents were then allowed to dry. Double extractions, 18, were obtained from 8 - 10 days old nestlings, but only when gullets were plentiful. In any case, using simple or double extractions we are getting fewer 20 % of plentiful gullets. We then separated the contents according to food type. Animal prey, mainly aphids (Homoptera, Aphidae) and Coleoptera larvae, and seeds were separated. Seeds of the same species were put together. We dried each sample at 103 ºC for two hours (APHA, AWVA, WEF, 1992) to assess their dry weight. After drying, each food type from a nest was weighed. The dry weight varied between 2.63 and 81.9 mg of dry weight per nest. However, there were different ranges in April (2.63 - 23.83 mg), May (6.11 - 81.95 mg) and June (5.55 - 22.98 mg).

Effects of the method on the nestlings

To test possible effects of nestling manipulation on survival we compared breeding success between manipulated (n = 31) and non-manipulated nests (n = 31 nests). Manipulated nests were those nests where food samples were taken from the nestlings. Non-manipulated nests presented similar hatching dates (less than

5 days of difference and same year) to nests from which we took food samples. Comparisons were made considering as the first day the day when nestlings were three days old. To compare the daily survival rates between manipulated and non-manipulated nestlings, we used the Mayfield method (Mayfield, 1975). We give the standard error of the Mayfield estimator (Johnson, 1979).

Data analysis

Due to the differences in dry weight obtained from each nest, we standardised the weight data. To achieve this, we adjusted the weights for each sample using the smallest sample size (2.63 mg) as a reference. For instance, the nest with the smallest sample size presented three food types: *Sonchus oleraceus* (2 mg), grass (0.56 mg) and aphids (0.07 mg); and the nest with higher sample size with the same food types presented 79.12 mg, 2.56 mg and 0.27 mg, respectively. The standardised weight data in the higher sample size presented the next new weight: 2.54, 0.08, 0.01 mg, respectively. Thus, each nest presents the same contribution. After this reduction, the frequency of every type found in the 31 nests was determined. We then measured the contribution, in dry weight, of the different food types to the nestling's diet. Both seed and animal items were identified in laboratory using a binocular magnifying glass.

We fitted least-squared quadratic regressions to the data on the dry weight of each seed species to describe seasonal trends. We used quadratic equations because these are the simplest polynomials that could describe the seasonal changes, including the curvilinear rise and fall of each seed species. We also used regression analysis to associate with breeding season progression (Braak and Looman, 1995). For this analysis, we used the standardised weight values, varying between 0 and 2.63 mg of dry weight. The independent variable was the sampling date from 6 April and 30 June (day 6 - 91; 1 = 1 April). This ordination of dates has been widely used (Crick *et al*., 1993, Gil-Delgado *et al*., 2005). Statistical analyses were done using SPSS/PC+ statistical package (SPSS Inc., 1998).

RESULTS

Ten species of seeds were found in the crops of goldfinches, with two, sow-thistle *Sonchus oleraceus* and thistle *Carduus pycnocephalus* being most common. The other eight species were present only during part of the breeding season and in few nests (Table 1). In fact, four species were sampled in only one nest and another four species of seeds were only found over a period of 30 days. Food of animal origin and green leaves of grass were found in the samples during the three months of the study (Table 1).

To analyze the variation in the diet throughout the season we considered five types of food: i) sow-thistle *S. oleraceus*, ii) *C. pycnocephalus*, iii) all other seeds, iv) green leaves of grass and v) animal items (aphids and Coleoptera larvae).

The amount of sow thistle found in crops decreased between April and May and then increased until the end of the breeding season ($Y_{S.\ oleraceus} = 2.736 - 0.064X_{Date} + 0.001X^2_{Date}$; $R^2 = 0.249$; $F_{28} = 4.63$, $P = 0.018$; Fig. 2a). The other thistle showed an opposite trend, increasing its contribution until the middle of breeding season and then decreasing until disappearance in June ($Y_{C.\ pycnocephalus} = -0.846 + 0.072X_{Date} - 0.001X^2_{Date}$; $R^2 = 0.244$, $F_{28} = 4.52$, $P = 0.02$; Fig. 2b). The contribution of animal foods in the diet decreases as the season progresses, and a linear regression was the best fit for the data ($Y_{Animal} = 0.335 - 0.004X_{Date}$, $R^2 = 0.152$, $F_{29} = 5.22$; $P = 0.03$; Fig. 2c).

For the other food types no obvious temporal trends were found (Grass leaves: $R^2 = 0.023$, $F_{28} = 0.33$, $P > 0.05$; Other seeds: $R^2 = 0.06$, $F_{28} = 0.08$; $P > 0.05$).

TABLE 1

Food types of goldfinch nestlings. The first column shows the ten seed-producing plant species, grass and arthropod matter. Second and third columns show the percentages of each food type in relation to the number of nests sampled (%), but also in relation to the biomass in dry weight (BDW). Total BDW = 31 x 2.63 mg. The fourth column gives the dates of first (D1) and last (D2) day when each food type was found. *[Tipos de alimento en los pollos de jilguero. La primera columna muestra las especies de plantas cuyas semillas forman parte de la dieta, los tallos y los tipos de artrópodos. La segunda y tercera columna muestra los porcentajes de cada tipo de alimento en relación al número de nidos muestreados (%), y en relación con la biomasa en peso seco (BDW). El BDW total = 31 x 2,63 mg. La columna cuarta muestra el primer (D1) y último (D2) día de la estación reproductora en que encontramos cada tipo de alimento.]*

Food Type	**%**	**BDW (%)**	**D1/D2**
Sonchus oleraceus	96.8	61.7	6-April / 30-June
Carduus pycnocephalus	38.7	20.4	6-April / 2-June
Poa annua	9.7	< 1.0	18-April / 6-May
Erodium cicutarium	16.1	1.8	6-April / 9-May
Alopecurus myosuroides	9.7	2	20-April / 2-June
Setaria spp.	19.4	2.3	3-June / 28-June
Sonchus asper	3.2	< 1.0	4-June
Parietaria judaica	3.2	< 1.0	18-April
Papaver dubium	3.2	< 1.0	7-May
Amaranthus spp	3.2	< 1.0	17-June
Grass leaves	58.1	3.8	15-April / 5-June
Aphids	67.7	3.5	6-April / 30-June
Coleoptera larvae	22.6	2.6	15-April / 9-May
TOTAL	31	81.53 mg	

Effect on nestling survival

The first manipulations were on three days old nestlings, as this day was used as 0. The number of three days old nestlings in manipulated (3.6, SD = 1.1; n = 82 nestlings (23 nests)) and un-manipulated (3.8, SD = 0.8; n = 95 nestlings of 25 nests) nests no present significant differences ($t_{46} = 0.7$, $P > 0.05$). The number of fledglings of manipulated (2.9, SD = 0.9) and non- manipulated nests (3.1, SD = 1.1) did not differ ($t_{46} = 0.64$, $P > 0.05$). Nests that failed by cutting and predation were not considered in these analyses. Thus, the manipulated and non-manipulated nests presented losses of 0.7 nestlings between 3 - 12 days old.

The daily survival rates was similar between manipulated nests (0.963 < 0.975 < 0.987) and non-manipulated ones (0.966 < 0.977 < 0.9877).

DISCUSSION

Direct sampling of food from the crops of nestlings permits direct comparison of the relative importance of seeds and animal food in the diet of goldfinches. Another advantage is that direct extraction facilitates the identification of different seeds. In addition, the use of a spoon-spatula tool may present less danger to birds than other techniques, such as emetics (see Poulin and Lefevre, 1995; Barba and Mon-

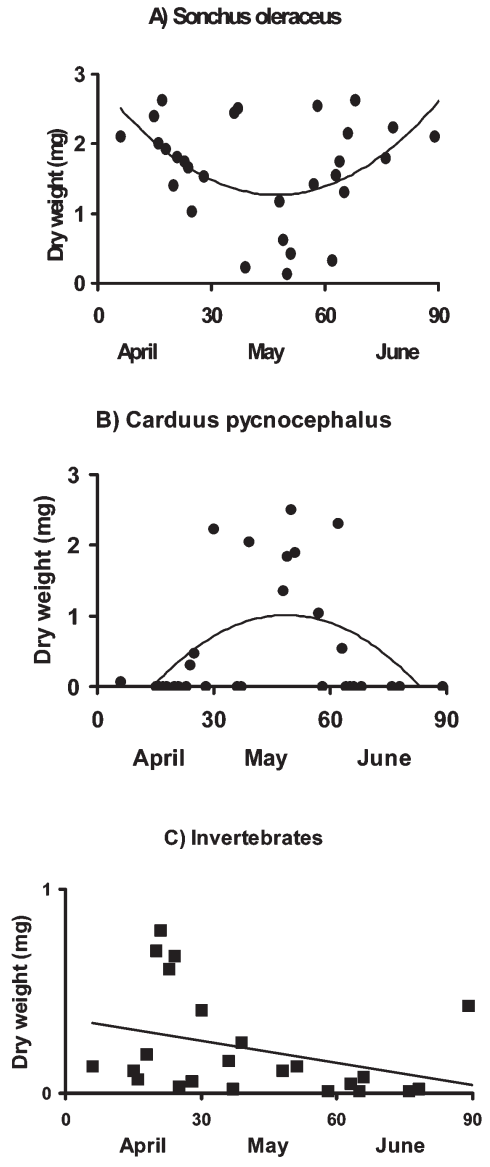

FIG. 2.—Scatterplots showing temporal change in prevalence crops of goldfinch nestlings of three food types. A) *Sonchus oleraceus*; B) *Carduus pycnocephalus*; C) Invertebrates. Curves were fitted using quadratic (A, B) or linear (C) regressions. *[Variación de tres tipos de alimento durante la temporada reproductora. A)* Sonchus oleraceus*; B)* Carduus pycnocephalus*; C) Invertebrados. Los mejores ajustes fueron obtenidos mediante ecuaciones cuadráticas (A, B), y regresión lineal (C).]*

rós, 1999; Johnson *et al*., 2002; Duraes and Marini, 2003). Indeed, our results show that daily survival rates of sampled nestlings were not different from those unmanipulated.

Among different techniques used to analyse the diet of birds, some such as the use of stomach contents can be used when birds were found dead because shooting birds to determine the diet is considered unacceptable (Sutherland, 2004). Other techniques need additional material such as nest cameras (Cowie and Hinsley, 1988), but analyzing films can be tedious and previous observations are needed to see whether the camera affects the behaviour of the birds (Sutherland, 2004**).** Emetics and stomach flushing do not usually kill but these methods are considered too invasive and with emetics some birds died (Poulin *et al*., 1994**).** Furthermore, three days old nestlings were too small to emetic supply.

Dropping and faecal analysis present hard remains and the identification presents more difficulties than stomach contents and stomach flushing, although the low level of intrusion makes dropping useful. However, dropping and faecal analysis present a problem in relation to the different digestibility between food types (Tigar and Osborne, 2000). Further, it limits in quantifying the importance of each type of food (Litviatis, 2000; Donald *et al.*, 2001).

Our technique applied on plentiful gullet need not the collar that is used in ligature method. Furthermore, the food quantification is more exact than in the content identification of the translucent skin of the neck (Newton, 1967; Glück, 1985), and allows us to measure the biomass of different food types directly. Thus, samples obtained with the spoon could be used to calculate the energy intake (Robel *et al*., 1995).

The main foods given to goldfinch nestlings were seeds of two thistle species, *S. oleraceus* and *C. pycnocephalus*. They showed opposite trends in use by goldfinches with the presence of *S. oleraceus* decreasing from April to May

and then increasing until the end of breeding season, and *C. pycnocephalus* increasing from April to May and then decreasing in the last third of the season. The availability of both thistles was not monitored, but the higher contribution of *C. pycnocephalus* during May might suggest that this thistle preferred when available**.** The preference by certain seeds plant species in Sagunto agrees with Newton (1967). During breeding, the goldfinch uses seeds of many plant species and Newton (1967) pointed out that the seasonal changes in the diet could not be attributed to changes in relative abundance of the different food plants.

Glück (1985) pointed out that, in Germany, goldfinch parents preferred not to feed their chicks with *S. oleraceus*, although available. He suggested that other species were selected because of their higher energy contents. *S. oleraceus* presents a low energetic content but it has higher water content (> 70 %) than other seeds consumed (Glück, 1985). In England nestlings of linnets *Carduelis cannabina* consumed seeds of *S. oleraceus* (Moorcroft *et al.*, 1997). In Germany, Glück (1985) pointed out that *S. oleraceus* and other seeds of plants with high water content were consumed outside the breeding season.

In the Mediterranean region, birds may face problems such as hyperthermia and risk of dehydration. In our study area, the breeding season of goldfinches lasts from mid-March to July (Gil-Delgado *et al.*, 1991), with mean July temperatures close to 30 ºC (Gil-Delgado *et al.* 2002). Therefore, there is a higher water requirement in eastern Spain than in Germany, which may explain why goldfinches in Sagunto preferred seeds rich in water content. The increase of *S. oleraceus* at the end of the season can be explained by higher water requirements as temperature increase, June was the warmest month, or by the lack of other thistle species.

Other eight seed species were obtained in a few nests, between 1 - 3 nests out of 31. Further, present usually lower values (< 5 %) of biomass. In Britain, Newton (1972) reported seeds of ten genus during breeding season. In Germany, the number of different seeds consumed by goldfinches was higher than Sagunto (Glück, 1985).

The amount of animal prey declined as the season progressed. This pattern was similar to that described by Newton (1972). It is not clear whether aphids and Coleoptera larvae were ingested incidentally with seeds and grass. The decline in animal food seems not to have any effect on nestling growth, given that the parents can rear their nestling in the final third of the breeding season without apparent difficulties (Gil-Delgado *et al.*, 1991). On the other hand, availability of animal food may decline as season progresses. This decline could be a consequence of the use of rotovating machines, insecticides and herbicides to eliminate the herbaceous plants in the orange groves.

Therefore, in orange groves of eastern Spain goldfinches fed their nestlings with seeds of a few plants. Two species, *S. oleraceus* and *C. pycnocephalus* presented a higher contribution (82 %).

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Jose A. Gil-Delgado is a Professor of Ecology in the University of Valencia but he is also a permanent research of the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia. Since 1975, he studies the breeding birds in a plot in an orange plantation of Sagunto. In this plot, breeding birds are monitored each year. He is currently working on breeding and population ecology of birds and recently also the mammals of orange groves. **Daniel Guijarro** was a student of Biology. Today works in Gandia (Valencia) but in jobs without relation in biology.