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

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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 mouling. 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.
Keywords: energy budget, fat stores, moult migration, phenotypic plasticity, wing-moult

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

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

The North American Black-necked Grebe Podiceps nigricollis is a well-known moult migrant (Jehl & Henry 2010), with up to two million individuals breeding in North America drawing from extensive prairie breeding-areas to-and moult on just two alkaline lakes, Mono Lake (California) and Great Salt Lake (Utah), where they feed on abundant brine shrimps Artemiafranciscana safe from natural predators (Storer & Jehl 1985, Jehl 1997, Jehl & Henry 2010). At least one such
aggregation was known in Eurasia, with up to 186,000 recorded moulting at the hypersaline alkaline lake Burdur Gölü in Turkey (Hagermeir & Blair 1997). This lake has been subject to considerable hydrological change (see Green et al. 1996) and currently supports no obviously abundant food source, nor similar numbers of moulting birds in recent years (Girgin et al. 2004, Gülle et al. 2010).

The attraction of such large numbers of birds to rich feeding sources, where extreme water chemistry ensures low species diversity but high food biomass and a general lack of predators of flightless waterbirds suggests that the Black-necked Grebe has no need to exploit endogenous stores to balance energetic and nutritional budgets during wing feather replacement. However, the pattern of body mass variations in moulting Black-necked Grebes is different from those reported for waterfowl, and even another grebe species (Piersma 1988). Indeed, Black-necked Grebes in North America start fattening just after arriving at moulting sites, and continue to do so during moult (Jehl 1988). These North American studies showed that the grebes also doubled the mass of the liver, stomach and intestines, as well as increasing the heart mass and showing the coordinated atrophy/hypertrophy pattern of breast muscle and hypertrophy/atrophy of leg muscle consistent with their patterns of use during flightless moult (Jehl 1997). It has been suggested that the body reserves of fat act as an insurance against the annual collapse of the main prey of grebes, brine shrimps, in late autumn (Jehl 1988). Although brine shrimps are small, they are super-abundant where they occur and are rich in highly digestible lipids and protein (Caudell & Conover 2006a, Varo et al. 2011). If body mass gain is an anticipatory response to sudden prey disappearance, it may be expected that gains in body mass should be greater as the season advances, as the probability of the collapse of the prey population increases with date (Cooper et al. 1984, Jehl 1988, Caudell & Conover 2006b). In this paper, we test this hypothesis using flightless Black-necked Grebes caught in salt ponds at the Odiel marshes, southern Spain, where more than 10,000 birds from breeding
areas across Europe congregate from August to December to moult remiges, and where prey populations collapse after the end of October (Sánchez et al. 2006a, Varo et al. 2011).

**METHODS**

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

The moulting period of Black-necked Grebes at Odiel extended from mid-summer (late June) until early winter (mid-December, Varo et al. 2011). During this period brine shrimp *Artemia parthenogenetica*, the main prey of grebes obtained by sub-surface diving, suffers a sharp decline in abundance at a variable date in early winter (from late October onwards) related to cold winter temperatures (see Sánchez et al. 2006a, Varo et al. 2011). We used a database generated by a ringing programme at the Odiel Marshes, southern Spain initiated in 1993 by the Monitoring Team of the Doñana Biological Station. During ringing operations, captured Black-necked Grebes were aged according to iris coloration following Storer and Jehl (1985) and body mass was measured to
the nearest 5 g. Depending on the state of the remiges, birds were assigned to one of six categories (modified from Storer & Jehl 1985): old (i.e. unmoulted) remiges, moult 1 (recently shed), moult 2 (remiges less than half-grown), moult 3 (remiges around ¾), moult 4 (remiges almost fully grown, but with remainders of sheath) and new (i.e. fully regrown) remiges. Although some young of the year were captured (identified by a pale eye), these were not included in this study as they do not undergo wing-moult in their first winter. On this basis, data from 5680 grebes captured and weighed between 3 August and 16 December from 2000 to 2010 inclusive were available for analyses. Although many of these birds were recaptured, only the data from initial capture were used to avoid any potential risk of capture trauma affecting body mass and to avoid pseudoreplication. Of these birds, 549 had old feathers and were yet to commence moulting, 1251 were moulting and 3880 had new feathers and had thus completed moult. Day of capture (based on number of days after 1 January each year) ranged from 215 to 350 (mean 283.1 ± 0.386 SE). Of these, 5597 grebes were sexed visually by one experienced observer (Luis Garcia), based on head shape (molecular sexing has shown this to have an accuracy of 85%, \( n = 307 \), unpubl. data). Combined head and bill length plus wing length (from the folded carpel joint to the tip of the longest regrowing/grown primary feather) were also measured with callipers to the nearest mm for a reduced set of 2640 individuals. Results for this smaller dataset are not fully presented, as they were entirely consistent with those for the complete dataset.

Variation in log transformed body mass was analysed as the dependent variable using a General Linear Model. Predictor variables included year, moult status and sex as fixed factors, and date of capture and head-bill length as continuous variables (note not all predictors were applied in all models, see below). Models pooling all moulting birds together in one level of a factor were conducted, as well as those separating the four moult sub-classes defined above. We also subdivided
captured individuals into ‘early’ and ‘late–moulters’ based on whether the individual’s date of
capture fell before or after the mean capture date (i.e. day 283, see above), to test whether mass
differed between these two groups. Because earlier tests detected differences between the sexes, we
use GLM and Tukey LSD tests to test for differences in mass at different stages of moult in males
and females, early- and late-moulters. Body mass was natural log transformed to remove
heteroscedasticity. Analyses were conducted in Statistica 11 package (StatSoft, Inc. 2012).

RESULTS

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

Both raw data and the GLM estimates indicated that body mass was lowest prior to moult, peaked
during moult, then declined after moult (Table 1, Fig. 1). When all moultng birds were pooled in
the same level of a factor in a separate GLM (otherwise equivalent to that of Table 1), moultng
grebes were significantly heavier than those which had completed moult, which in turn were
significantly heavier than birds which had yet to commence moult ($P< 0.001$ for all pairwise LSD
post-hoc tests). Early-moulters were significantly lighter at all stages than late-moulters in both sexes and mean mass of moulting birds was significantly greater during moult than before (Fig. 1).

Birds captured with new feathers post moult were significantly lighter than during moult (Fig. 1). When a date x moult status interaction was added to the pooled model, it was highly significant (Table 2). Although mass increased with date for all three classes of grebes, the difference in mass between early- and late-moulting birds yet to moult was greater than between early- and late-moulting birds that had moulted \( (t = 4.03, \text{df} = 5580, P = 0.0007) \). Mass difference between early- and late-moulting birds that had moulted was greater than between early- and late-moulting birds which were mouling \( (t = 4.78, \text{df} = 5580, P = 0.0003) \). Repeating this GLM using untransformed body mass for guidance gave similar results and estimates indicated that for every day later that they were caught, birds were 0.47 g (± 0.046 se) heavier when they have not yet moulted, 0.10 g (± 0.043) heavier when they were mouling and 0.30 g (± 0.030) heavier when they completed moult.

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

Neither year, sex and capture date contributed to the model with head-bill length as the dependent variable, hence change in body mass was not associated with the arrival of birds of different structural size.
DISCUSSION

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

We were able to detect a strong seasonal trend with higher body mass in later moulting grebes (see also Jehl 1988 and Caudell & Conover 2006b), including those pre-moulting, during moult and post-moult. Although body mass is strongly influenced by structural size (Peig & Green 2009), we infer that the trends in body mass in our case reflect a change in the size of energy stores, since there was no change over time in the head-bill length of the birds. This may reflect the general trend of accumulating mass while feeding on the abundant brine shrimps, i.e. all individuals may tend to put on mass over time while they stay at Odiel. Alternatively, birds arriving with greater mass could potentially moult later, irrespective of local food availability, but sequential recaptures of grebes suggest this was not the case (unpubl. data). Generally, birds caught later in the season may have spent more time at Odiel prior to capture, and thus put on more mass. Alternatively, birds moulting later may have arrived later, but commenced moult at a higher mass having accumulated greater fat stores given the seasonal increase in probability of a sudden decline in prey availability before
moult is complete. The day to day change in mass of pre-moulting birds may be particularly rapid because they increase feeding rates in response to water temperature (see Varo et al. 2011 for extensive data), prey density, day-length or a combination of all these. Certainly Black-necked Grebes at Odiel Marshes show a strong correlation between prey density and time spent feeding, increasing foraging effort from 19-40% of all daylight activities in August-October to 53-74% in November-December in the face of falling prey densities (Varo et al. 2011) as is known from Great Salt Lake in North America (Caudell & Conover 2006b). Hence, moult migrants arriving late to the site could accumulate more fat stores prior to moult potentially as a buffer against a later prey crash. This is supported by the greater body mass of grebes bearing old feathers progressively through the moult period, although (as explained below) detailed information about individual behaviour would be required to determine how rates of mass accumulation change in relation to timing of moult. Furthermore, grebes at the site had highest body mass in 2009, a year with higher Artemia abundance and water temperatures, suggesting the acquisition of fat stores is influenced by energetic gains when thermoregulation costs are low and the food supply is good (Varo et al. 2011). Inevitably, we could not distinguish between the grebes caught early in the season that were about to moult and those that would delay feather replacement and accumulate additional mass to moult in the late-season, so we require sequential recapture histories of individually marked birds to know if the lower mass amongst pre-moulting grebes early in the season was due to birds arriving with depleted body stores that would acquire fats stores at the site and moult much later. Such recapture histories would have to account for any potential effects of the stress of recapture on body mass. Nevertheless, changes in the mass of grebes undergoing moult confirm that earlier moulting birds were consistently lighter than those initiating moult later.
The data presented suggest that Black-necked Grebes do not strongly catabolize somatic lipid stores to meet energy demands during moult at this site, since body mass either became greater as the season progressed (in the early stages of early-moulters) or was stable (late-moulters) supporting the results from a previous study (Jehl 1988). The protein needs of feather growth are modest compared to changes in fat stores during moult, and even if met from stored protein accumulated prior to wing moult, its effect on overall body mass (together with any change in water content) would equate to a few grams (Jehl 1988).

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

Knowledge of moult amongst grebe species remains poor, but Piersma (1988) found that the body mass of Great Crested Grebes *Podiceps cristatus* reached its lowest at any time throughout the annual cycle during wing moult. Fat mass of females increased during early moult and then levelled off, the fat content of males did not change, and Piersma (1988) found some evidence for
reduced feeding rates during moult. He concluded that growing feathers were soft and easily
broken especially before emerging from the feather sheath, and that as a result, grebes dived less
often and less deep to avoid damage. Nevertheless, the lack of change in fat content shows that even
under these circumstances, the Great Crested Grebes could meet their short term energy needs from
exogenous sources. The differences in the moulting ecology of these two grebe species may be
related to prey type and temporal availability of food (Jehl 1990).

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

We may reject an alternative hypothesis that the mass change in grebes is driven by the cestode
parasite, *Confluariapodicipina*, which uses *Artemia* as an intermediate host and grebes as the final
hosts (Georgiev *et al*. 2005). The prevalence and infestation rate of *Confluaria* in brine shrimps
increases from June to October, falling in December (Sánchez *et al*. submitted). *Artemia* infested
with cestodes tend to show positive phototaxis and increased surface behaviour, occurring higher in
the water column (Gabrionet *et al*. 1982, Sánchez *et al*. 2006b). Bright red infected *Artemia* are more
attractive to feeding birds than paler, uninfected individuals (Thiéry et al. 1990, Sánchez et al. 2006b, 2009a) and are also more energetically profitable to grebes because of their elevated triglyceride content (Amat et al. 1991, Sánchez et al. 2009b) associated with a diet-shift in infected shrimps (Sánchez et al. 2013). Hence, Black-necked Grebes at Odiel should accumulate more mass earlier in moult because of the increasing parasite loads of *Artemia* which enhances their accessibility, profitability and attractiveness as prey for birds, but our results do not support this. Besides, the cost of parasite infestation would very likely have fitness consequences for grebe individuals, since increasing parasite loads would probably elevate energy expenditure in grebe hosts, as well as potentially causing the growth of poorer quality wing feathers, since parasite infestation may affect feather growth and quality in birds (e.g. Hill et al. 2003, Amat et al. 2007, Pap et al. 2011). The influence of internal parasites on moult requires further research, not least to test whether energy expenditure, wing feather growth and quality are affected by parasite loads in moulting grebes and if so, whether there is some fitness advantage to moulting later to avoid exposure to parasites, despite the increasing risk of the collapse of food supply later in the season.

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

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

REFERENCES


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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

Figure S1. Body mass of Black-necked Grebes caught during the moult season at Odiel Marshes, Spain July-December 2000-2010. Symbols indicate the moult status at time of capture for each individual.

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**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 ($r^2 = 0.170, F_{17,5579} = 67.29, P < 0.0001$).

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</table>

**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 ($r^2 = 0.173, F_{16,5580} = 72.81, P < 0.0001$).
Figure 1. Mean body mass (mean ± 95% confidence intervals) according to moult 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 moult period for each moult category. There were significant differences between the mean body mass of males versus females and early- versus late-moulters in each moult 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 moult stage based on Tukey LSD tests ($P < 0.05$).