



HAL
open science

Stable isotopes reveal captive vs wild origin of illegally captured songbirds in France

Frédéric Jiguet, Kevin J Kardynal, Keith A Hobson

► **To cite this version:**

Frédéric Jiguet, Kevin J Kardynal, Keith A Hobson. Stable isotopes reveal captive vs wild origin of illegally captured songbirds in France. *Forensic Science International*, 2019, 302, pp.109884. 10.1016/j.forsciint.2019.109884 . mnhn-02552945

HAL Id: mnhn-02552945

<https://mnhn.hal.science/mnhn-02552945>

Submitted on 24 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Stable isotopes reveal captive vs wild origin of illegally captured songbirds in France**

2 Frédéric Jiguet^{a*}, Kevin J. Kardynal^b, Keith A. Hobson^{b,c}

3

4 *corresponding author: frederic.jiguet@mnhn.fr, tel +33(0)140793423

5

6 ^a CESCO, UMR7204 MNHN-CNRS-Sorbonne Université, CP135, 43 Rue Buffon, 75005 Paris, France

7 ^b Environment and Climate Change Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan, S7N

8 3H5, Canada

9 ^c Dept. Biology and Environment and Climate Change Canada, University of Western Ontario, Room

10 2025 BGS Building, 1151 Richmond St. N., London, Ontario, N6A 5B7, Canada

11

12

13 **Abstract**

14 Naturally occurring stable isotopes have become important tracers in the study the wildlife ecology
15 including the identification of origins for migration research, investigations of trophic relationships,
16 and in the forensic determination of illegally harvested and captive fauna. Extensive illegal trapping for
17 use as decoys and in cuisine has contributed to drastic declines of Ortolan Bunting *Emberiza hortulana*
18 populations breeding and migrating through France. We contrasted feather hydrogen isotope ($\delta^2\text{H}_f$)
19 values in illegally captured and subsequently confiscated Ortolan Buntings ($n = 234$), including feathers
20 known to have grown in captivity ($n = 34$) or of unknown growing environment (wild or cage), with
21 $\delta^2\text{H}_f$ values in birds legally caught (ringed and released) in the wild ($n = 40$). We sought to determine if
22 these bird groups could be differentiated based on this single isotope. Feathers grown in captivity had
23 considerably lower $\delta^2\text{H}_f$ relative to feathers of wild birds, which is potentially indicative of tap water
24 consumption and the use of different diets in captive birds. Further, applying mixing models to $\delta^2\text{H}_f$

25 values revealed similar proportions of captive vs. wild origins for birds illegally captured in 2012, 2014
26 and 2015, and a larger proportion of individuals with feathers grown in the wild in 2013. This
27 potentially mirrors the confiscation of birds at poaching sites only in the former years, but also of
28 recently caught wild buntings kept captive in 2013. Our results show that even a single stable isotope
29 ($\delta^2\text{H}$) with good association with origins where feather keratins are produced may be useful in
30 understanding origins of captive birds and may advance the monitoring of illegally captured birds.

31

32 **Keywords**

33 Bird; *Emberiza hortulana*; Illegal hunting; Ortolan Bunting; Poaching; seized birds

34

35 **Highlights**

- 36 - The ortolan bunting is a protected migratory passerine poached in south-west France for
37 gastronomic purposes
- 38 - Captive-grown feathers of ortolan buntings have very low deuterium concentrations
39 compared to wild-grown feathers
- 40 - This difference is used to estimate the relative proportion of recently captured vs. long caged
41 ortolan buntings seized by the police from poachers
- 42 - Stable isotopes can be useful tools to infer the wild vs. captive origin of seized birds and further
43 adapt the care time before being released in the wild

44

45 **1. Introduction**

46 Naturally occurring patterns of stable isotopes across large geographic areas enable the tracking of
47 bird migration. This is based on the principle that stable isotope ratios in food and water consumed by
48 birds can be correlated with those values in growing tissues (e.g. feathers, blood) following well-
49 described processes. Protium (^1H) and its heavier isotope, deuterium (^2H), occur naturally in
50 environmental waters, and the ratio of the heavier to lighter isotope ($^2\text{H}/^1\text{H}$ measured as $\delta^2\text{H}$) in

51 precipitation has been shown to change predictably at continental scales (i.e. “isoscares”) [1]. Indeed,
52 $\delta^2\text{H}$ values in precipitation ($\delta^2\text{H}_p$) generally have a latitudinal structure across continents [2] and these
53 patterns form the basis of tracing animal movements because such patterns are transferred up
54 foodwebs [3]. Foodweb isotopic signatures are reflected in the tissues of organisms, and such
55 signatures can vary spatially based on a variety of biogeochemical processes. Organisms moving
56 between isotopically distinct foodwebs carry with them information on the location of previous
57 feeding (Hobson 1999). Migration connectivity can thus be studied by tracking chemicals contained in
58 tissue, notably stable isotopes in keratins (Hobson & Wassenaar 2008). As feathers grow during a short
59 time period during a precise phase of the annual cycle, they are preferred to the continuously growing
60 claws for inferring geographical origins. Determination of migratory origins using isotopes does not
61 require sacrificing individuals, just sampling a feather of known growth timing. Migrants wearing
62 feathers grown on breeding grounds can be attributed to probabilistic breeding areas by comparing
63 the concentrations of some stable isotopes in their feathers and the geographical variations of the
64 same isotopes in the natural environments where the feathers could have grown. The same holds true
65 if analyzing feathers of breeding individuals that have molted on their wintering grounds.

66 Inferring the origin of migratory animals in terrestrial systems can be based on various stable
67 isotopes, especially nitrogen, hydrogen and carbon. Nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes are mainly used
68 to determine the trophic level of a species. Hydrogen (^1H) and deuterium (^2H) has revolutionized stable
69 isotope analysis in the study of animal migration. Deuterium ratios vary strongly with weather
70 conditions, resulting in highly predictable spatial variation across continents (Hobson & Wassenaar
71 2008). Constructing continental maps that predict deuterium levels is relatively straightforward, given
72 the large amount of existing data on continental weather patterns, and biologists further use such
73 maps to determine probabilistic migratory origins of species. Deuterium ratios in feathers are effective
74 indicators of breeding latitude in European birds (Hobson & Wassenaar 2008, Bearhop et al. 2003).
75 Carbon isotopic signatures ($^{13}\text{C}/^{12}\text{C}$) are used to distinguish plants that use C3, C4 and CAM modes of
76 photosynthesis, as the C4 and CAM pathways lead to lower carbon fractionation than C3

77 photosynthesis (Karasov & Martínez del Rio 2007). Carbon isotopes can thus be used to reconstruct
78 migratory routes, if the geographical distribution of C3, C4 and CAM plants and the diet preferences
79 of the study species are known (Hobson 1999). As C3 and C4 plants have not the same occurrence rate
80 north and south of the Sahara, carbon isotopic signatures in winter-grown feathers can discriminate
81 individual birds that have overwintered south and north of the Sahara. Clearly, these isotopes provide
82 rougher estimates of origins than deuterium, while C and N should be avoided when studying farmland
83 species growing their feathers or hair in environments receiving agricultural fertilizers, disturbing
84 natural concentrations of their isotopes.

85 The Ortolan Bunting *Emberiza hortulana* is a long-distance migrant Palaearctic songbird, that
86 breeds across Eurasia and winters south of the Sahara. It breeds mainly in farmed habitats, while its
87 migratory strategy has been confirmed by isotopic investigations of deuterium concentrations in
88 winter-grown feathers [4]. The species is protected in the European Union and listed in Appendix I of
89 the Birds Directive (1979/409/EEC), and formally protected in France since 1999. However, this species
90 was illegally harvested in southwest France for culinary interest, as migrants have the outstanding
91 capacity to double their mass, primarily as fat, within a short period prior to their fall migration. While
92 bird conservation organizations have repeatedly denounced this practice, hunters, local politicians and
93 leading French Michelin-starred chefs [5] have defended the persistence of this hunting and
94 gastronomic practice as part of traditional cultural heritage. The European Commission referred France
95 to the Court of Justice of the European Union (EU) in December 2016 on the charge of illegal hunting
96 of Ortolan Buntings, as France could not prove it was satisfactorily taking action to stop the poaching
97 activities. In this context, the French nature police intervened in the field by interrogating some alleged
98 offenders and seizing captive Ortolan Buntings. These illegally captured birds were taken into care and
99 later released in the wild before the end of the migration season.

100 In all birds, the renewal of feathers through moult is necessary to maintain the functions of
101 these keratinous appendages. Songbirds typically renew flight feathers once each year and body
102 feathers twice each year. Adult Ortolan Buntings moult the body feathers twice each year [6], on the

103 breeding grounds in June to August, before the autumn migration to Africa, and on the wintering
104 grounds before the spring migration. Their flight feathers are moulted only once, during the post-
105 breeding moult in Europe. First-year individuals have feathers grown in the nest on the breeding
106 grounds. Hence, during the fall migration, all individuals have a complete set of feathers recently grown
107 on their breeding grounds regardless of their age.

108 Bird feathers grown at a given location generally have $\delta^2\text{H}$ values ($\delta^2\text{H}_f$) proportional to local
109 amount-weighted mean $\delta^2\text{H}_p$ [7]. However, this is not necessarily true for feathers grown in captivity.
110 Captive birds are assumed to be provided tap water and commercial food of unknown and varied
111 origins, which can have different $\delta^2\text{H}$ values than local precipitation or wild grown seeds. This
112 discrepancy in $\delta^2\text{H}$ is especially true if tap water does not correspond well to amount-weighted growing
113 season precipitation where feathers are grown and if food provided to the captive birds is not issued
114 from local production [2],[8]. Under a global research program dedicated to unravel the migration
115 strategy of the Ortolan Bunting [4], we had access to numerous individuals captured illegally during
116 four consecutive years. These birds included recently captured wild individuals and caged decoys
117 placed on hunting sites. Caged decoys could be either individuals kept year-long in captivity (i.e. with
118 captive-grown feathers), or recently captured wild individuals (i.e. wild-grown feathers) placed in cages
119 to complement/renew the stock of long-captive decoy birds. All seized first-calendar individuals (i.e.
120 hatch year) were of conclusively wild origin, thus having wild-grown feathers, since captive breeding
121 of the species is not possible. Most other individuals could have feathers of wild or captive origin, but
122 the plumage of a few adults displayed color aberration with either a lack of or an excess of dark
123 pigmentation (leucism or melanism), signaling captive-grown feathers [9].

124 Within the poached and seized birds, our main aim was to study $\delta^2\text{H}_f$ values in order to
125 estimate the proportion of individuals that were recently captured compared to those individuals
126 captured in earlier years and further kept in captivity. All seized birds hatched in the wild, as there is
127 no captive breeding of the species, so our aim was to address the question of the temporality of their
128 capture, either recent or older. By first considering obvious caged buntings, we first assessed $\delta^2\text{H}_f$

129 values for cage-grown feathers to determine if $\delta^2\text{H}_f$ could be used to separate recently captured and
130 caged birds. We expected birds with suspected cage-grown feathers to have potentially different $\delta^2\text{H}_f$,
131 either lower or higher. As most Ortolan Buntings migrating by southwest France come from Poland
132 and Scandinavia [4] where deuterium concentrations in the environment are lower than in France, we
133 expected higher $\delta^2\text{H}_f$ in caged birds. We then analyzed the distributions of $\delta^2\text{H}_f$ in feathers collected
134 from Ortolan Buntings illegally captured during four consecutive hunting seasons to estimate the
135 proportions of individuals of wild vs captive origins. We finally propose possible interpretations for the
136 observed inter-annual variations in these proportions, in terms of confiscation efforts by the police.

137

138 **2. Materials and methods**

139 *2.1 Sample collection and hydrogen isotopic measurements*

140 We collected feathers from Ortolan Buntings seized by the nature police in Landes department,
141 southwestern France, when interrogating owners of illegal trapping sites. Seized birds were placed in
142 wildlife care centers where licensed forensic ringers processed them before release. Seized birds could
143 include caged and recently trapped birds found at capture sites, and potentially birds kept in captivity
144 to be fattened for consumption. All surviving confiscated individuals were ringed and feather-sampled
145 prior to release in the wild before the end of the autumn migration season. We organized the collection
146 of one tail feather from each bird cared for in 2012 (n=50), 2013 (n=18), 2014 (n=53) and 2015 (n=113).
147 Individual birds were classified into three groups: 1) decoys (n=34) that were illegally captured birds
148 with plumage abnormalities or highly developed feet scabies, 2) illegally captured birds from each year
149 (2012, 2013, 2014 and 2015; these groups include the previously cited obvious decoys, but also illegally
150 captured first-calendar year individuals), and 3) wild birds legally captured and ringed in autumn
151 (n=40). For birds with tail feathers that were too heavily damaged from being in captivity, we instead
152 sampled one tertial feather. We stored feathers in individual envelopes at ambient temperature until
153 isotopic analysis.

154 Feathers were cleaned in 2:1 chloroform:methanol solvent rinse and prepared for $\delta^2\text{H}$ analysis
155 at the Stable Isotope Laboratory of Environment Canada, Saskatoon, Canada. The $\delta^2\text{H}$ of the non-
156 exchangeable hydrogen of feathers was determined using the method described by Wassenaar &
157 Hobson [10] based on two calibrated keratin hydrogen-isotope reference materials (CBS: -197 ‰, KHS:
158 -54.1 ‰). We performed hydrogen isotopic measurements on H_2 gas derived from high-temperature
159 (1350 °C) flash pyrolysis (Eurovector 3000; Milan, Italy) of 350 ± 10 ug feather subsamples and keratin
160 standards loaded into silver capsules. Resultant separated H_2 was analysed on an interfaced Isoprime
161 (Crewe, UK) continuous-flow isotope-ratio mass spectrometer. Measurement of the two keratin
162 laboratory reference materials corrected for linear instrumental drift were both accurate and precise
163 with typical within-run measurement error < 2 ‰. All results are reported for non-exchangeable H
164 expressed in the typical delta notation, in units of per mil (‰), and normalized on the Vienna Standard
165 Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale.

166

167 *2.2 Statistical analyses*

168 We first tested if $\delta^2\text{H}_f$ values measured from each bird group followed normal distributions by
169 performing Shapiro-Wilk tests. We also performed a general linear model to compare $\delta^2\text{H}_f$ values
170 among five groups (wild birds and the four annual datasets). We then applied the normalmixEM
171 function of the ‘mixtools’ package in R v3.5.2 [11] to the datasets for each year (2012, 2013, 2014 and
172 2015) to obtain the mixing proportions, means and standard deviations of both estimated mixed
173 normal distributions. We could have pre-defined the means and standard deviations of the two mixed
174 normal distributions using values estimated from the obvious wild and decoy birds. However, we did
175 not do so as we expected that inter-annual variations in meteorological conditions during migration
176 (e.g. predominance of eastern vs. western winds) and relative breeding productivity across the range
177 (i.e. origins) should create variation in the annual distribution of $\delta^2\text{H}_f$ in wild birds migrating through
178 southwestern France. This expectation was supported by a year effect in an analysis of variance

179 predicting $\delta^2\text{H}_f$ values by year of capture for birds captured and ringed in the wild by forensic ringers
180 ($F_{2,37} = 28.3$, $P < 0.001$).

181

182 **3. Results**

183 *3.1 Normality*

184 We report the mean \pm s.d. values of $\delta^2\text{H}_f$ for the wild individuals and obvious decoys in Table 1, while
185 Figure 1 shows the boxplot of the different groups studied here (including obvious decoys). The boxplot
186 illustrates that illegally captured birds include individuals of wild and captive origins in unknown
187 proportions. Shapiro-Wilk's tests confirmed the normal distribution of the deuterium values for the
188 following datasets: wild birds ($W=0.967$, $P=0.051$), obvious decoys ($W=0.961$, $P=0.268$), and 2013 birds
189 ($W=0.925$, $P=0.161$), but not for birds confiscated in 2012 ($W=0.900$, $P<0.001$), 2014 ($W=0.878$, $P <$
190 0.001) and 2015 ($W=0.899$, $P < 0.001$). A general linear model performed on data from wild birds and
191 all years revealed that all annual groups differed from the wild sample (year effects predicting $\delta^2\text{H}_f$
192 compared to the wild sample defined as the intercept; intercept estimate \pm s.d. = -88.18 ± 2.69 ‰):
193 2012 (-11.18 ± 3.46 ‰, $t=-3.10$, $P=0.002$); 2013 (-13.06 ± 4.83 ‰, $t=-2.71$, $P=0.007$); 2014 (-11.30 ± 3.41
194 ‰, $t=-3.56$, $P=0.002$), 2015 (-12.16 ± 3.13 ‰, $t=-3.89$, $P<0.001$). Fig. 1 illustrates the differences
195 between these groups.

196

197 *3.2 Mixing proportions*

198 Fitting normal mixture densities to the annual datasets, we identified for each year two mixed normal
199 distributions closely matching those of obvious decoys and of wild individuals (Table 1 and Fig. 2). The
200 estimated proportion of birds with captive- vs. wild-grown feathers were relatively balanced in 2013
201 (45% vs 55%) but were skewed in 2012 (61% vs 39%), 2014 (75% vs 25%) and 2015 (72% vs 28%), with
202 a larger proportion of caged birds.

203

204 **4. Discussion**

205 Ortolan Buntings illegally captured in France in 2012-2015 included some individuals that were obvious
206 decoys with plumage abnormalities, leucism or melanism, which are likely linked to nutrient
207 deficiencies [9],[12]. All of these individuals were captive for long periods prior to sampling and so they
208 grew their feathers in captivity. The feathers sampled on these birds provided a good opportunity to
209 obtain estimates of the $\delta^2\text{H}_f$ concentrations in captive-grown feathers, and further compare them to
210 similar measures obtained from Ortolan feathers grown in the wild. The latter came from active
211 migrants captured in nature by licensed forensic ringers. The decoys had notably lower $\delta^2\text{H}_f$
212 concentrations than wild birds which is consistent with the expectation that captive birds, presumably
213 drinking tap water and eating commercial food, can have different $\delta^2\text{H}_f$ than those of wild birds derived
214 from diets and drinking water driven by local precipitation at the site of feather development. Here,
215 by analyzing $\delta^2\text{H}_f$ concentrations of seized Ortolans, we were able to predict their wild or captive origin,
216 as long as the captive birds consumed only minimal rainwater and wild-grown seeds while growing
217 their feathers. For birds that have not molted feathers while in captivity, analysis of $\delta^2\text{H}$ in other
218 chemically active tissues (e.g. toenails, blood) with differing turnover periods may provide further
219 insights into their capture history and requires further study [14]. However, the expectation of a
220 difference of isotopic concentrations in feathers of wild vs. captive individuals will be entirely case
221 specific and depend very much on the molt origin of wild birds compared to captive birds and their
222 artificial diets [2],[8],[13].

223 Within the datasets obtained for the different years, 2013 appears distinct, with balanced
224 proportions of wild and captive birds, while the other three years included more captive than wild
225 birds. The difference could arise from variations in confiscation efforts and procedures, concerning
226 mainly long-lasting or recently captured decoys present on field poaching stations, possibly
227 complemented by additional wild and recently captured individuals moved indoors for fattening. If the

228 police operated mainly at field capture sites, confiscated birds would concern only caged decoys, with
229 a majority of long-caged individuals with feathers grown in captivity, and fewer recently captured
230 individuals kept in the field to complement the available decoys surviving from the precedent year. If
231 the police further confiscated the wild individuals captured recently but then moved indoor for
232 fattening, we would expect a higher proportion of individuals wearing wild-grown feathers. The first
233 scenario would produce a larger proportion of birds of captive origin (see 2012, 2014, 2015), while the
234 second scenario would produce a comparatively larger proportion of wild individuals (as in 2013).

235 While isotope profiling or fingerprinting are often used in food quality controls or criminal
236 cases [15], there is a growing literature showing that stable isotope analyses are a powerful tool in
237 forensic tracing of the origins of legally or illegally traded animals and plants [16],[17]. Studying the
238 African Grey Parrot *Psittacus erithacus*, Alexander et al. [18] found a systematic difference in feather
239 carbon ($\delta^{13}\text{C}_f$) and deuterium ($\delta^2\text{H}_f$) values for known wild and captive birds, and a match between
240 isotopic ratios obtained for imported birds that deceased in captivity and for wild birds, attesting that
241 these imported birds had been captured illegally in the wild. Castelli and Reed [19] identified unique
242 patterns of three isotopic ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) to distinguish wild and pen-raised Northern
243 Bobwhite *Colinus virginianus*. Dittrich et al. [20] investigated the isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and
244 $\delta^{18}\text{O}$) of legally traded frog legs from suppliers in Vietnam and Indonesia, and segregated naturally
245 born from intensively farmed frogs. Indeed, the use of stable isotope analyses can definitely improve
246 the monitoring of legally and illegally traded wildlife species across the globe.

247 Analysis of stable isotopes in recently grown feathers of illegally captured birds in Europe is a
248 promising forensic tool to help authorities identify wild poached individuals of protected species that
249 are otherwise bred legally in captivity. This could be highly efficient to segregate legally from illegally
250 traded Goldfinches *Carduelis carduelis*, which suffers from a long-term poaching pressure that has
251 resulted in a drastic range retraction in North Africa [21]. International illegal trade of poached
252 Goldfinches has also recently increased across southern Europe, following the decline in North African

253 breeding numbers [21]. Indeed, French authorities have recurrently seized Goldfinches in recent years,
254 and analyzing the stable isotope concentrations (notably $\delta^2\text{H}$) in their feathers could help to determine
255 the captive vs wild origin of seized birds, and the geographic origin of wild seized individuals [22]. As
256 the principal strategy used to deal with animals recovered from illegal traffic is to release them back
257 into nature [23], this would allow rapid release of individuals with recently wild-grown feathers, and
258 give longer recovery time to individuals having been captive for longer durations.

259 As a conclusion, we acknowledge that differences in isotopic concentrations in bird feathers
260 grown in captivity and in the wild are generally case specific, though if attested as was the case for
261 ortolan buntings in southwest France, they can provide a useful tool to determine the duration of
262 previous captivity for seized birds, and therefore adapt their rehabilitation period before an optimal
263 release back in the wild. The information on the relative proportion of wild and recently captured
264 individuals, vs. individuals captured since at least one year, can further inform on the efficiency of the
265 confiscation efforts to target wild birds whose captures impeded directly the fate of wild populations [4].

266

267 **References**

- 268 [1] J.B. West, G.J. Bowen, T.E. Dawson, K.P. Tu (Eds.), *Isoscapes: understanding movement, pattern,*
269 *and process on Earth through isotope mapping*, Springer, Dordrecht, The Netherlands, 2010,
270 ISBN: 9789048133536
- 271 [2] G.J. Bowen, L.I. Wassenaar, K.A. Hobson, *Global application of stable hydrogen and oxygen*
272 *isotopes to wildlife forensics*, *Oecologia* 143 (2005) 337–348.
- 273 [3] K.A. Hobson, L.I. Wassenaar, *Tracking Animal Migration using Stable Isotopes*. *Handbook of*
274 *Terrestrial Ecology Series*, Academic Press / Elsevier, Amsterdam, 2018, ISBN: 9780128147238
- 275 [4] F. Jiguet, A. Robert, R. Lorrillière, K.A. Hobson, K.J. Kardynal, R. Arlettaz, F. Bairlein, V. Belik, P.
276 Bernardy, J.L. Copete, M.A. Czajkowski, S. Dale, V. Dombrowski, D. Ducros, R. Efrat, J. Elts, Y.

277 Ferrand, R. Marja, S. Minkevicius, P. Olsson, M. Pérez, M. Piha, M. Raković, H. Schmaljohann, T.
278 Seimola, G. Selstam, J.-P. Siblet, M. Skierczyński, A. Sokolov, J. Sondell, C. Moussy, Unravelling
279 migration connectivity reveals unsustainable hunting of the declining ortolan bunting, Scientific
280 Advances 5 (2019) aau2642

281 [5] A. Darroze, Touch' pas mon ortolan. Atlantica Editions, Biarritz, 2000, ISBN: 2843941946

282 [6] F. Jiguet, K.J. Kardynal, M. Piha, T. Seimola, J.L. Copete, M.A. Czajkowski, V. Dombrowski, R. Efrat, S.
283 Minkevicius, M. Raković, M. Skierczyński, K.A Hobson, Stable isotopes reveal the systematic winter
284 moult of central tail feathers in the Ortolan Bunting *Emberiza hortulana*, J. Ornithol. (submitted)

285 [7] K.A. Hobson, S.L. Van Wilgenburg, L.I. Wassenaar, K. Larson, Linking Hydrogen ($\delta^2\text{H}$) Isotopes in
286 Feathers and Precipitation: Sources of Variance and Consequences for Assignment to Isoscapes,
287 PLoS One 7(4) (2012) e35137.

288 [8] J.L. Bowen, K.D. Kroeger, G. Tomasky, W.J. Pabich, M.L. Cole, R.H. Carmichael, I. Valiela, A review
289 of land-sea coupling by groundwater discharge of nitrogen to New England estuaries: Mechanisms
290 and effects, Appl. Geochem. 22 (2007) 175-191.

291 [9] B.L. Sage, The incidence of albinism and melanism in British birds, British Birds 56 (1963) 409-416.

292 [10] L.I. Wassenaar, K.A. Hobson, Comparative equilibration and online technique for determination
293 of non-exchangeable hydrogen of keratins for use in animal migration studies, Isotopes Environ.
294 Health Stud. 39 (September (3)) (2003) 211-7.

295 [11] T. Benaglia, D. Chauveau, D.R. Hunter, D. Young, Mixtools: An R Package for Analyzing Finite
296 Mixture Models, J. Stat. Software 32 (2009) 1–29.

297 [12] G.E. Hill, Proximate Basis of Variation in Carotenoid Pigmentation in Male House Finches, Auk
298 109 (1992) 1–12. doi.org/10.2307/4088262

299 [13] Y. Jameel, S. Brewer, S.P. Good, B.J. Tipple, J.R. Ehleringer, G.J. Bowen GJ, Tap water isotope ratios
300 reflect urban water system structure and dynamics across a semiarid metropolitan area, Water
301 Resour. Res. 52 (2016) 5891-5910. doi.org/10.1002/2016WR019104

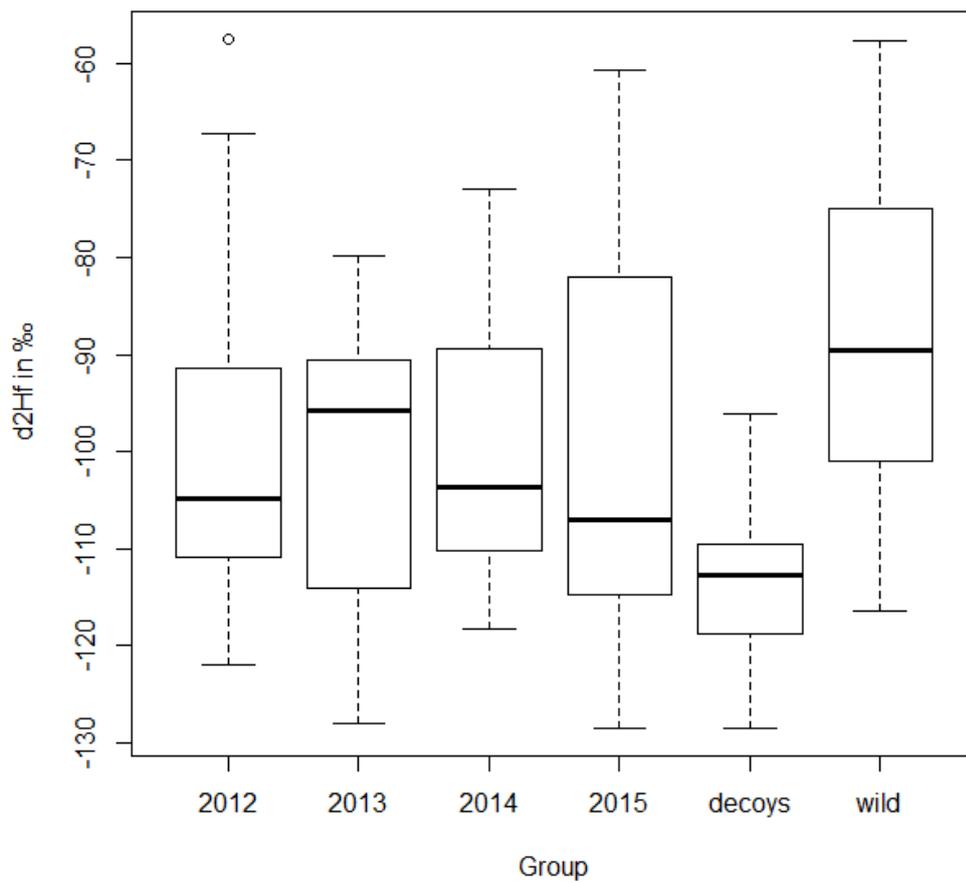
- 302 [14] D.F. Mazerolle, K.A. Hobson KA, Patterns of differential migration in white-throated sparrows
303 evaluated with isotopic measurements of feathers, *Can. J. Zool.* 85 (2006) 413-420.
- 304 [15] W. Meier-Augenstein, *Stable Isotope Forensics: An Introduction to the Forensic Application of*
305 *Stable Isotope Analysis*, 2010, ISBN: 9780470517055
- 306 [16] K. Retief, A.G. West, M.F. Pfab, Can stable isotopes and radiocarbon dating provide a forensic
307 solution for curbing illegal harvesting of threatened cycads?, *J. Forensic Sci.* 59 (2014) 1541–1551.
- 308 [17] E.S. Oliveira, D. de Freitas Torres, R.R. da Nóbrega Alves, Wild animals seized in a state in Northeast
309 Brazil: Where do they come from and where do they go? *Environ. Dev. Sustain.* (2018)
310 doi.org/10.1007/s10668-018-0294-9
- 311 [18] J. Alexander, C.T. Downs, M. Butler, S. Woodborne, C.T. Symes, Stable isotope analyses as a
312 forensic tool to monitor illegally traded African grey parrots, *Animal Conserv.* (2018)
313 doi.org/10.1111/acv.12445
- 314 [19] P.M. Castelli, L.M. Reed, Use of stable isotopes to distinguish wild from pen-raised northern
315 bobwhite, *Wildl. Soc. Bull.* 41(1) (2017) 140-145. doi.org/10.1002/wsb.746
- 316 [20] C. Dittrich, U. Struck, M.-O. Rödel, Stable isotope analyses—A method to distinguish intensively
317 farmed from wild frogs, *Ecol. Evol.* 7(8) (2017) 2525-2534. doi.org/10.1002/ece3.2878
- 318 [21] R. Khelifa, R. Zebssa, H. Amari, M. Khalil Mellal, S. Bensouilah, A. Laouar, H. Mahdjoub, Unravelling
319 the drastic range retraction of an emblematic songbird of North Africa: potential threats to Afro-
320 Palearctic migratory birds, *Sci. Rep.* 7 (2017) 1092. doi.org/10.1038/s41598-017-01103-w
- 321 [22] A. Kelly, R. Thompson, J. Newton, Stable hydrogen isotope analysis as a method to identify illegally
322 trapped songbirds, *Sci. Justice* 48 (2008) 67–70.
- 323 [23] L. Macedo Magroski, A. do Nascimento Pessoa, W. Guedes de Lucena, A. Loures-Ribeiro, C. Barros
324 de Araújo, Where to release birds seized from illegal traffic? The value of vocal analyses and
325 ecological niche modeling, *Perspect. Ecol. Conserv.* 15 (2017) 91-101.

326 Table 1. Mixing proportions, mean (\bar{x}) and standard deviation (SD) of deuterium concentrations in
 327 Ortolan Bunting feathers ($\delta^2\text{H}_f$) for each of the mixed normal distribution. The last line provides the
 328 mean and standard deviation for obvious dummies and wild birds also reported in Fig. 1.

Year	Proportion 1 (in %)	$\delta^2\text{H}_f \bar{x} 1$	SD 1	Proportion 2 (in %)	$\delta^2\text{H}_f \bar{x} 2$	SD 2
2012	60.1	-109.01	5.87	39.9	-84.82	14.16
2013	44.6	-115.24	5.87	55.4	-89.98	4.67
2014	74.9	-106.42	6.63	25.1	-78.75	4.15
2015	72.2	-111.21	8.98	27.8	-72.14	7.14
References	Decoys (n=34)	-114.1	8.50	Wild (n=40)	-85.73	15.32

329

330 Figure 1. Boxplot of deuterium concentration values in Ortolan Bunting feathers ($\delta^2\text{H}_f$, in ‰) for
331 annual datasets of confiscated birds, for confiscated obvious decoys and for individuals captured
332 legally captured in the wild by ringers. Each boxplot reports median, 1st and 3rd quartiles, min and
333 max values.



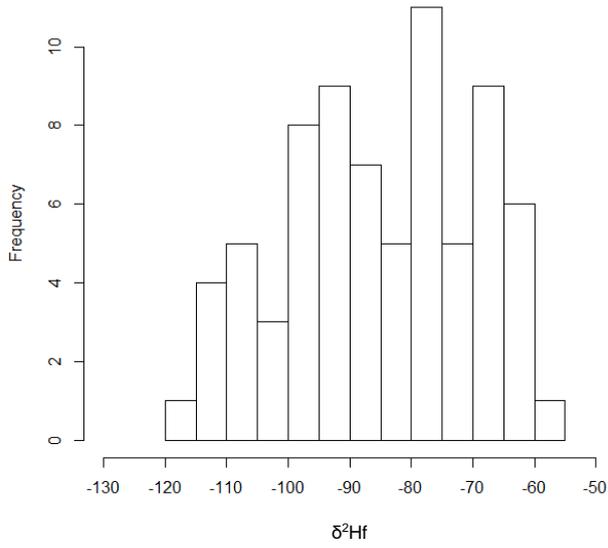
334

335

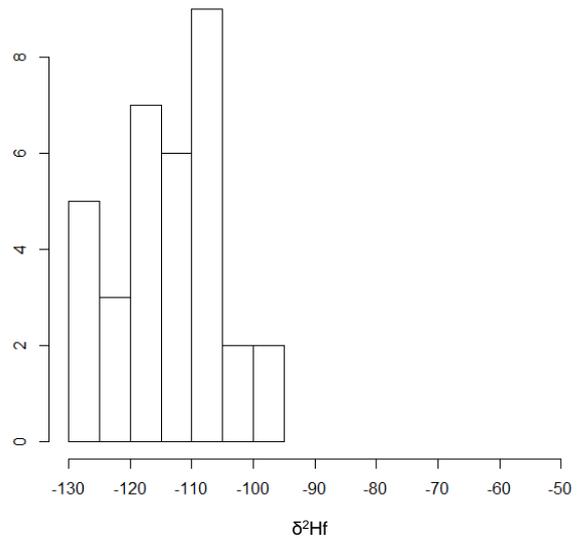
336 Figure 2. Distributions of deuterium concentrations in Ortolan Bunting feathers ($\delta^2\text{H}_f$, in ‰) for (A)
337 wild birds (upper left, n=40) and (B) obvious decoys (upper right, n=34) (in frequency), then (C-D-E-F)
338 as obtained from fitting normal mixture densities to the annual datasets (with global and mixed
339 density curves).

340

A Wild birds



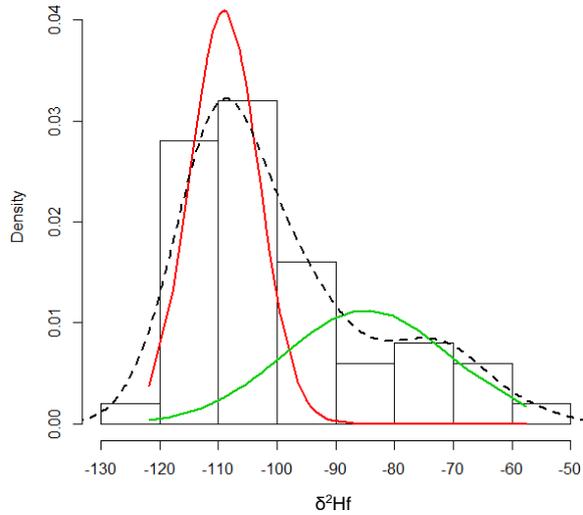
B Obvious decoys



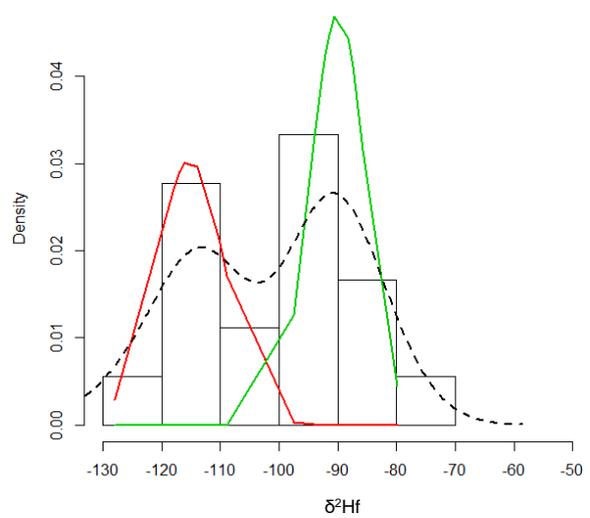
346

347

C Captures 2012



D Captures 2013



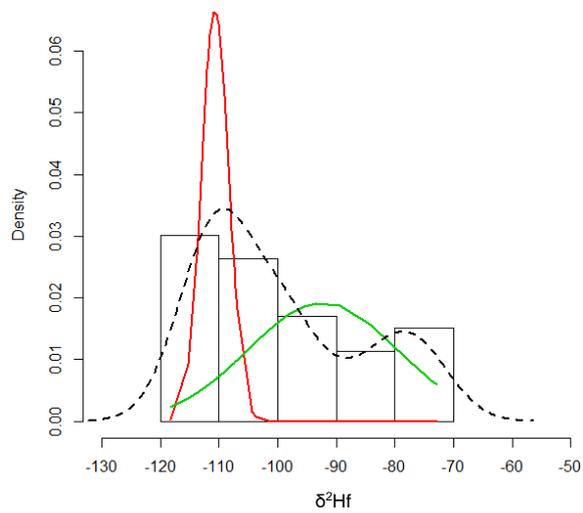
348

349

350

351

E Captures 2014



F Captures 2015

