Serial sampling provides chronological evidence that endogenous protein is used for primary growth in a molt-migrant goose

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Running title: Isotopic change in feather time

1 **ABSTRACT**

2 This is a proof of concept paper based on chronological samples of growing feathers

3 from geese thought to be molt-migrants. When molt-migrant birds initiate molt

4 shortly after migrating to a new isoscape, isotope values measured along the length

5 of their feathers should change continuously. To assess long-term changes and daily

6 cycling in $\delta^{15}N$ and $\delta^{13}C$ values, we serially sampled growing primaries of three

7 presumed molt-migrant geese. Two showed changing $\delta^{15}N$ signatures along the

8 length of their growing primaries, indicating they were molt-migrants, while the

9 third, presumably a resident, showed no change. We then resampled these feathers

10 at closer intervals for evidence of the predicted diel cycle in the use of exogenous

11 and endogenous protein for feather growth, generated by the diel feeding cycle of

12 these geese. As predicted, the two geese that were equilibrating to a new isoscape

13 showed oscillations of approximately 24-hour periodicity in $\delta^{15}N$ values, measured

14 along the length of their primaries. In contrast, the goose that was not equilibrating

15 to a new isoscape showed no 24-hour periodicity in its $\delta^{15}N$ values. Our results

16 demonstrate that chronological sampling along the length of individual primaries

17 holds great potential for identifying individuals that are molt-migrants.

18

19

20   **INTRODUCTION**

21   Molt-migrants require time for their endogenous protein reserves to come

22   into equilibrium with local isoscapes (Martinez del Rio and Anderson-Sprecher

23   2008). Thus, feathers grown during this period of equilibration should show a

24   steady change in isotopic signature along the length of their feathers. This follows

25   because feathers grow at a more or less constant rate throughout the 24-hour cycle

26   (Murphy and King 1986; Schieltz and Murphy 1995, Lillie and Wang 1940); thus,

27   when exogenous protein from local foraging is exhausted, endogenous protein

28   reserves, which are coming into equillibrium with the new isoscape, supply the

29   protein needed to build feathers during daily periods of fasting (Murphy and King

30   1990).

31   These facts conspire to make two predictions that help identify molt migrant

32   birds that initiate feather growth shortly after moving to a new isoscape. First, a

33   steady change in isotope signature along the length of flight feathers is expected for

34   molt-migrants that begin molt before equilibrating to a new isoscape. This

35   prediction is easily tested with course sampling along the length of flight feathers.

36   Second, fine sampling along feathers, should reveal a roughly 24-hour periodicity

37   representing the alternating use of exogenous and endogenous protein sources for

38   feather construction. The amplitude of this cycle is likely driven by the size of the

39   protein pool in the blood relative to the amount of protein being withdrawn for

40   feather synthesis. If the fraction of blood protein used for feather synthesis is small,

41   then daily cycling associated with feeding and fasting may be damped and difficult

42   to measure, but it still should be present.

43   Greylag Geese (*Anser anser*) that breed in terrestrial, freshwater habitats in

44   Sweden are known from sightings of marked individuals (Nilsson et al. 2001), to

45   migrate in late summer to the maritime saltmarshes of the island of Saltholm

46   (55°38'N 12°45'E) in Denmark where they graze saltmarsh plants (Fox et al. 1998)

47   and undergo their annual molt (Fox et al. 1995). Marine environments are well

48   known to have very different $\delta^{15}N$ signatures than freshwater environments, and

49   they may have slightly different $\delta^{13}C$ signatures. Fox et al. (2009) confirmed that

50   newly grown flight feathers of Greylag Geese showed stable isotope values that

51   were intermediate between those derived from a terrestrial plant diet (i.e. in

52   Sweden prior to the molt-migration) and a diet of saltmarsh plants from Saltholm.

53   To explore expected changes in $\delta^{15}N$ and $\delta^{13}C$ along the length of primaries

54   and to examine the potential for 24-hour cycling in the use of exogenous and

55   endogenous protein for feather growth, we serially sampled a single primary

56   feathers from each of three Greylag Geese from Saltholm; these feathers (and no

57   others) were available from the study of Fox et al. (2009). Because feathers are

58   generated from tip to base and composed of non-living keratin, primaries grown by

59   geese that migrated to Saltholm for their molt should change chronologically from

60   more positive (freshwater) $\delta^{15}N$ signatures at the feather tip to more negative

61   (marine) $\delta^{15}N$ signatures at the feather base. Further, because flightless Greylag

62   Geese forage only at night on Saltholm (Kahlert et al. 1996), a 24-h periodicity in the

63   $\delta^{15}N$ signature should be expected in serial samples from the flight feathers of geese

64   that were not in equilibrium with the Saltholm isoscape.

65   Our results show that serial samples taken along the length of primary flight

66   feathers readily show equilibration toward a new isoscape and, further, that

67   feathers sampled at fine intervals can reveal diel shifts in exogenous and

68   endogenous protein sources used for feather generation. We are aware of just two

69   prior studies of hair or feathers that used chronological samples to examine changes

70   in isotope signatures through time. Cerling et al. (2006) used Elephant hair to

71   demonstrate the movement of individual African elephants (*Loxodonta africana*) to

72   different foraging locations, and Church et al. (2006) used samples along the length

73   of a growing rectrix from a California Condor (*Gymnogyps californianus*) to show a

74   sudden deposition of lead that resulted in its death. Because our results are

75   unavoidably based on feathers from just three geese, their importance lies in the

76   new approaches we use to study expected changes in isotopic signatures for molt-

77   migrant birds.

78

79   **MATERIALS AND METHODS**

80   Feathers were obtained from Greylags in active molt on Saltholm, between

81   Copenhagen and Swedish Skania coast (Fox et al. 2009). The Danish Forest and

82   Nature Agency gave permission to catch and sample the geese and the landowners

83   of Saltholm gave permission to work on the island. The geese were caught under

84   the Copenhagen University Natural History Museum Ringing Permit A600 "DMU-

85   Kalø Ringmærkning."

86   The geese included in this study were not individually tracked, so we do not

87   know when they arrived at Saltholm, or how soon after arrival they started to molt,

88   or even if they were all molt-migrants to Saltholm. These limitations mean that we

89   could obtain useful information on periodicity only from birds that showed strong

90   changes in their $\delta^{15}N$ signatures along the length of the sampled primary, indicating

91   they were molt-migrants to Saltholm. Greylags are large geese that would require

92   considerably more than a month to come into equilibrium with a new isoscape as

93   different as that at Saltholm (Martinez del Rio and Anderson-Sprecher 2008).

94   Molting geese that were already in equilibrium with the salt marsh isoscape of

95   Saltholm should show no 24-hour cycling in isotopic signatures; these could be

96   either local, Saltholm, geese (e.g. failed breeders) or salt marsh breeders from a

97   similar isoscape. Although there is variation among individual feather growth rates,

98   Greylag Geese should grow their feathers at approximately 7 mm d^{-1}, as inferred

99   from a mean mass of 3509g (Dunning 2007) and the allometric relationship

100 between primary growth rate and body size (Rohwer et al. 2009).

101   The feathers used for this study were sampled twice, once at every 5mm to

102 plot the change in $\delta^{15}N$ and $\delta^{13}C$ over a longer period of feather time, measured as

103 mm from the tip of the primary, and a second time at 1 or 2mm intervals, in the

104 hope that we could detect a 24h periodicity in the protein source used for feather

105 generation. Which primary feather was used should not affect the results of this

106 study, as Greylag Geese, like most waterfowl, lose and replace their flight feathers

107 simultaneously (Hohman et al. 1992).

108   Methods for sample preparation and analysis generally follow those in

109 Paritte and Kelly (2009). Feathers were cleaned in dilute detergent followed by

110 repeated rinsing. After air drying the feathers were cleaned again in 2:1

111 chloroform:methanol and allowed to air dry before processing. Once feathers were

112 dried a research technician marked the rachis of each feather from its tip to the base

113   of the growing vane at 1, 2 or 5 mm intervals. The majority of the posterior vane

114   was then cut away, leaving only the few mm closest to the rachis. Then, using the

115   pen marks as a guide, an approximately 200μg sample of feather vane was cut

116   immediately adjacent to the pen mark. These samples were loaded into tin capsules

117   (3.5x5.5mm) and stored in an elisa plate until they were analyzed.

118   We analyzed samples in batch sequences of 49 samples and references,

119   referred to as autoruns. Each autorun typically analyzed 39 unknown samples and

120   8 laboratory reference samples in positions 1, 2, 7, 13, 19, 37, 43, 49. The laboratory

121   reference material was powdered Brown-headed Cowbird feather (*Molothorus*

122   *ater*), generated as described in Kelly et al. (2009).   Among sample variation in the

123   laboratory reference material was < 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. In addition, to

124   this laboratory reference we ran one sample each of two National Institute of

125   Standards and Technologies NIST reference materials (USGS 40 in autorun position

126   25 and USGS 41 in autorun position 31).   All stable isotope ratios are expressed in

127   standard δ notation, where $\delta^{13}C$ and $\delta^{15}N$ = [(isotope ratio sample/isotope ratio

128   standard) – 1] * 1000. Consequently, $\delta^{13}C$ and $\delta^{15}N$ are expressed in parts per

129   thousand (‰) deviation from a standard, which was Vienna Pee Dee Belemnite for

130    $\delta^{13}C$ and Air for $\delta^{15}N$. Isotope ratios were measured at the University of Oklahoma

131   using a Thermo Finnigan Delta V isotope ratio mass spectrometer connected to a

132   CosTech elemental analyzer.

133   For each autorun we corrected all measurements for instrumental drift

134   between the first and last sample. Instrumental drift corrections were based on the

135   slopes of best-fit lines for $\delta^{13}C$ and $\delta^{15}N$ values regressed against analysis time of

136   references within each autorun. A slope was calculated for the cowbird standard in

137   the run and this slope was used as the drift correction coefficient.

138   To determine if there was evidence of 24-hour cycling in the $\delta^{15}N$ values

139   along the length of the primaries, we used custom Matlab code to perform an

140   autocorrelation analysis after de-trending the data using linear regression and

141   removing the mean. We used this method to find correlation maxima and minima

142   that reveal periodicity in the $\delta^{15}N$ values. Using additional custom Matlab code, we

143   then developed a bootstrap method to test for the statistical significance of having

144 autocorrelation minima and maxima that correspond to a periodic pattern in

145 isotope values. To do so, we randomly permuted the data for each feather and

146 performed autocorrelation analyses of those permuted values. Out of 10,000

147 permutations, we asked what fraction of the data had both a minimum less than or

148 equal that observed in our original autocorrelation and a maximum spaced at the

149 appropriate interval.

150

151 **RESULTS**

152 **Evidence for equilibration following molt-migration**

153 Two geese, 501 and 508, showed a clear and steady decline in their $\delta^{15}N$ signatures

154 through time (P < 0.0001), while 509, showed no change (P = 0.34, Fig. 1). Both

155 geese that changed did so in a way consistent with the large shift of about 8 ‰ in

156 the $\delta^{15}N$ isoscapes suggested by the results of Fox et al. (2009). The third goose

157 showed no change in $\delta^{15}N$ along the length of its primary and had a mean of 7.6 ‰,

158 a value intermediate between the start and end values for the two geese that

159 showed a steady change in $\delta^{15}N$ with time (Fig. 1), which was puzzling. If this

160 individual had been on Saltholm long enough to be in equilibrium with the salt

161 marsh isoscape, then its mean $\delta^{15}N$ value should have been at or below the latest

162 values from the two geese that showed a steady change in their $\delta^{15}N$ values.

163 However, its mean of 7.6 ‰, was considerably higher than the lowest $\delta^{15}N$ values

164 found for those two geese (Fig. 1), suggesting that it was a resident goose that did

165 not feed in the saltmarshes of Saltholm. Some of the hayfields used by resident

166 Greylag Geese are less subject to marine influence than the saltmarshes where the

167 majority of migrants feed.

168   The $\delta^{13}C$ signature of goose 501 increased significantly along the length of its

169 primary (p= 0.0003), while $\delta^{13}C$ for goose 509 (p = 0.09) and goose 508 (p = 0.46)

170 showed no significant change (Fig. 1). Based on the results of Fox et al. (2009), the

171 $\delta^{13}C$ signatures of these geese should have declined if they had moved from Sweden

172 to Saltholm for the molt. Yet goose 501 showed a significant increase in its $\delta^{13}C$

173 during primary growth (Fig. 1); it also showed a highly significant decline in $\delta^{15}N$,

174 suggesting that it was indeed a molt-migrant to the island of Saltholm (Fig. 1).

175

176   **Evidence for 24 hour cycling in δ¹⁵N**

177   To explore the possibility of 24-hour cycling in the $\delta^{15}N$ values for these three geese,

178   we sampled their primaries as finely as possible, at 1 or 2mm intervals along the

179   length of the feather. Flightless Greylag Geese forage at night on Saltholm (Kahlert

180   et al. 1996). Thus, a 24-hour periodicity in the $\delta^{15}N$ signature should be recovered

181   from serial samples along the length of the feathers for geese 501 and 508, the two

182   indiviuduals that were coming into equilibrium with the Saltholm marine

183   environment (Fig. 2). In contrast, goose 509, which showed no change in $\delta^{15}N$ along

184   the length of its primary, was not expected to show a 24-hour periodicity in its $\delta^{15}N$

185   values because it apparently was in equilibrium with its Saltholm diet. The length of

186   these fine-resolution runs was shorter than the length of feather used to generate

187   Figure 1 because of sampling problems and a malfunction of the mass

188   spectrophotometer.

189   Autocorrelation results for $\delta^{15}N$ measured in these serial samples are

190   summarized in Figure 2. Note that, because there is significant sampling noise, the

191   signal is not purely periodic in any of the sampled feathers. Feather 508 shows a

192   minimum (most negative) autocorrelation value at the second autocorrelation lag.

193   Given a sampling interval of 2 mm, this corresponds to 4 mm of feather length.

194   Additionally, 508 showed positive autocorrelation values in the region of twice the

195   minimum, strongly indicating periodicity in the data. The bootstrap statistics

196   indicated that the probability of having the combination of a minimum at the lag of 2

197   and a max near the lag of 4 is P = 0.032. Feather 501 had a different sampling

198   interval and, accordingly, showed a more expanded autocorrelation function with a

199   minimum at lag of 3 and a maximum autocorrelation at twice that value. The

200   bootstrap probability of having that combined maximum and minimum was P= 0.01.

201   Thus, both feathers 501 and 508 show significant periodicity in their $\delta^{15}N$ values. In

202   contrast, feather 509, which showed no sign of coming into a new equilibrium for

203   $\delta^{15}N$, never showed a significant change in sign for the autocorrelation, suggesting,

204   as predicted, that there was no periodic signal underlying the data for this feather.

205

206 **DISCUSSION**

207 **The value of sampling feathers serially**

208 As far as we are aware, the data for $\delta^{15}N$ in Figure 1 constitute the first direct test of

209 a gradual change in the isotopic composition of feathers being grown while a molt-

210 migrant is coming into equilibrium with a new isoscape. Fox et al. (2009) inferred

211 this process by sampling food plants used by Greylag Geese on their breeding

212 grounds in southern Sweden, and on their saltwater molting grounds on the island

213 of Saltholm in Denmark. This inference was based on the assumption that

214 fractionation values for the conversion of $\delta^{15}N$ values in food plant to $\delta^{15}N$ values in

215 goose feather were accurately represented by the results of an experimental study

216 of Japanese Quail (*Coturnix japonica*) raised on a plant based diet (Hobson and Clark

217 1992a; Hobson and Clark 1992b). How well those values represent similar

218 processes in Greylag Geese is an unknown, as are the confidence intervals

219 associated with these transformations. Further, Fox et al. (2009) used only two food

220 plants from each locality to infer the expected changes in $\delta^{15}N$ and $\delta^{13}C$ values for

221 feathers, yet Greylag Geese probably use a larger diversity of plants at each of these

222 localities, as is known to be the case on Saltholm as the molt progresses (Fox et al.

223 1998). For these reasons, the direct measure of change along the length of the

224 feather that we have established for two of the three Greylag primaries in our

225 sample offers a powerful confirmation of the result obtained by Fox et al. (2009) for

226 $\delta^{15}N$. Their mean value for $\delta^{15}N$ of 8.4 ‰ for feathers from 12 molting geese is

227 reasonably close to the mean of 7.6 ‰ for the two geese in our sample that were

228 equilibrating with the Saltholm environment. The mean $\delta^{15}N$ for the goose that

229 showed no evidence of equilibrating was also 7.6‰ (509), considerably higher than

230 the latest (most proximal) $\delta^{15}N$ values for the geese that showed strong declines in

231 $\delta^{15}N$ (Fig. 1). The relatively high mean $\delta^{15}N$ for goose 509, together with the lack of

232 change in $\delta^{15}N$ along its feather, suggests that this goose had not arrived early and

233 delayed the start of its molt until reaching equilibrium with the Saltholm isoscape.

234 Possibly it was a Saltholm resident with a different diet.

235   Fox et al. (2009) suggested that $\delta^{13}C$ also changed in a way that suggested the

236 use of endogenous C in the generation of primary feathers during the molt.

237   However, the absolute difference in the expected values for $\delta^{13}C$ (again, generated

238   by sampling two food plants from the Swedish breeding grounds and two food

239   plants from the Saltholm molting grounds) was less than 2 ‰. While differences as

240   small as 2 ‰ can reliably be measured, predicting differences this small by applying

241   fraction values to the $\delta^{13}C$ values measured to samples of two food plants consumed

242   by geese at their breeding and molting sites seems hazardous. With their sample of

243   12 geese, Fox et al. (2009) did find the feather values to be intermediate between

244   the food values for Sweden and Saltholm, using the conversion figures for Japanese

245   Quail (Hobson and Clark 1992b). Our mean $\delta^{13}C$ value of 26.2 ‰ for the three

246   feathers we analyzed is close to their mean of 26.5 ‰ based on 12 geese (Fox et al.

247   2009). However, the difference in the inferred values for feather tissue generated

248   from Swedish reserves and from Saltholm plant material suggests that $\delta^{13}C$ values

249   should decline during primary growth. Yet, we found no evidence for such a decline

250   in the three feathers we examined: two geese showed no change, while the third

251   showed a significant increase in $\delta^{13}C$ along the length of its primary (Fig. 1).

252   Further, goose 501 that showed an increase in $\delta^{13}C$ values showed a strong decline

253   in $\delta^{15}N$ values along the length of its growing primary, so we know that this

254   individual was not yet in equilibrium with the Saltholm $\delta^{15}N$ isoscape. The positive

255   slope for $\delta^{13}C$ in this goose further suggests that the expected difference in feather

256   $\delta^{13}C$, estimated from food plants sampled in Sweden and Saltholm (Fox et al. 2009),

257   was not reliable.

258

259   **Stored reserves and 24-hour cycling**

260   As predicted the two geese that showed strong declines in $\delta^{15}N$ along the length of

261   their growing primaries also showed 24-hour cycling in their $\delta^{15}N$ values. Further,

262   the goose that showed no change in $\delta^{15}N$ along the length of its primary showed no

263   evidence of 24-hour cycling, a result we predicted because this goose was not

264   coming into equilibrium with the Saltholm $\delta^{15}N$ environment. These results support

265   the use of endogenous reserves for feather growth during parts of the 24-hour cycle

266   when geese do not forage and feathers continue to grow (Murphy and King 1990).

267   The periodicity of this cycling can also be estimated from the autocorrelation

268 analysis and that periodicity should match feather growth rates. Our

269 autocorrelation analyses revealed periodicities corresponding to growth rates of

270 roughly 8 and 6 mm d^{-1} for feathers 509 and 501, respectively. These periodicity

271 values accord well with the estimated primary growth rate of about 7 mm d^{-1} for a

272 bird the size of a Greylag Goose (Rohwer et al. 2009). Finer sampling than could be

273 achieved by our equipment would presumably eliminate the noise in our

274 autocorrelation results resulting from 1 or 2 mm sampling intervals, leaving only

275 noise associated with day-to-day differences in food intake and feeding times

276 (Moran et al. 2011).

277

278 **General**

279 Bridge et al. (2011) assessed the possible use of endogenous protein reserves for

280 molting by studying changes in δD and δ^{13}C in the primaries of Painted Buntings

281 (*Passerina ciris*). Like many other migrant song birds that breed in the central and

282 southern regions of western North America, Painted bunting from the Midwestern

283 breeding population migrate to northwest Mexico for their annual post-breeding

284 molt (Thompson 1991; Rohwer et al. 2005; Rohwer 2013). Here they exploit a food

285 flush generated by the late summer monsoon, which delivers most of the annual

286 precipitation to this region of northwest Mexico in July – September (Adams and

287 Comrie 1997; Comrie and Glenn 1998). Primary replacement in Painted Buntings in

288 Sinaloa is so rapid that it requires an average of only 30 and 34 days in adult females

289 and adult males, respectively (Rohwer 2013).

290   Bridge et al. (2011) showed that both δD and δ^{13}C values changed from

291 primary 1 to 9 in some Painted Buntings sampled in Sinaloa. They suggest that

292 birds that showed differences between primaries 1 and 9 should be individuals that

293 had initiated molt shortly after arriving in Sinaloa, before their endogenous protein

294 reserves reached equilibrium with the Sinaloa isoscape. Individuals that failed to

295 show strong differences between these primaries either may have delayed molt

296 until their endogenous protein reserves were in equilibrium with the Sinaloa

297 isoscape, or the food they consumed before migrating may have matched what they

298 were consuming on their Sinaloa molting grounds. Direct evidence of continuous

299   change in δD and $\delta^{13}C$ is needed to test the suggestion by Bridge et al. (2011) that

300   buntings showing strong differences in δD and $\delta^{13}C$ signatures between primaries 1

301   and 9 were coming into equilibrium with a new isoscape while molting. This could

302   now be accomplished by sampling across different primaries on the feather-time

303   axis developed by Rohwer and Broms (2012) spanning the replacement of all

304   primaries.

305   In general, measuring isotopic changes in serial samples taken at equal time

306   intervals from flight feathers offers a powerful tool for studying molt-migration. It

307   provides strong data for individual birds while avoiding the assumptions involved

308   with food sampling and using fraction estimates to compute expected tissue values

309   for isotopes. Samples from the primaries that represent equal time intervals can be

310   generated in two ways. For large birds, finely spaced serial samples taken along the

311   length of a primary can give such good data that the evidence of general changes can

312   even be confirmed by evidence of 24-hour periodicity in isotope measurements, as

313   we have shown here for Greylag Geese. For small birds, serially sampling single

314   flight feathers generates only a limited temporal series of samples and the flight

315   feathers of small birds grow too slowly (Rohwer et al. 2009) for traditional sampling

316   methods to achieve a sample density sufficient to detect 24-hour cycling. However,

317   samples representing approximately equal time intervals across the full primary

318   molt can be taken from different primaries (Rohwer and Broms 2012), thus greatly

319   extending the sampling period to the time required to replace all 9 or 10 primaries.

320   Such sampling, coupled with information on how soon after arrival molt-migrants

321   initiate primary replacement, could give excellent empirical data on the time

322   required to reach equilibrium following changes in isoscapes by small wild birds.

323

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332

333   **REFERENCES**

334   Adams, D. K., and A. C. Comrie. 1997. The North American Monsoon. *Bulletin of the*

335   *American Meterological Society* 78:2197-2213.

336   Bridge, E. S., F. A. M., K. J. F., C. A., and S. Rohwer. 2011. Causes of bimodal stable

337   isotope signatures in the feathers of a molt-migrant songbird. *Canadian Journal*

338   *of Zoology* 89:951-959.

339   Cerling, T. E., G. Wittemyer, H. B. Rasmussen, F. Vollrath, C. E. Cerling, T. J. Robinson,

340   and I. Douglas-Hamilton. 2009. Stable isotopes in elephant hair document

341   migration patterns and diet changes. *Proceedings of the National Academy of*

342   *Science, USA* 103:371-373.

343   Church, M. E., R. Gwiazda, R. W. Risebrough, K. Sorenson, C. P. Chamberlain, S. Farry,

344   W. Heinrich, B. A. Rideout, and D. R. Smith. Ammunition is the principal source

345   of lead accumulated by California Condors re-introduced into the wild.

346   *Environmental Science and Technology* 40:6143-6150.

347   Comrie, A. C., and E. C. Glenn. 1998. Principal components-based regionalization of

348   precipitation regimes across the southwest United States and northern Mexico,

349   with an application to monsoon precipitation variability. *Climate Research*

350   10:201-215.

351   Dunning, J. B., Jr. 2007. CRC Handbook of Avian Body Masses, Second ed. CRC Press,

352   London.

353   Fox, A. D., K. A. Hobson, and J. Kahlert. 2009. Isotopic evidence for endogenous

354   protein contributions to Grelag Goose *Anser anser* flight feathers. *Journal of*

355   *Avian Biology* 40:108-112.

356   Fox, A. D., J. Kahlert, and H. Ettrup. 1998. Diet and habitat use of moulting Greylag

357   Geese Anser anser on the Danish island of Saltholm. *Ibis* 140:676-683.

358   Fox, A. D., J. Kahlert, H. Ettrup, L. Nilsson, and J.-P. Hounisen. 1995. Moulting Greylag

359   Geese on the Danish island of Saltholm; numbers, phenology, status and origins.

360   *Wildfowl* 46:16-30.

361   Hobson, K. A., and R. G. Clark. 1992a. Assessing avian diets using stable isotopes. 2.

362   Factors influencing diet-tissue fractionation. *Condor* 94:189-197.

363   Hobson, K. A., and R. G. Clark. 1992b. Assessing avian diets using stable isotopes. I:

364   Turnover of carbon-13 in tissues. *Condor* 94:181-188.

365   Hohman, W. L., C. D. Ankney, and D. H. Gordon. 1992. Ecology and management of

366   postbreeding waterfowl. Pages 128-189 in Ecology and management of

367   breeding waterfowl. (B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H.

368   Johnson, J. A. Kadlek, and G. L. Krapu, Eds.). University of Minnesota Press.,

369   Minneapolis.

370   Kahlert, J., A. D. Fox, and H. Ettrup. 1996. Nocturnal feeding in moulting Greylag

371   Geese Anser anser - an anti-predator response? *Ardea* 84:15-22.

372   Kelly, J. F., E. S. Bridge, A. M. Fudickar, and L. I. Wassenaar. 2009. A test of

373   comparative equilibration for determining non-exchangeable stable hydrogen

374   isotope values in complex organic materials. *Rapid Communications in Mass*

375   *Spectrometry* 23:2316-2320.

376   Lillie, F. R., and W. H. 1940. Physiology of development of the feather: IV. The diurnal

377   curve of growth in the brown leghorn fowl. *Proceedings of the National Academy*

378   *of Science, USA* 26:67-85.

379   Martinez del Rio, C., and R. Anderson-Sprecher. 2008. Beyond the reaction progress

380   variable: the meaning and significance of isotope incorporation data. . *Oecologia*

381   156:765-772.

382   Moran, J. J., M. K. Newburn, M. L. Alexander, R. L. Sams, J. F. Kelly, and H. W. Kreuzer.

383   2011. Laser ablation isotope ratio mass spectrometry for enhanced sensitivity

384   and spatial resolution in stable isotope analysis. *Rapid Communications in Mass*

385   *Spectrometry* 25:1282–1290.

386   Murphy, M. E., and J. R. King. 1986. Diurnal constancy of feather growth-rates in

387   White-crowned Sparrows exposed to various photoperiods and feeding

388   schedules during the postnuptial molt. *Canadian Journal of Zoology-Revue*

389   *Canadienne De Zoologie* 64:1292-1294.

390   Murphy, M. E., and J. R. King. 1990. Diurnal changes in tissue glutathione and protein

391   pools of molting White-crowned Sparrows - the influence of photoperiod and

392   feeding schedule. *Physiological Zoology* 63:1118-1140.

393   Nilsson, L., J. Kahlert, and H. Persson. 2001. Moult and moult migration of Greylag

394   Geese Anser anser from a population in Scania, South Sweden. *Bird Study*

395   48:129-138.

396   Paritte, J. M., and J. F. Kelly. 2009. Effect of cleaning regime on stable-isotope ratios

397   of feathers in Japanese Quail (Coturnix japonica). *Auk* 126:165–174.

398   Rohwer, S. 2013. Molt Intensity and Conservation of a Molt-migrant (*Passerina ciris*)

399   in Northwest Mexico. *Condor* 115:421-433.

400   Rohwer, S., and K. Broms. 2012. Use of feather lopss intervals to estimate molt

401   duration and to sample feather vein at equal time intervals throughout the

402   primary replacement. **Auk** 129:653-659.

403   Rohwer, S., L. K. Butler, and D. R. Froehlich. 2005. Ecology and demography of east-

404   west differences in molt scheduling in Neotropical migrant passerines. Pages

405   87-105 in Birds of Two Worlds (R. Greenberg, and P. P. Marra, Eds.). Johns

406   Hopkins University Press, Baltimore.

407   Rohwer, S., R. E. Ricklefs, V. G. Rohwer, and M. M. Copple. 2009. Allometry of the

408   duration of flight feather molt in birds. *PLoS Biology* 7:e1000132.

409   Schieltz, P. C., and M. E. Murphy. 1995. Diurnal variation in oxygen consumption by

410   molting and nonmolting sparrows. *Comparative Biochemistry and Physiology a-*

411   *Physiology* 112:265-272.

412   Thompson, C. W. 1991. The sequence of molts and plumages in Painted Buntings

413   and implications for theories of delayed plumage maturation. *Condor* 93:209-

414   235.

415

416

Figure 1. Values for δ15N and δ13C measured in serial samples along the length of the primary. Feather vein was sampled at 5mm intervals near the rachis of the growing primary for its full length, starting at the tip of the feather.
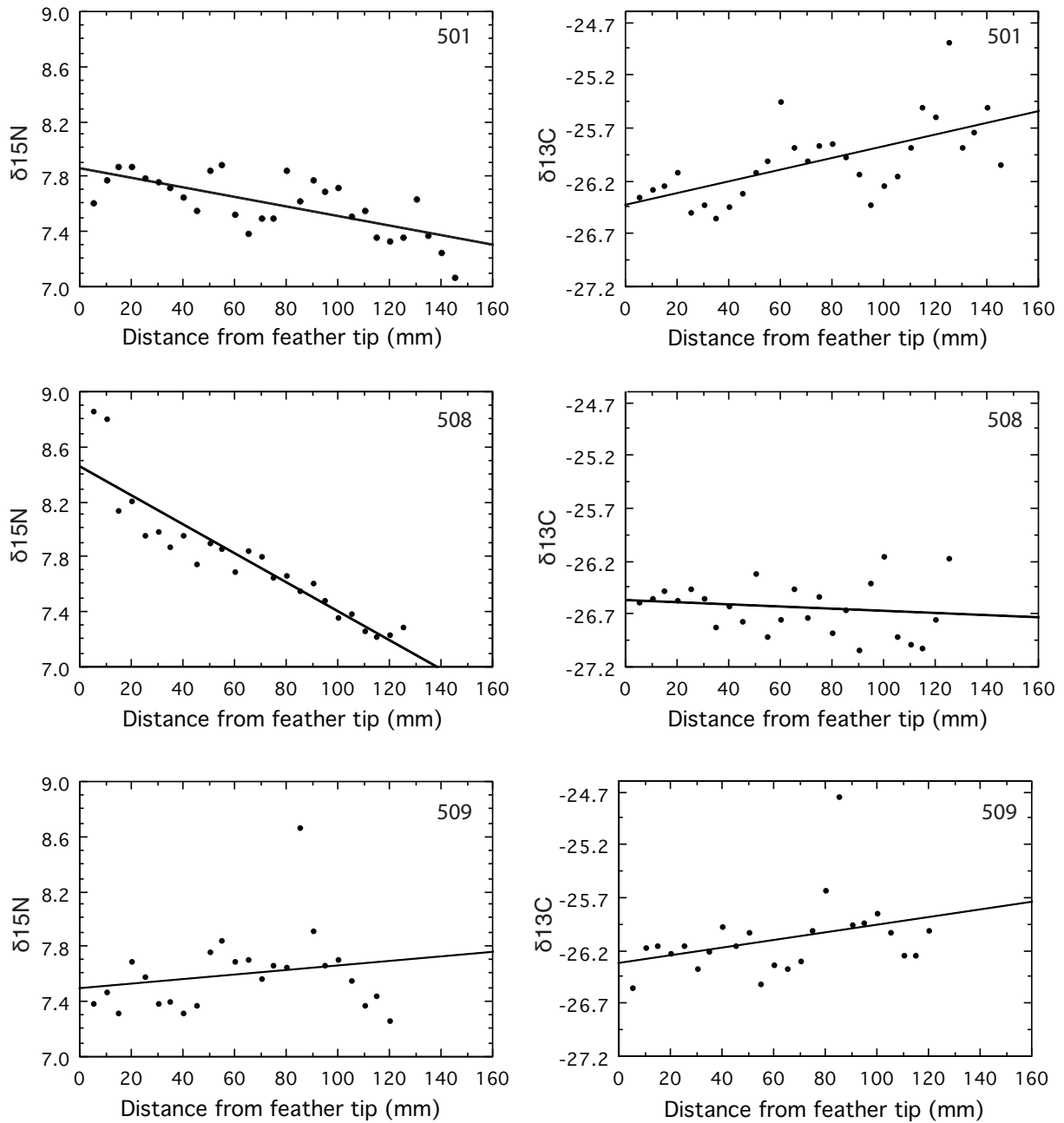
Figure 2. Autocorrelation and regression results for δ15N measured at 1 or 2 mm intervals from the tips of growing primaries. Greylag Geese 501 and 508 showed decreasing δ15N values, indicating they were equilibrating with the Saltholm isoscape as their primaries were growing, and both showed significant autocorrelations (p = 0.01 and 0.03, respectively). Goose 509 showed no change in its δ15N values and no autocorrelation (p > 0.25).