Running head: *Mass of moulting Black-necked Grebes*

Late-moulting Black-necked Grebes *Podiceps nigricollis* show greater body mass in the face of failing food supply

ANTHONY D. FOX^{1*} CRISTINA RAMO,² NICO VARO,² MARTA I SÁNCHEZ,² JUAN A. AMAT,² & ANDY J. GREEN²

¹Department of Bioscience, Aarhus University, Kalø, Grenåvej 14, DK-8410 Rønde, Denmark,

²Department of Wetland Ecology, Estación Biológica de Doñana, C.S.I.C., Calle Américo Vespucio s/n, E-41092 Sevilla, Spain

*Corresponding author.

Email: tfo@dmu.dk

From August to December, thousands of Black-necked Grebes *Podiceps nigricollis* concentrate in salt ponds in the Odiel Marshes, southern Spain during the flightless moult period, where they feed on brine shrimps *Artemia parthenogenetica*. We hypothesised that because grebes moulted in a food-rich, predator-free environment, there would be no net loss of body mass caused by the use of fat stored to meet energy needs during remigial feather replacement (as is the case for some other diving waterbirds). However, because the food resource disappears in winter, we predicted that grebes moulting later in the season would put on more body mass prior to moult, because of the increasing risk of an *Artemia* population crash before the moult period is completed. Body mass determinations of thousands of grebes captured during 2000-2010 showed that grebes in active wing-moult showed greater mass with date of capture. Early-moulting grebes were significantly lighter at all stages than later moulting birds. Grebes captured with new feathers post-moult were significantly lighter than those in moult. This is the first study that supports the hypothesis that individual waterbirds adopt different strategies in body mass accumulation according to timing of moult: early-season grebes were able to acquire an excess of energy over expenditure and accumulate fat stores whilst moulting. Delayed moulters acquired greater fat stores in advance of moult to contribute to energy expenditure for feather replacement and retained extra stores later, most likely as a bet hedge against the increasing probability of failing food supply and higher thermoregulatory demands late in the season. An alternative hypothesis, that mass change is affected by a trophically transmitted cestode using brine shrimps as intermediate host and the grebes as final host, was not supported by the data.

30

31 **Keywords:** energy budget, fat stores,moult migration, phenotypic plasticity,wing-moult

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34 Many waterbirds undergo simultaneous replacement of their wing feathers in a fashion that renders
35 them completely flightless for a period (Woolfenden 1967). Such a flightless period during
36 remigial replacement is critical in the annual cycle, and in many waterbirds is associated with a
37 moult migration to geographically remote, but specific habitats where the risk of predation is
38 substantially reduced (Salomonsen 1968, Jehl 1990). Feathers that must sustain flight throughout
39 the year must be regrown simultaneously, in safety, without damage. This synchronous feather
40 replacement denies the individual powers of flight and therefore (i) constrains them to a locality
41 where they potentially deplete the local food supply and (ii) denies escape flights from predators.
42 Many waterbirds undergoing simultaneous wing-moult also show physiological adaptations to meet
43 the elevated energy demands during this period, including changes in organ mass (Fox & Kahlert
44 2005), flight and leg muscle atrophy and hypertrophy (Ankney 1979, 1984) and increased metabolic
45 rate (Portugal *et al.* 2007). Several dabbling duck species, such as the Mallard *Anas platyrhynchos*
46 (Panek & Majewski 1990, Fox *et al.* 2013), Teal *A. crecca* (Sjöberg 1988), Wigeon *A. penelope*,
47 Gadwall *A. strepera* (King & Fox 2012) and Mottled Duck *A. fulvigula* (Moorman *et al.* 1993)
48 accumulate fat prior to the flightless period which they deplete during moult (e.g. Moorman *et al.*
49 1993, Fox & Kahlert 2005,Fox *et al.* 2013). Such adaptations to supplementing energy needs from
50 body stores during flightlessness are likely long established: it is estimated that the extinct 70-kg
51 raptor, *Argentavis magnificens*, could only have undergone simultaneous moult by living off fat
52 reserves for the duration of the flightless period (Rohwer *et al.* 2009). Use of such energy stores to
53 at least partially meet existence energy demands could allow birds to reduce activities and hence

54 reduce exposure to predation and predators, even though exogenous energy sources fulfil the
55 majority of their needs during moult.

56

57 In the case of a single female Pochard *Aythya ferina*, however, the stores of endogenous fat were
58 almost sufficient to meet her entire existence energy needs during the flightless moult period (Fox
59 & King 2011). Studies suggest that many Anatidae species reduce activities considerably during
60 moult (Döpfner 2009, Portugal *et al.* 2010), so depletion of fat stores acquired prior to flightlessness
61 may represent a strategy to minimising risk of predation during remigial growth. However, some
62 diving duck species show no significant change in body mass throughout remigial moult (e.g.
63 Redheads *Aythya americana*, Bailey 1981, 1985; Ring-necked Duck *Aythya collaris* males, Hohman
64 *et al.* 1988; Common Scoter *Melanitta nigra* males, Fox *et al.* 2008) and continue to dive for food
65 (e.g. in the Common Eider *Somateria mollissima* Guillemette *et al.* 2007). In these cases, it would
66 seem that flightless birds are able to re-grow flight feathers in the shortest possible time by
67 exploiting low-predation-risk habitats in which to moult, but which provide adequate exogenous
68 food resources to meet the specific nutritional demands of feather replacement. Under these
69 circumstances, moulting birds have no need to exploit endogenous stores to balance energetic and
70 nutritional budgets during flightlessness, and suffer no net loss of body mass before regaining the
71 powers of flight.

72

73 The North American Black-necked Grebe *Podiceps nigricollis* is a well-known moult migrant (~~Jehl~~
74 ~~& Henry 2010~~), with up to two million individuals breeding in ~~North America drawing from~~
75 extensive prairie ~~breeding~~ areas ~~to and~~ moulting on just two alkaline lakes, Mono Lake (California)
76 and Great Salt Lake (Utah), where they feed on abundant brine shrimps *Artemia franciscana* safe
77 from natural predators (Storer & Jehl 1985, Jehl 1997, Jehl & Henry 2010). At least one such

78 aggregation was known in Eurasia, with up to 186 000 recorded moulting at the hypersaline alkaline
79 lake Burdur Gölü in Turkey (Hagermeir & Blair 1997). This lake has been subject to considerable
80 hydrological change (see Green *et al.* 1996) and currently supports no obviously abundant food
81 source, nor similar numbers of moulting birds in recent years (Girgin *et al.* 2004, Gülle *et al.* 2010).
82 The attraction of such large numbers of birds to rich feeding sources, where extreme water
83 chemistry ensures low species diversity but high food biomass and a general lack of predators of
84 flightless waterbirds suggests that the Black-necked Grebe has no need to exploit endogenous stores
85 to balance energetic and nutritional budgets during wing feather replacement. However, the pattern
86 of body mass variations in moulting Black-necked Grebes is different from those reported for
87 waterfowl, and even another grebe species (Piersma 1988). Indeed, Black-necked Grebes in North
88 America start fattening just after arriving at moulting sites, and continue to do so during moult (Jehl
89 1988). These North American studies showed that the grebes also doubled the mass of the liver,
90 stomach and intestines, as well as increasing the heart mass and showing the coordinated
91 atrophy/hypertrophy pattern of breast muscle and hypertrophy/atrophy of leg muscle consistent with
92 their patterns of use during flightless moult (Jehl 1997). It has been suggested that the body reserves
93 of fat act as an insurance against the annual collapse of the main prey of grebes, brine shrimps, in
94 late autumn (Jehl 1988). Although brine shrimps are small, they are super-abundant where they
95 occur and are rich in highly digestible lipids and protein (Caudell & Conover 2006a, Varo *et al.*
96 2011). If body mass gain is an anticipatory response to sudden prey disappearance, it may be
97 expected that gains in body mass should be greater as the season advances, as the probability of the
98 collapse of the prey population increases with date (Cooper *et al.* 1984, Jehl 1988, Caudell &
99 Conover 2006b). In this paper, we test this hypothesis using flightless Black-necked Grebes caught
100 in salt ponds at the Odiel marshes, southern Spain, where more than 10 000 birds from breeding

101 areas across Europe congregate from August to December to moult remiges, and where prey
102 populations collapse after the end of October (Sánchez *et al.* 2006a, Varo *et al.* 2011).

103

104 METHODS

105 The Odiel marshes (37°14'N, 6°57'W) comprise an estuarine complex of 7185 ha at the mouths of
106 the rivers Odiel and Tinto in Huelva Province, southern Spain. They include 1174 ha of salt pans,
107 including 1118 ha of intensively managed areas, where sea water is pumped from primary
108 to secondary evaporation areas and finally to crystallizers, successively increasing water salinity (see
109 Sánchez *et al.* 2006a for more details). In this study, grebes were mainly caught in four ponds (80 cm
110 mean deep) within the secondary evaporation zone, where the brine shrimp is the most abundant
111 invertebrate, attracting most moulting grebes (Sánchez *et al.* 2006a). Grebes are absent
112 from crystallization ponds and less abundant in the primary than secondary evaporation ponds. In the
113 following analyses, data from caught birds were pooled from all secondary ponds because they were
114 adjacent, hydrologically connected, showed similar salinity levels and experienced regular
115 interchange of both grebes and shrimps.

116

117 The moulting period of Black-necked Grebes at Odiel extended from mid-summer (late June) until
118 early winter (mid-December, Varo *et al.* 2011). During this period brine shrimp *Artemia*
119 *parthenogenetica*, the main prey of grebes obtained by sub-surface diving, suffers a sharp decline in
120 abundance at a variable date in early winter (from late October onwards) related to cold winter
121 temperatures (see Sánchez *et al.* 2006a, Varo *et al.* 2011). We used a database generated by a
122 ringing programme at the Odiel Marshes, southern Spain initiated in 1993 by the Monitoring Team
123 of the Doñana Biological Station. During ringing operations, captured Black-necked Grebes were
124 aged according to iris coloration following Storer and Jehl (1985) and body mass was measured to

125 the nearest 5 g. Depending on the state of the remiges, birds were assigned to one of six categories
126 (modified from Storer & Jehl 1985): old (i.e. unmoulted) remiges, moult 1 (recently shed), moult 2
127 (remiges less than half-grown), moult 3 (remiges around $\frac{3}{4}$), moult 4 (remiges almost fully grown,
128 but with remainders of sheath) and new (i.e. fully regrown) remiges. Although some young of the
129 year were captured (identified by a pale eye), these were not included in this study as they do not
130 undergo wing-moult in their first winter. On this basis, data from 5680 grebes captured and weighed
131 between 3 August and 16 December from 2000 to 2010 inclusive were available for analyses.
132 Although many of these birds were recaptured, only the data from initial capture were used to avoid
133 any potential risk of capture trauma affecting body mass and to avoid pseudoreplication. Of these
134 birds, 549 had old feathers and were yet to commence moulting, 1251 were moulting and 3880 had
135 new feathers and had thus completed moult. Day of capture (based on number of days after 1
136 January each year) ranged from 215 to 350 (mean 283.1 ± 0.386 SE). Of these, 5597 grebes were
137 sexed visually by one experienced observer (Luis Garcia), based on head shape (molecular sexing
138 has shown this to have an accuracy of 85%, [n = 307](#), unpubl. data). Combined head and bill length
139 plus wing length (from the folded carpal joint to the tip of the longest regrowing/grown primary
140 feather) were also measured with callipers to the nearest mm for a reduced set of 2640 individuals.
141 Results for this smaller dataset are not fully presented, as they were entirely consistent with those
142 for the complete dataset.

143

144 Variation in log transformed body mass was analysed as the dependent variable using a General
145 Linear Model. Predictor variables included year, moult status and sex as fixed factors, and date of
146 capture and head-bill length as continuous variables (note not all predictors were applied in all
147 models, see below). Models pooling all moulting birds together in one level of a factor were
148 conducted, as well as those separating the four moult sub-classes defined above. We also subdivided

149 captured individuals into ‘early’ and ‘late-moulters’ based on whether the individual’s date of
150 capture fell before or after the mean capture date (i.e. day 283, see above), to test whether mass
151 differed between these two groups. Because earlier tests detected differences between the sexes, we
152 use GLM and Tukey LSD tests to test for differences in mass at different stages of moult in males
153 and females, early- and late-moulters. Body mass was natural log transformed to remove
154 heteroscedasticity. Analyses were conducted in Statistica 11 package (StatSoft, Inc. 2012).

155

156 RESULTS

157 Body mass of grebes was found to depend strongly on date of capture, moult status and sex (Table
158 1). Males were heavier than females and mass was greater as the season progressed. Fitting of non-
159 linear LOWESS regression lines for the plot of date against mass confirmed a linear increase in
160 mass over time. The addition of a date squared term ~~intodid not enter~~ the GLM revealed a slight
161 deviation from a strictly linear trend, ~~however,~~ date ($F_{1,5578} = 151.4, P < 0.0001$) had a much stronger
162 partial effect than date squared ($F_{1,5578} = 11.4, P < 0.001$) when both were included in a GLM.
163 Addition of date squared had very little impact on the effect of the full model (change in $r^2 = 0.170$
164 to 0.171) ~~with a significant effect and is not presented here, further indicating that the so,~~ for
165 simplicity, data-date squared was excluded from further analyses ~~effect was linear~~. A plot of body
166 mass against date for all years combined for both sexes is provided in Supporting Information
167 Figure 1.

168 Both raw data and the GLM estimates indicated that body mass was lowest prior to moult, peaked
169 during moult, then declined after moult (Table 1, Fig. 1). When all moulting birds were pooled in
170 the same level of a factor in a separate GLM (otherwise equivalent to that of Table 1), moulting
171 grebes were significantly heavier than those which had completed moult, which in turn were
172 significantly heavier than birds which had yet to commence moult ($P < 0.001$ for all pairwise LSD

173 post-hoc tests). Early-moulters were significantly lighter at all stages than late-moulters in both
 174 sexes and mean mass of moulting birds was significantly greater during moult than before (Fig. 1).
 175 Birds captured with new feathers post moult were significantly lighter than during moult (Fig. 1).
 176 When a date x moult status interaction was added to the pooled model, it was highly significant
 177 (Table 2). Although mass increased with date for all three classes of grebes, the difference in mass
 178 between early- and late-moulting birds yet to moult was greater than between early- and late-
 179 moulting birds that had moulted ($t = 4.03$, $df = 5580$, $P = 0.0007$). Mass difference between early-
 180 and late-moulting birds that had moulted was greater than between early- and late-moulting birds
 181 which were moulting ($t = 4.78$, $df = 5580$, $P = 0.0003$). Repeating this GLM using untransformed
 182 body mass for guidance gave similar results and estimates indicated that for every day later that
 183 they were caught, birds were 0.47 g (± 0.046 se) heavier when they have not yet moulted, 0.10 g
 184 (± 0.043) heavier when they were moulting and 0.30 g (± 0.030) heavier when they completed
 185 moult.

186 GLMs for a smaller dataset with full morphometric data showed the head-bill length was a strong
 187 predictor of body mass, and its inclusion meant that sex was no longer a significant predictor of
 188 body mass. A GLM with moult status, year, head-bill length and capture date as predictors produced
 189 a full model with an r^2 of 0.263 (detailed results not shown). The reduced sample size meant that
 190 fewer of the pairwise differences between moult categories and between levels of the moult x date
 191 interaction were statistically significant. Otherwise, the model estimates were very similar.

192 ~~Neither year, sex and capture date contributed to the model with~~When head-bill length was made
 193 the dependent variable with year, sex and capture date as predictor variables, there was no evidence
 194 of a change in bill length with date (GLM, $F_{1,2568} = 0.12$, $P = 0.73$), hence change in body mass was
 195 not associated with the arrival of birds of different structural size.

196

197 DISCUSSION

198 These results show that later moulting Black-necked Grebes of both sexes at Odiel Marshes were
199 consistently heavier than those moulting earlier in the season. This supports the hypothesis that the
200 greater body mass in the early stages of moulting (regardless of date) could be a strategy to ensure
201 the acquisition of sufficient body stores to meet the energetic demands needed to complete the
202 moulting process if an unforeseen decrease in the temperature causes a decline in shrimp abundance
203 (Jehl 1988), a risk that increases in probability through the season. An alternative interpretation
204 could be that birds of different body mass simply moult at different times, but nevertheless, there is
205 an advantage to maintaining higher body mass later in the season as a hedge against the collapse of
206 the food supply.

207

208 We were able to detect a strong seasonal trend with higher body mass in later moulting grebes (see
209 also Jehl 1988 and Caudell & Conover 2006b), including those pre-moulting, during moult and
210 post-moult. Although body mass is strongly influenced by structural size (Peig & Green 2009), we
211 infer that the trends in body mass in our case reflect a change in the size of energy stores, since
212 there was no change over time in the head-bill length of the birds. This may reflect the general trend
213 of accumulating mass while feeding on the abundant brine shrimps, i.e. all individuals may tend to
214 put on mass over time while they stay at Odiel. Alternatively, birds arriving with greater mass could
215 potentially moult later, irrespective of local food availability, but sequential recaptures of grebes
216 suggest this was not the case (unpubl. data). Generally, birds caught later in the season may have
217 spent more time at Odiel prior to capture, and thus put on more mass. Alternatively, birds moulting
218 later may have arrived later, but commenced moult at a higher mass having accumulated greater fat
219 stores given the seasonal increase in probability of a sudden decline in prey availability before

220 moult is complete. The day to day change in mass of pre-moulting birds may be particularly rapid
221 because they increase feeding rates in response to water temperature (see Varo *et al.* 2011 for
222 extensive data), prey density, day-length or a combination of all these. Certainly Black-necked
223 Grebes at Odiel Marshes show a strong correlation between prey density and time spent feeding,
224 increasing foraging effort from 19-40% of all daylight activities in August-October to 53-74% in
225 November-December in the face of falling prey densities (Varo *et al.* 2011) as is known from Great
226 Salt Lake in North America (Caudell & Conover 2006b). Hence, moult migrants arriving late to the
227 site could accumulate more fat stores prior to moult potentially as a buffer against a later prey crash.
228 This is supported by the greater body mass of grebes bearing old feathers progressively through the
229 moult period, although (as explained below) detailed information about individual behaviour would
230 be required to determine how rates of mass accumulation change in relation to timing of moult.
231 Furthermore, grebes at the site had highest body mass in 2009, a year with higher *Artemia*
232 abundance and water temperatures, suggesting the acquisition of fat stores is influenced by
233 energetic gains when thermoregulation costs are low and the food supply is good (Varo *et al.* 2011).
234
235 Inevitably, we could not distinguish between the grebes caught early in the season that were about
236 to moult and those that would delay feather replacement and accumulate additional mass to moult in
237 the late-season, so we require sequential recapture histories of individually marked birds to know if
238 the lower mass amongst pre-moulting grebes early in the season was due to birds arriving with
239 depleted body stores that would acquire fats stores at the site and moult much later. Such recapture
240 histories would have to account for any potential effects of the stress of recapture on body mass.
241 Nevertheless, changes in the mass of grebes undergoing moult confirm that earlier moulting birds
242 were consistently lighter than those initiating moult later.

243

244 The data presented suggest that Black-necked Grebes do not strongly catabolize somatic lipid stores
245 to meet energy demands during moult at this site, since body mass either became greater as the
246 season progressed (in the early stages of early-moulters) or was stable (late-moulters) supporting
247 the results from a previous study (Jehl 1988). The protein needs of feather growth are modest
248 compared to changes in fat stores during moult, and even if met from stored protein accumulated
249 prior to wing moult, its effect on overall body mass (together with any change in water content)
250 would equate to a few grams (Jehl 1988).

251

252 Although moulting waterbirds take to the safety of water to avoid predation during the flightless
253 moult, in certain situations they are exposed to risk from aquatic predators (e.g. Moore 2001, Elsey
254 2004, Fox *et al.* 2010), but in the case of the Black-necked Grebe, the flat trophic structure that
255 characterises saline/hypersaline lakes favoured for moult migration sites results in (i) exceptionally
256 high food abundance and (ii) lack of large, upper trophic level predators (Williams 1998) which are
257 generally absent at Odiel Marshes. This combination of high energy and high protein food supply
258 and lack of predators presumably explains the spectacular gatherings of moult migrant Black-
259 necked Grebes at Mono and Great Salt Lakes from large parts of North America. In addition, the
260 long-term probability of needing reserves at a rather predictable time may have favoured the
261 evolution of an endogenous regulation of reserves in the Black-necked Grebe, in contrast to
262 situations in which variations in food availability are unpredictable (Lovvorn 1994).

263

264 Knowledge of moult amongst grebe species remains poor, but Piersma (1988) found that the body
265 mass of Great Crested Grebes *Podiceps cristatus* reached its lowest at any time throughout the
266 annual cycle during wing moult. Fat mass of females increased during early moult and then
267 levelled off, the fat content of males did not change, and Piersma (1988) found some evidence for

268 reduced feeding rates during moult. He concluded that growing feathers were soft and easily
269 broken especially before emerging from the feather sheath, and that as a result, grebes dived less
270 often and less deep to avoid damage. Nevertheless, the lack of change in fat content shows that even
271 under these circumstances, the Great Crested Grebes could meet their short term energy needs from
272 exogenous sources. The differences in the moulting ecology of these two grebe species may be
273 related to prey type and temporal availability of food (Jehl 1990).

274

275 Our study is the first to find support for the hypothesis that individual waterbirds adopt different
276 strategies with regard to timing of moult at the same site. We demonstrate that early season
277 moulters exploit a predictable food supply, and acquire an excess of energy over expenditure, which
278 permits an accumulation of fat stores, at least during the earliest stages of feather replacement.
279 Later, moulting birds acquired greater fat stores in advance of moult, presumably as a hedge against
280 the impending (but unpredictable) failing food supply and increasing thermoregulatory demands
281 that inevitably increase as the season progresses. This further demonstrates the remarkable degree
282 of phenotypic plasticity shown by different species in meeting energy needs during the
283 simultaneous replacement of wing feathers of waterbirds, even in this case when undertaken in a
284 predator-free environment.

285

286 We may reject an alternative hypothesis that the mass change in grebes is driven by the cestode
287 parasite, *Confluariapodicipina*, which uses *Artemia* as an intermediate host and grebes as the final
288 hosts (Georgiev *et al.* 2005). The prevalence and infestation rate of *Confluaria* in brine shrimps
289 increases from June to October, falling in December (Sánchez *et al.* submitted). *Artemia* infested
290 with cestodes tend to show positive phototaxis and increased surface behaviour, occurring higher in
291 the water column (Gabrion *et al.* 1982, Sánchez *et al.* 2006b). Bright red infected *Artemia* are more

292 attractive to feeding birds than paler, uninfected individuals (Thiéry *et al.* 1990, Sánchez *et al.*
293 2006b, 2009a) and are also more energetically profitable to grebes because of their elevated
294 triglyceride content (Amat *et al.* 1991, Sánchez *et al.* 2009b) associated with a diet-shift in infected
295 shrimps (Sánchez *et al.* 2013). Hence, Black-necked Grebes at Odiel should accumulate more mass
296 earlier in moult because of the increasing parasite loads of *Artemia* which enhances their
297 accessibility, profitability and attractiveness as prey for birds, but our results do not support this.
298 Besides, the cost of parasite infestation would very likely have fitness consequences for grebe
299 individuals, since increasing parasite loads would probably elevate energy expenditure in grebe
300 hosts, as well as potentially causing the growth of poorer quality wing feathers, since parasite
301 infestation may affect feather growth and quality in birds (e.g. Hill *et al.* 2003, Amat *et al.* 2007, Pap
302 *et al.* 2011). The influence of internal parasites on moult requires further research, not least to test
303 whether energy expenditure, wing feather growth and quality are affected by parasite loads in
304 moulting grebes and if so, whether there is some fitness advantage to moulting later to avoid
305 exposure to parasites, despite the increasing risk of the collapse of food supply later in the season.

306
307 These results are the first to address variation in moult patterns between early- and late-moulting
308 waterbirds and suggest state-dependent individual variation in moult strategies, with potential
309 fitness consequences for those individuals. Earlier studies have shown individual differences in
310 speed of moult, rate of body mass loss and degree of behavioural change during moult, some of
311 which seem to be state dependent, amongst waterbirds that generally commence moult at the same
312 time (van de Wetering & Cooke 2000, Portugal *et al.* 2011). These combined results suggest
313 considerable inter- and intra-specific variation in accumulation and depletion of fat stores,
314 phenotypic plasticity of organ size and behavioural adaptation shown by individual waterbirds to
315 meet the demands of the flightless period of remigial feather replacement, which potentially have

316 consequences for feather quality, overwinter survival and other fitness measures, as demonstrated in
317 passerines (Dawson *et al.* 2000).

318

319 We thank all volunteers from Estación Biológica de Doñana and SEO/BirdLife that, led by Luis García, trapped and
320 measured grebes over the years. Thanks to Enrique Martínez, Director of Paraje Natural Marismas del Odiel for
321 permission to use the study site and facilities to conduct fieldwork. Financial support was received from Consejería de
322 Innovación, Ciencia y Empresa, Junta de Andalucía (project P07-CVI-02700) with EU-ERDF support. Thanks to Steve
323 Portugal, an anonymous referee and Ruedi Nager for suggestions that improved an earlier draft.

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510
511 **SUPPORTING INFORMATION**

512 Additional Supporting Information may be found in the online version of this article:
513 **Figure S1.** Body mass of Black-necked Grebes caught during the moult season at Odiel
514 Marshes, Spain July-December 2000-2010. Symbols indicate the moult status at time of capture
515 for each individual.

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TABLES

Variable	Level	Estimate	se	df	F
Intercept		5.789008	0.0163	1	126141.2
Date		0.000754	0.000059	1	161.6
Moult	Old	-0.052592	0.00481	5	31.4
	Moult 1	0.010299	0.006907		
	Moult 2	0.030765	0.005344		
	Moult 3	0.013872	0.005754		
	Moult 4	0.016196	0.006181		
Sex	Male	0.033609	0.001596	1	443.2
Year				10	30.5

Table 1. Results of a GLM of body mass ($\log_e$ transformed) of Black-necked Grebes from 2000-2010, subdividing moulting birds into four subclasses. Degrees of freedom (df) of each numerator are shown, that of the denominator was 5579. All partial effects are highly significant ($P < 0.0001$). Females and birds with new feathers are aliased (i.e. estimates would be 0). Details of estimates for each year are not presented. The full model was highly significant ($r^2 = 0.170$, $F_{17,5579} = 67.29$, $P < 0.0001$).

Variable	Level	Estimate	se	df	F
Intercept		5.772492	0.02067	1	77992.23
Date		0.000743	0.000075	1	97.45
Moult Status	Old	-0.161542	0.03206	2	18.67
	Moulting	0.175645	0.029229		
Moult Status x Date	Old	0.000468	0.000116	2	11.78
	Moulting	-0.00052	0.000109		
Sex	Male	0.033614	0.001594	1	444.87
Year				10	31.53

Table 2. Results of a GLM of body mass ($\log_e$ transformed) of Black-necked Grebes from 2000-2010, pooling moulting birds and considering the interaction between moult status and date (days counted from 1 January). Degrees of freedom (df) of each numerator are shown, that of the denominator was 5580. All partial effects are highly significant ($P < 0.0001$). Females and birds with new feathers are aliased. Details of estimates for each year are not presented. The full model was highly significant ($r^2 = 0.173$, $F_{16,5580} = 72.81$, $P < 0.0001$).

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FIGURE

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Figure 1. Mean body mass (mean $\pm$ 95% confidence intervals) according to moulting status amongst Black-necked Grebes captured at Odiel Marshes, southern Spain August-December 2000-2010 (see text for details). Each point represents the mean of raw mass data from male and female grebes caught in the first or second half of the moulting period for each moulting category. There were significant differences between the mean body mass of males versus females and early- versus late-moulting in each moulting category based on GLM analysis of natural log transformed mass data (early females, $F_{11,5585} = 63.0$, $P < 0.0001$). Separate GLM analyses (also on transformed data, but here represented as means of raw mass values) within each category (early females, $F_{2,1197} = 24.1$, $P < 0.0001$; late females, $F_{2,1193} = 6.8$, $P = 0.0012$; early males, $F_{2,1690} = 38.8$, $P < 0.0001$; late males, $F_{2,1509} = 4.27$, $P = 0.0141$) showed significant differences between mean body mass (indicated by differing letters within each category) at each moulting stage based on Tukey LSD tests ($P < 0.05$).