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Changes in eggshell thickness and ultrastructure in the Bearded Vulture (*Gypaetus barbatus*) Pyrenean population: A long-term analysis

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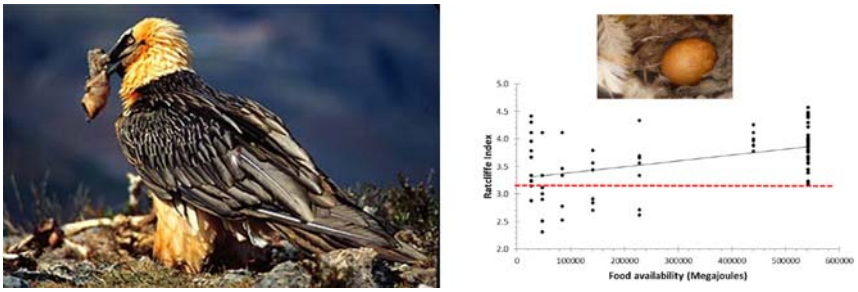
HIGHLIGHTS

- Shell thickness of Bearded Vulture eggs obtained from 1989 to 2012 was compared with eggs collected from 1858 to 1911.
- Bearded Vulture eggs exhibit significant variability in gross and ultrastructural biometry.
- The OC and PCB levels found in Bearded vulture eggs were lower than those considered critical for their survival.
- A decrease in the Ratcliffe Index and eggshell thickness was observed in eggs collected since 2001.
- Changes in egg and eggshell parameters seem to be the result of extrinsic factors as food availability and quality.

GRAPHICAL ABSTRACT



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ABSTRACT

The Spanish Bearded Vulture (*Gypaetus barbatus*) population has suffered from negative trends in a number of reproductive parameters that could jeopardize its long-term viability. From 1989 to 2012, 27 entire eggs and 63 eggshell fragments were collected from nests after breeding failure and/or fledging. Longer-term changes in eggshell thickness were made by examining 69 eggs collected in Spain from 1858 to 1911, and now held in European museums. Low levels of contamination with organochlorine pesticides and polychlorinated biphenyls were found in whole eggs and in conjunction with the high fertility rates observed in the field (66.7%) do not indicate a population suffering from the effects of organochlorine contamination. However, a decrease in the Ratcliffe Index and eggshell thickness were observed in eggs collected since 2001, increasingly so in samples post-2004, indicating an abrupt loss of egg quality. We found no significant relationship between organochlorine residues and eggshell in any of the variables measured. In contrast, we found a positive relationship between food availability and the Ratcliffe Index, eggshell thickness and eggshell surface area. A density-dependent explanation of reduced egg quality could arise from ecological constraints as the decrease of food resources. The impact of sudden changes in food availability due to sanitary regulations between 2006 and 2011 could be related with the loss of Bearded Vulture egg quality.

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1. Introduction

Contamination by OC pesticides has had serious consequences for bird populations, mainly in raptors through increased reproductive failure (Wiemeyer and Porter, 1970; Newton and Bogan, 1974), Reduction of eggshell thickness was one of the earliest recorded effects of dichlorodiphenyl dichloroethylene (DDE) on bird reproduction (Hickey and Anderson, 1968; Ratcliffe, 1970; Peakall and Kiff, 1979). Decreases in breeding success are related to DDE concentrations in the parent birds (Bowerman et al., 1995) leading to a potential relationship between DDE level and productivity (Wiemeyer et al., 1984: Helander et al., 2002; Hernández et al., 2008).

Measurements of eggshell thickness therefore provide a useful tool to assess the DDE contamination level of a threatened species as a precursor to improved management and conservation measures. A case in point is the Bearded Vulture (*Gypaetus barbatus*), an endangered species of conservation concern in Europe (Annex I, EU Wild Birds Directive 79/409/EEC, Appendix II of the Bern Convention, Bonn Convention and CITES), which has recovered since the 1990s to about 230 European breeding territories in 2016. Bearded Vultures are a long-lived species, with a low annual reproductive rate and a pronounced deferral of age of first breeding (Antor et al., 2007; Margalida et al., 2015). Factors which negatively impact on demographic parameters can have extremely detrimental effects on their population dynamics (Oro et al., 2008; Margalida et al., 2015).

In recent decades, the Pyrenean population of the Bearded Vulture increased progressively (from 57 territories in 1989 to 174 in 2016) but has suffered decreasing breeding success in a density-dependent productivity depression scenario (Carrete et al., 2006). Productivity fell from 0.6 young/territorial pair in the 1980s to 0.30 young/territorial pair currently (Carrete et al., 2006; Margalida et al., 2014) and its prospects of survival are reduced (Oro et al., 2008; Margalida et al., 2014). Recent studies have revealed high adult mortality as a result of the increased incidence of illegal poisoning (Margalida et al., 2008; Berny et al., 2015), a factor which seriously threatens the survival of the population (Margalida et al., 2015). For example, between 1990 and 2010 a total of 53 cases of Bearded Vultures illegally poisoned were reported in Spain (Margalida, 2012).

The number of studies on these parameters in Bearded Vultures contrasts with the lack of studies on pesticide contamination of the Pyrenean population (Hernández, 2005a, 2005b), although the OC levels found in eggs and carcasses seem to be, as yet, insufficient to cause breeding impairment or reduced reproductive rates (Elliot and Norstrom, 1998; Hernández et al., 2008; Odsjö and Sondell, 2014).

The recent outbreak of bovine spongiform encephalopathy (BSE) led to changes in sanitary regulations that have resulted in significant food shortages for scavenging animals (Donázar et al., 2009; Margalida et al., 2010). The reduction of available food resources (by c. 80%, see Cortés-Avizanda et al., 2010; Margalida et al., 2014) as a consequence of the retrieval and incineration of livestock carcasses, and the closure of several vulture feeding stations between 2006 and 2011, has been shown to have affected the breeding ecology and behaviour of some vulture species through damaging changes to their breeding and demographic parameters (Donázar et al., 2009; Margalida et al., 2014) and their behavioural characteristics (Zuberogoitia et al., 2010; Margalida et al., 2011). The impact of sudden changes in food availability, and therefore in habitat quality, could affect population trends through changes in life-history parameters (Margalida et al., 2014). Moreover, the effects of organochlorine compounds on egg quality have not been fully examined.

In this study, we used material from a long-term study of eggs and eggshells of the Pyrenean Bearded Vulture population to assess the influence of these reductions in food availability on eggshell parameters and egg quality. We evaluated several egg and eggshell parameters, including eggshell thickness, and their trends over the last 24 years (1989–2012) as well as the levels of OCs present in the eggs. We compared these results from the field with measurements taken from Bearded Vulture eggs added to museum collections between 1858 and 1911 to look at the longer-term changes in eggshell thickness.

2. Material and methods

2.1. Study area and population monitoring

Samples taken from Bearded Vulture populations in Spain and France (130 and 43 breeding territories in 2016, respectively) were studied. The study was carried out within the framework of the conservation plans for this species in France and the Autonomous Communities in Spain. Programs to monitor population trends, breeding parameters and survival rates (including a capture-mark-resighting program) have been performed (Oro et al., 2008; Margalida et al., 2014).

Between 1989 and 2012, all known territories were visited two or three times each month to search for signs of occupancy, territorial and/or courtship activity, and nest building, and to record reproductive parameters (Margalida and Bertran, 2000). Observations began in September–October each year, coinciding with the start of nest-building and sexual activity, and ended during the fledging period (June–August). Nests were observed using 20–60× telescopes and video cameras. During the egg-laying period, 14–20 pairs were selected each year for detailed daily monitoring to determine the date of egg-laying. Egg-laying was confirmed when an incubation shift between parents was observed, or once the eggs could be directly observed in the nest. Failing these methods, clutch size (a simple, one-egg clutch vs a two-egg clutch) was determined by visiting the nest after a confirmed breeding failure and/or after the breeding season, recovering the egg remains. In these cases, we only included the data when entire eggs were found or when the nest inspection was carried out as soon as possible after breeding failure, to avoid possible loss of eggs due to predation by Common Ravens (*Corvus corax*), or after fledging.

2.2. Sample collection

Addled or abandoned eggs or eggshell fragments were collected from the nests of Bearded Vultures breeding in the Pyrenees from 1989 to 2012, after confirmation of breeding failure and/or after fledging. Eggs and eggshells were removed from nests by authorized staff and official agents working under the framework rules of this species' recovery plans in the Autonomous Communities of Spain. Entire eggs were transported to rehabilitation centres in portable incubators and, upon arrival, were weighed, measured (length and breadth), and, when necessary, artificially incubated for some days before confirming breeding failure.

A total of 27 addled eggs, and eggshell fragments from a further 63 eggs were collected and analyzed (a total of 90 samples collected from 49 pairs). Fifty of the eggs were from one-egg clutches and 40 were from two-egg clutches. Although eggs may have been collected from the same territory or nest in different years the adults involved may have been different individuals because adult birds were not marked.

In cases where eggshell fragments were the only material retrieved from a nest after breeding failure, they were classified according to their size and external appearance before examination using a scanning electron microscope (SEM; Phillips Model KX-20 electron microscope, Philips Electronics Nederland, Eindhoven, The Netherlands). In 18 eggs from two-egg clutches (28.6% of the total) the fragments were large enough to distinguish between the pieces belonging to each egg. Only eggshell fragments from the equator of the egg were studied, to