# Testing the use/disuse hypothesis: pectoral and leg muscle changes in captive barnacle geese *Branta leucopsis* during wing moult

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## SUMMARY

**Previous studies on wild moulting waterfowl have demonstrated that flight and leg muscles experience periods of hypertrophy and atrophy. This is thought to be in response to the change in use of the locomotor muscles as described in the use/disuse hypothesis. We tested this hypothesis using captive barnacle geese. Forty geese were dissected before, during and after wing moult, to determine the changes in mass and functional capacity of the flight and leg muscles. Physiological cross sectional areas (*PCSA*) and mean fascicle lengths of functional muscle groups were calculated to ascertain the force-producing capabilities of the flight and leg muscles. At the onset of moult, flight muscle mass was at a minimum, having atrophied by 35% compared with pre-moult levels, but it returned to pre-moult levels by the end of wing moult. By contrast, the leg muscles hypertrophied during wing moult by 29%, and the *PCSA* of individual muscle groups increased substantially. Increases in mass, *PCSA* and fascicle length of individual leg muscle groups during moult suggest that, when flightless, the leg muscles are functionally adapted to provide greater force and/or manoeuvrability to the birds, to aid ground-based escape from predators. Through studying captive animals that are unable to fly, it has been possible to conclude that the major changes in leg and flight muscle in moulting captive geese cannot be explained through use or disuse. Instead, changes seem to be compensatory or to occur in anticipation of changes in locomotor patterns.**

Key words: Barnacle geese, moult, muscle atrophy and hypertrophy, physiological cross sectional area.

## INTRODUCTION

The use/disuse hypothesis contends that high-resistance exercise will cause hypertrophy of skeletal muscle whereas failure to exercise a muscle group for prolonged periods will lead to atrophy (Alexander and Goldspink, 1977). Most species of waterfowl undergo a simultaneous wing feather moult to replace all flight feathers, which renders them flightless and dependent on terrestrial locomotion for a period of approximately 28 days (Hohman et al., 1992). Thus, wing moult in waterfowl is a period where the functional demands on muscle groups are substantially different to those at other times of year.

Studies on wild waterfowl have shown that during wing moult, birds lose body mass (Sjöberg, 1986; Van der Jeugd et al., 2003), alter their behaviour (Kahlert et al., 1996; Adams et al., 2000) and significantly increase their metabolic rate (e.g. Guozhen and Hongfa, 1986). Furthermore, a common observation in wild moulting waterfowl is atrophy of the major flight muscles at the onset of the flightless period of wing moult, coupled with hypertrophy of the leg muscles, which has been linked to increased levels of terrestrial locomotion (Ankney, 1979; Ankney, 1984; Gaunt et al., 1990; Fox and Kahlert, 2005). Towards the end of wing moult, flight muscles of wild moulting waterfowl hypertrophy (Ankney, 1979). In some simultaneous moulting bird groups such as grebes, hypertrophy of the flight muscles coincides with an increase in use through wing flapping behaviour (Piersma, 1988; Jehl, 1997), although this is not normally detected in waterfowl (e.g. Ankney, 1979; Ankney, 1983) or waders (Dietz et al., 1999), suggesting that alternative mechanisms may be involved in these groups.

Previous studies on atrophy and hypertrophy in wild moulting waterfowl have focused on changes in absolute mass of flight muscles and total leg musculature (e.g. Ankney, 1984; Fox and Kahlert, 2005). However, such studies may result in an incomplete understanding of muscle function because muscles of equal mass may differ dramatically in architecture and thus in the forces and velocities that can be produced (Wickiewicz et al., 1983). Atrophy and hypertrophy also occur to differing degrees and at different rates according to the function and fibre type profile of each muscle (Alexander and Goldspink, 1977). Therefore, in addition to muscle mass, two further variables are crucial to understanding muscle function: physiological cross-sectional area (*PCSA*), which reflects the number of sarcomeres in parallel, and fascicle length (fascicle being a bundle of fibres visible to the naked eye), which reflects the number of sarcomeres in series (Alexander and Goldspink, 1977; Wickiewicz et al., 1983; Thorpe, 1997). An increased *PCSA* reflects an increase in a muscle's maximum force generating potential whereas an increased fascicle length allows force generation over a wider range of motion and an increase in the velocity of shortening of the muscle (Thorpe et al., 1999). A more detailed analysis of muscle architecture is therefore necessary to understand fully the functional changes that occur in the muscles of the locomotor system during wing moult and the possible impact of such changes on locomotor performance.

Recent work on captive barnacle geese, *Branta leucopsis*, found that, despite having constant access to food and protection from predators, their physiological and behavioural responses to wing moult were similar to those of their wild conspecifics (Portugal et al., 2007). The present study expands on that analysis by exploring

whether waterfowl that have been held captive and unable to fly (flight feathers were regularly clipped) for their entire lives would show cycles of hypertrophy and atrophy that were consistent with their wild counterparts, even though such changes cannot be explained by the result of the use/disuse hypothesis. Although the captive geese used in the present study were dependent on terrestrial locomotion throughout their annual cycle, we predict that the leg and breast muscles will show compensatory adjustments in anticipation of the different forms of locomotion that are undertaken by their wild conspecifics. In particular, we hypothesise that both the *PCSA* and fascicle length of the leg muscles will increase during moult to enhance the ability of flightless, moulting geese to employ rapid running or swimming to escape from potential predators.

## MATERIALS AND METHODS
### Birds
A captive population of 40 barnacle geese *B. leucopsis* Bechstein 1803 obtained as 3-week-old goslings was maintained under natural light in large outdoor aviaries at the University of Birmingham, UK. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK), which has held a self-sustaining captive population of this species since 1982. At the time of sampling, all birds were at least two years old. The geese were fed with a 50–50 diet (Lilico, Surrey, UK) of mixed poultry corn (4% fat, 12% protein and 71% carbohydrate) and poultry growers pellets (3% fat, 16% protein and 61% carbohydrate), and water was available *ad lib*. The aviary ($30\,m \times 25\,m$) consisted of three pools, areas of shrubs and trees and open grass.

Four birds were sampled each fortnight from the 10th July (one week prior to the commencement of wing moult) to the 3rd November (when wing moult had been completed for two months) 2006, with extra sampling periods at the end of July and beginning of August during peak wing moult. Each bird was anaesthetised with 5% isofluorane gas mixed with a 4:1 ratio of air and oxygen, and then injected with a lethal dose of pentobarbitone ($140\,mg\,kg^{-1}$) into the intertarsal vein, to avoid damaging the muscles required for analysis. Final body mass was measured ($\pm5\,g$) before the carcasses were double-bagged and frozen at $-20°C$ until the dissections were performed.

### Moult score
The stage of moult was assigned using a 5-point classification score system (e.g. Bridge, 2004; Portugal et al., 2007). Moult score was defined as: (1) pre-wing moult, (2) primaries and secondaries remain, new primary pin visible, (3) all primaries and secondaries missing, (4) new primaries visible well beyond primary coverts and secondaries visible beyond secondary coverts (small flight feathers covering the primaries and secondaries) and (5) post wing-moult.

The primary feathers are attached to the manus and are primarily responsible for thrust during flight whereas the secondary feathers are corrected to the ulna and are used for lift. The captive geese reached moult scores 2–4 (wing moult) in July and August. All birds sampled in November, were moult score 5 (post wing-moult).

### Dissection protocol
Frozen carcasses were thawed for 24 h in a refrigerator and reweighed (<1% change in mass) prior to dissection. In total, the two major flight muscles and 29 leg muscles were removed from the birds; muscles were identified based on Howell (Howell, 1938) and Vanden-Berge and Zweers (Vanden-Berge and Zweers, 1993). During dissection, the skin of each bird was opened along the keel to expose the breast muscles, and pectoralis major and supracoracoideus were excised from the right side of the bird. Skin was removed from the entire right leg and right side of the abdomen and the individual muscles identified. Muscles were dissected free from fascia, taken out individually and the external tendons removed before being weighed on electronic scales (Sartorius, Göttingen, Germany) to the nearest 0.001 g.

Muscle belly length was obtained by measuring the distance from the most proximal fascicles to the most distal fascicles (e.g. Thorpe et al., 1999). For parallel fibred muscles, fascicles were revealed by making an incision from the origin to the insertion of the muscle belly. Pennate fibred muscles were first cut transversely, then one half cut longitudinally in such a way that muscle fascicles lay in the plane of the cut, and muscle fascicle lengths were sampled from the distal and proximal parts (Thorpe et al., 1999). At least three measurements of fascicle length were taken except for muscles where fascicle length was equal to belly length (e.g. iliotibialis cranialis), which were only measured once.

### Muscle analysis
Major leg and flight muscles were grouped into functional categories (Alexander et al., 1981; Vanden-Berge and Zweers, 1993), the composition of which are detailed in Table 1. Muscle mass and *PCSA* values for all muscle groups were doubled (e.g. Jehl, 1997) to account for the side that was not dissected (assuming the muscles were symmetrical).

The physiological cross-sectional area of the muscle can be obtained from:

$$PCSA = m / (pl), \qquad (1)$$

where $m$=muscle mass, $p$=density [$1.06\,kg\,m^{-3}$ (Méndez and Keys, 1960)] and $l$ is fascicle length. The proportion of muscle force transmitted to the tendon depends on the angle of pennation of the muscle fibres (such that $PCSA=mlpl \times \cos\theta$, where $\theta$ is the angle of pennation of the fibres with respect to the line of pull of the muscle).

Table 1. Muscle composition of the major flight and leg muscle groups

| Muscle group | Muscles within group |
| --- | --- |
| Flight | Pectoralis, supracoracoideus |
| Ankle dorsiflexors | Tibialis anterior |
| Ankle plantarflexors | Gastrocnemius medialis and lateralis |
| Deep ankle flexors and extensors | Deep flexors 1–8 and deep extensors 1–3 |
| Hip abductors | Iliotibialis lateralis, iliotrochantericus cranialis, iliotrochantericus caudalis |
| Knee extensors | Femorotibialis, femeortibialis lateralis |
| Hip extensors and adductors | Iliotibialis cranialis, pubo-ischio-femoralis, caudofemoralis, ambiens, iliofibularis, flexor crusis lateralis, flexor crusis medialis, ischiofemoralis |

Muscle names and functions are based on Howell (Howell, 1938) and Vanden-Berge and Zweers (Vanden-Berge and Zweers, 1993). Note muscles are grouped according to their primary function.

Pennation angle was not included in our estimates of *PCSA*. Smith et al. measured pennation angles in the hindlimb muscles of the ostrich, *Struthio camelus,* and found all angles to be close to 30 deg. (Smith et al., 2006). The cosine of 30 deg. is 0.866 and would thus reduce the estimations of *PCSA* by approximately 13%.

Masses and *PCSA*s were calculated as group totals, which are the sum of constituent muscles in each functional group (Alexander et al., 1981; Thorpe et al., 1999). As some muscles form a greater percentage of the total mass of a group than others, fascicle length of muscle groups was calculated as a weighted harmonic mean (Alexander et al., 1981; Thorpe et al., 1999; Payne et al., 2006). This was achieved by weighting the fascicle length of each individual muscle by the mass of the muscle. Hence:

$$L = \Sigma m_j \, / \, \Sigma (m_j/l_j), \qquad (2)$$

where *L* is the fascicle length for a group of muscles of which the *j*th member has a mass $m_j$ and fascicles of length $l_j$ (Alexander et al., 1981).

### Behaviour

Behavioural data were taken from Portugal et al. (Portugal et al., 2007). The behavioural data were recorded from the same flock of birds one year prior to the current study. This means that it was not possible to link directly each individual bird's activity profile to the changes in muscle architecture. However, it allowed us to obtain an indication of whether captive barnacle geese increase locomotor behaviour, particularly wing flapping, towards the end of moult in 'anticipation' of the resumption of flight. The activity budgets of the captive barnacle geese were recorded at three points during the year (June, August and November). Behaviour was recorded onto paper at three different times of the day: morning (07:00–12:00 h GMT), afternoon (12:00–17:00 h GMT) and evening (17:00–22:00 h GMT). Observations were made from a shed a short distance (approximately 6 m) away from the birds and were restricted to periods of good weather. An individual goose was selected and observed for a total period of 5 min, and activities were recorded at 15 s intervals. If there was any disturbance during the 5 min observation, the data were not used. The number of individuals sampled each day ranged from 7 to 14. In total 105 observation sessions were conducted.

Twenty separate behaviours were recorded during the study and pooled into six categories (Austin and Frederickson, 1987; Adams et al., 2000): foraging (feeding and pausing), resting (which includes loafing and sleeping), maintenance (preening, scratching, stretching and splash bathing), locomotion (tail wagging, walking, swimming, wing-flapping and scooting), social (agonistic and courtship) and alert (head raising and inactivity to scan the immediate area).

### Statistics

All data were analysed using general linear model (GLM) with Tukey *post-hoc* testing. Preliminary analysis revealed that mass changed significantly during the study period [analysis of variance (ANOVA), $F_{(4, 35)}=9.5$, $P<0.001$] (Fig. 1). Body mass at moult score 3 was significantly lower than those at moult stages 1, 4 and 5. The difference in mean body mass between moult scores 1 and 3 was approximately 700 g, a decrease of over 30%. This change in body mass had a substantial effect on muscle mass, both in terms of total locomotory muscle mass (flight and leg muscles combined) and flight and leg muscles considered separately (Fig. 2). Similar effects were noted on *PCSA* and fascicle length. As a result, body mass was a covariate in all subsequent analyses
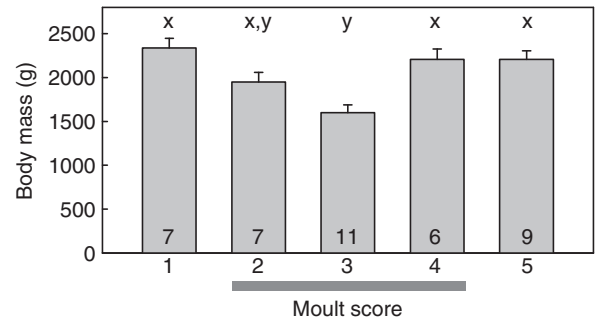


Fig. 1. Mean (±s.e.m.) body mass in captive barnacle geese during their annual wing moult. Sample sizes are shown at the base of each bar. Body mass changed significantly between moult score categories. See text for classification into moult scores. *Post-hoc* testing revealed significant differences between moult score categories. Moult score categories not sharing the same letter above the bar were significantly different. The dark horizontal bar represents moult scores during the wing moult period (Jul–Aug).

of muscle masses, *PCSA* and fascicle length (see Packard and Boardman, 1988). Data reported in figures are corrected for the significant effects of body mass and, where appropriate, a significant interaction between body mass and moult score. The two locomotor muscle groups (flight muscle mass and total leg muscle mass) were compared to determine if changes in muscle mass were compensatory. Percentage data for each behavioural category from each sampling session were arcsin transformed and two-way ANOVA (time of day and month) with *post-hoc* Tukey HSD tests ($P<0.05$) performed to determine whether there were differences in the mean proportion of time dedicated to each category of behaviour between the three different times of year and between morning, afternoon and evening observations. All tests are considered significant at $P<0.05$. Values given are means ± s.e.m.

### RESULTS
### Muscle group changes

Body mass adjusted (BMA) total locomotor muscle mass changed significantly during the study period [GLM, $F_{(4, 30)}=5.53$, $P<0.01$] (Fig. 3A). *Post-hoc* testing revealed that total locomotor muscle mass was significantly greater at moult score 5 (537±20 g) than at moult score 2 (445±17 g) but no other differences were significant. These changes were the result of opposing significant changes in leg muscle mass [GLM, $F_{(4, 30)}=3.6$, $P<0.05$] (Fig. 3B) and flight muscle mass [GLM, $F_{(4, 34)}=10.76$, $P<0.001$] (Fig, 3C). BMA leg muscle mass increased significantly by 29% between moult score 1 and 2. BMA flight muscle mass showed the opposite trend, declining significantly by 35% between moult score 1 and 2, before increasing significantly again at moult score 3 and returning to pre-moult values by moult score 5.

Body mass had a significant effect on *PCSA* for total locomotor muscle combined and total leg muscle but not in the flight muscles. BMA locomotor muscle *PCSA* changed significantly during the study period [GLM, $F_{(4, 30)}=7.91$, $P<0.01$] (Fig. 4A) primarily as a result of the changes in leg muscle *PCSA* [GLM, $F_{(4, 30)}=2.99$, $P<0.05$] (Fig. 4B). BMA leg muscle *PCSA* increased significantly from pre-moult values (7330±843 mm$^2$, moult score 1) reaching a maximum in the middle of wing moult (10153±1328 mm$^2$, moult score 3) before decreasing significantly by 53% in late moult (4807±772 mm$^2$). There was no significant change in flight muscle
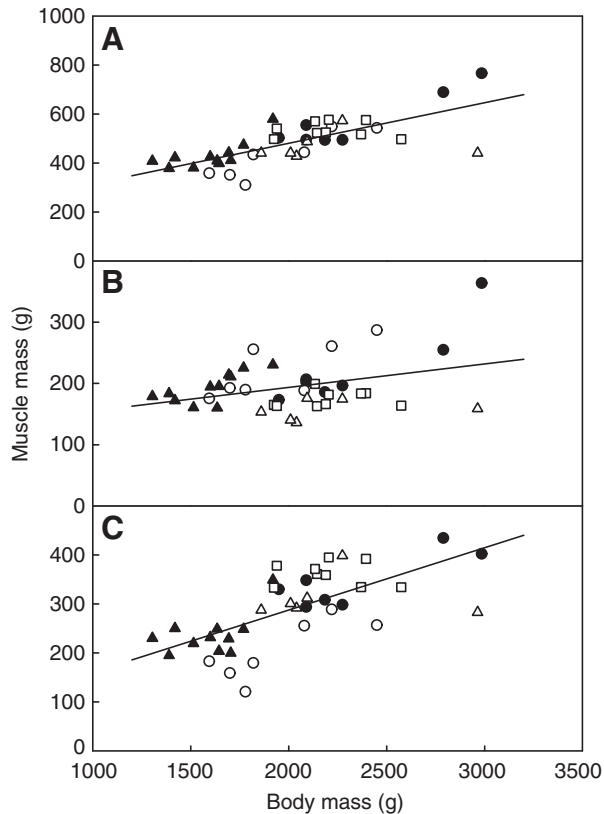
Fig. 2. Relationship between body mass and (A) total locomotory muscle mass, (B) leg muscle mass and (C) flight muscle mass in captive barnacle geese during their annual wing moult. Geese were destructively sampled with moult scored sequentially as 1 (filled circles), 2 (open circles), 3 (filled triangles), 4 (open triangles) and 5 (open squares). See text for details of classification into moult scores. In each case, there was a significant linear relationship between body mass and muscle mass: total muscle mass=149+(0.17×body mass) ($P<0.001$, $R^2$=0.52), leg muscle mass=117+(0.04×body mass) ($P<0.05$, $R^2$=0.13), flight muscle mass=32+(0.13×body mass) ($P<0.001$, $R^2$=0.45).



Fig. 3. Mean (±s.e.m.) (A) total locomotory muscle mass, (B) leg muscle mass and (C) flight muscle mass in captive barnacle geese during their annual wing moult. All data shown are adjusted for changes in body mass during the moult. Total locomotory muscle mass, leg muscle mass and flight muscle mass all changed significantly between moult score categories. *Post-hoc* testing revealed significant differences between moult score categories. See text for classification into moult scores. Moult score categories not sharing the same letter above the bar were significantly different. The dark horizontal bar represents moult scores during the wing moult period (Jul–Aug).

$PCSA$ [GLM, $F_{(4, 34)}$=1.73, $P$=0.17] (Fig. 4C). Body mass had a significant effect on fascicle length in the leg muscles and for all locomotor muscles combined but not in the flight muscles. There was no significant differences between moult scores of any of the measures of fascicle length: BMA locomotor muscle fascicle [GLM, $F_{(4, 34)}$=1.24, $P$=0.31] (Fig. 5A), BMA leg muscle fascicle length [GLM, $F_{(4, 34)}$=0.97, $P$=0.44] (Fig. 5B), flight muscle fascicle length [GLM, $F_{(4, 34)}$=0.83, $P$=0.51] (Fig. 5C).

### Detailed leg muscle architecture

Body mass had a significant effect on leg muscle mass for every muscle group except the ankle dorsiflexors. Accounting for this effect, the mass of all leg muscle groups except ankle dorsiflexors changed significantly between moult score categories (GLM, all $P<0.05$) (Fig. 6). Overall, the masses of all the leg muscle groups followed a similar pattern of mass gain and loss (Fig. 6), with muscle mass being higher during moult scores 1–3 than 4 and 5. Leg muscle groups atrophied significantly between moult scores 3 and 4 by the following amounts: 58% (knee extensors), 48% (ankle plantarflexors), 43% (hip abductors) and 56% (hip extensors and adductors). A non-significant decrease was noted in the ankle dorsiflexors (37%). Body mass had a significant effect on $PCSA$ for every leg muscle group except the
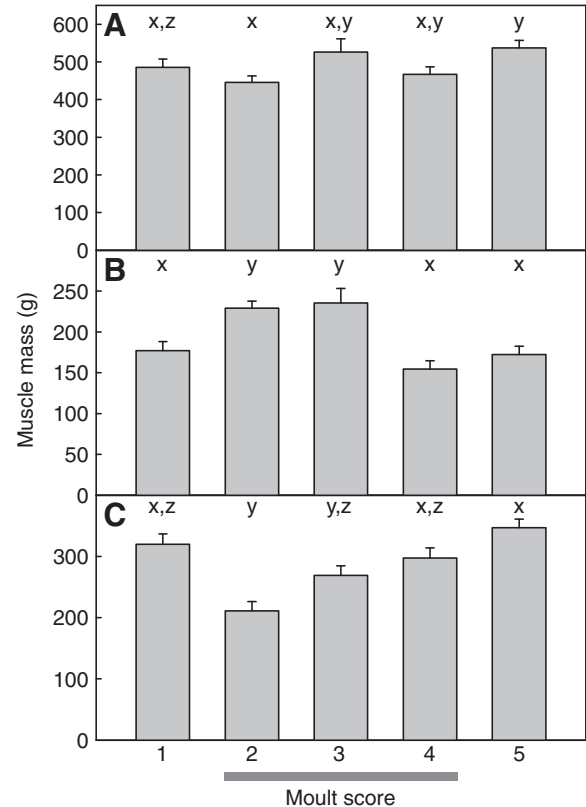
knee extensors. $PCSA$ of the knee extensors and BMA $PCSA$ of all other leg muscle groups, except hip abductors, changed significantly over the study period (GLM, all $P<0.05$) (Fig. 6B). The largest difference occurred between moult scores 3 and 4, with each leg muscle group $PCSA$ decreasing significantly by the following amounts: 58% (knee extensors), 48% (ankle plantarflexors), 56% (hip extensors and adductors), 57% (deep extensors and flexors) and 56% (ankle dorsiflexors). For the leg muscle groups, fascicle length only changed significantly in the ankle dorsiflexor group, with fascicle length being significantly higher at moult stage 4 (14.2±1.5 mm) than moult stage 1 (8.6±1.5 mm) (Fig. 6C).

### Behavioural observations

The greater flight muscle mass from moult stage 3 onwards was not accompanied by an increase in wing-flapping behaviour (repeated-measures ANOVA, $P$=0.61). The overall time budgets of the captive geese recorded during moult did not change significantly (Fig. 7), and there was no increase in foraging or locomotor behaviour (Portugal et al., 2007). If we assume the behavioural response of the geese to moult is consistent between years, the data suggest that the significant changes in muscle mass and function observed in the captive geese were not associated with changes in behaviour.
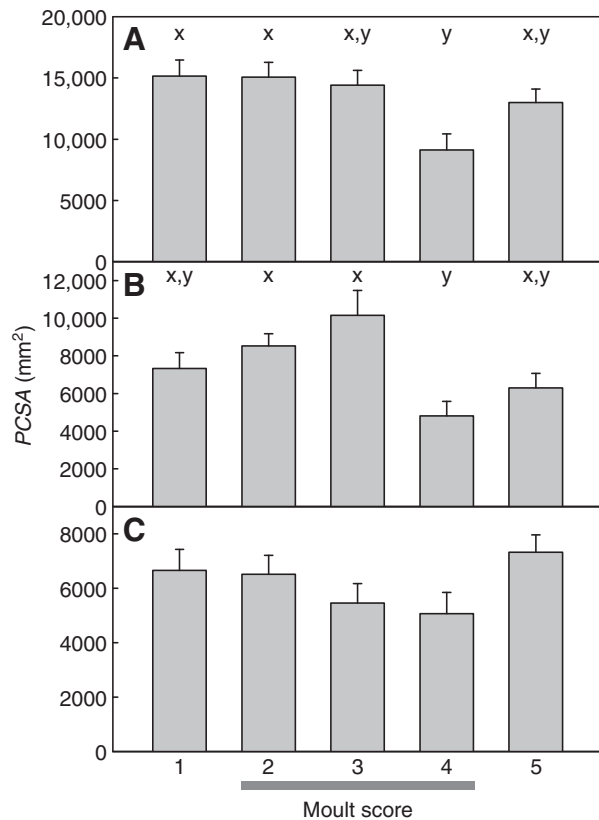
Fig. 4. Mean (±s.e.m.) (A) total locomotory muscle physiological cross sectional area (*PCSA*), (B) leg muscle *PCSA* and (C) flight muscle *PCSA* in captive barnacle geese during their annual wing moult. All data shown are adjusted for changes in body mass during the moult where appropriate. Flight muscle *PCSA* did not change significantly between moult score categories, although both total locomotory muscle *PCSA* and flight muscle *PCSA* did. *Post-hoc* testing revealed significant differences between moult score categories. See text for classification into moult scores. Moult score categories not sharing the same letter above the bar were significantly different. The dark horizontal bar represents moult scores during the wing moult period (Jul–Aug).

Fig. 5. Harmonic mean (±s.e.m.) (A) total locomotory muscle fascicle length, (B) leg muscle fascicle length and (C) flight muscle fascicle length in barnacle geese during their annual wing moult. All data shown are adjusted for changes in body mass during the moult where appropriate. Fascicle length did not change significantly between moult score categories, for any of the muscle groups. See text for classification into moult scores. The dark horizontal bar represents moult scores during the wing moult period (Jul–Aug).

## DISCUSSION
### Changes in flight and leg muscle mass

Despite the fact that the geese in the present study had never flown, the results show that captive barnacle geese still experience significant functional changes in flight and leg musculature throughout the period of wing feather moult. The approximate 35% atrophy of the flight muscles at the onset of moult observed in the present study is consistent with that reported from wild waterbirds, with values ranging from 17% in the mallard *Anas platyrhynchos* (Young and Boag, 1982) to 33% for black-necked grebes *Podiceps nigricollis* (Jehl, 1997) [for full reviews of muscle atrophy values see Piersma and also see Hohman et al. (Piersma, 1988; Hohman et al., 1992)]. Greylag geese *Anser anser* and brant geese *Branta bernicla* exhibited reductions in flight muscle of 30% and 32%, respectively, at the onset of wing moult (Fox and Kahlert, 2005; Ankney, 1984) but Ankney (Ankney, 1984) noted that in moulting brant geese, the pectoral muscles did not return to 'normal' mass until, or after, the birds were fully capable of flight. Many species of geese are effectively also behaviourally flightless during the breeding season, because they are incubating eggs or rearing goslings (Ankney, 1984). Furthermore, in wild snow geese, hypertrophy of the leg muscles starts prior to the wing moult and its associated flightless phase. This
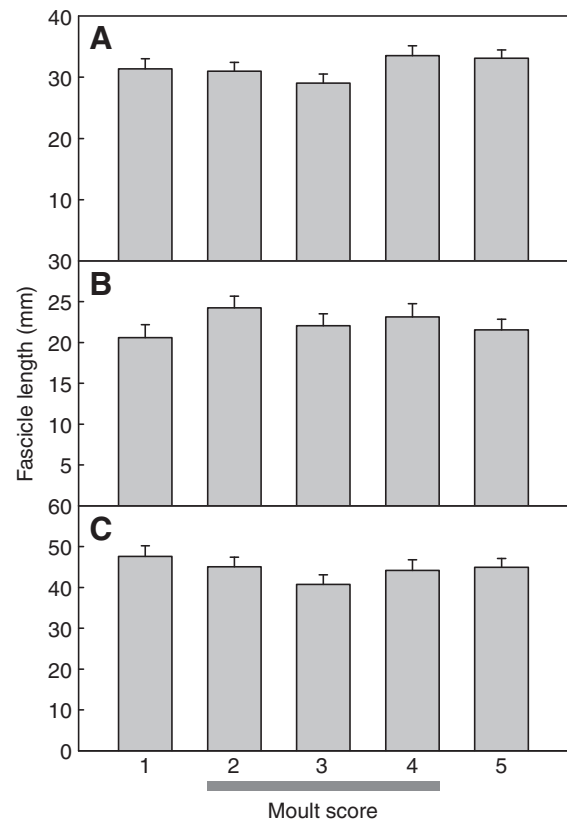
hypertrophy prior to moult in wild birds, as a result of breeding, may have implications for how the results of the present study are interpreted. Therefore, it is possible that in the captive barnacle geese, changes in muscle mass and architecture may have occurred prior to the start of wing moult, during what would be the breeding season (31% of the flock were paired and attempting to breed). Therefore, it is likely that moult score 5 (October–November), not 1 (pre-moult, typically May–June), might be more representative of the non-breeding or non-moulting period for the captive geese.

The sudden atrophy of the leg muscles of captive barnacle geese from moult score 3 onwards may support the proposal of Brown and Saunders (Brown and Saunders, 1998) that leg muscles would need to atrophy before the re-commencement of flight, otherwise the large mass of the leg muscle may hinder flight. Brown and Saunders also suggested there would be an optimum cross over point at mid-moult where leg muscles can assist with take off to reduce the requirement of the recovering pectoral muscle (Brown and Saunders, 1998). It is possible therefore that slightly increased leg muscle mass is beneficial as it may assist with take off, in the early stages of regaining flight. Wild moulting greylag geese exhibit a similar pattern of atrophy and hypertrophy in the leg muscles to the captive geese in the present study, with a period of hypertrophy
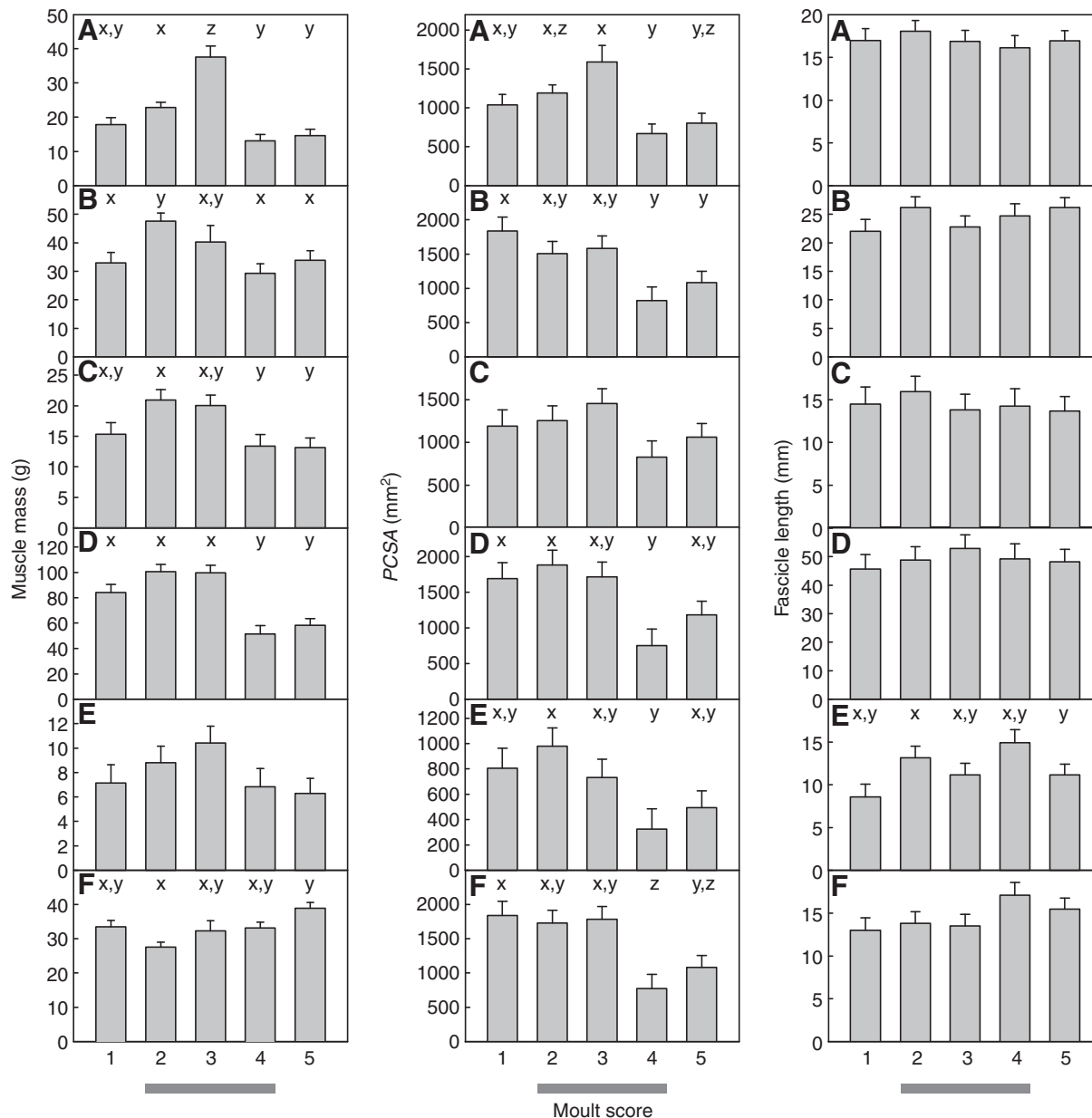
Fig. 6. Mean (±s.e.m.) muscle mass, physiological cross sectional area (*PCSA*) and fascicle length for six muscle groups in the legs of barnacle geese during their annual wing moult. The six groups were: (A) knee extensors, (B) ankle plantarflexors, (C) hip abductors, (D) hip extensor and adductors, (E) ankle dorsiflexors and (F) extensors and flexors in barnacle geese. All data shown are adjusted for changes in body mass during the moult. Ankle dorsiflexor muscle mass and hip abductor *PCSA* did not change significantly between moult score categories but muscle mass and *PCSA* of all other muscles groups did. Fascicle length changed significantly between moult score categories in the ankle dorsiflexors only. *Post-hoc* testing revealed significant differences between moult score categories. See text for classification into moult scores. Moult score categories not sharing the same letter above the bar were significantly different. The dark horizontal bar represents moult scores during the wing moult period (Jul–Aug).

(36%) followed by atrophy (11%) (Fox and Kahlert, 2005). In comparison, increases in leg muscle mass during moult of 16%, 41%, 27% and 30% have been reported for wild mallard, giant Canada geese *Branta canadensis maxima*, snow geese and gadwall *Anas strepera*, respectively (Hay, 1974; Ankney, 1979; Young and Boag, 1982; Rosser and George, 1985).

**Physiological cross-sectional area and fascicle length**

If we assume moult score 5 to be indicative of the non-breeding season, all leg muscle groups bar the hip abductors show an increased capacity for powering leg-dominated locomotion when flightless,

be it behaviourally flightless when guarding goslings (pre-moult) or functionally flightless during wing moult. Therefore, the changes observed in the *PCSA* of the leg muscle groups suggest that the muscle architecture of the hindlimb may be modified to increase force production to facilitate faster running, which is likely to aid escape when moulting birds are flightless. Geese take off by running along land or water or by jumping. The only muscle group to have a *PCSA* that did not change significantly throughout moult was the hip abductors, which are responsible for rotating, flexing and abducting the hip (Vanden-Berge and Zweers, 1993). In guineafowl, *Numida meleagris,* the iliotibialis lateralis, which is part of the hip
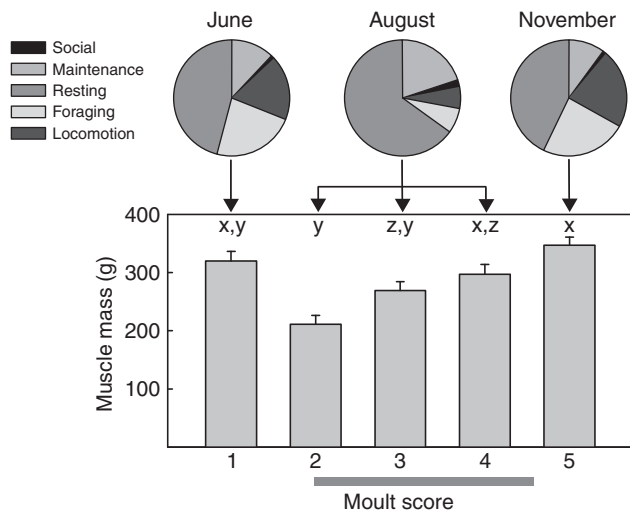
Fig. 7. Behavioural data from 14 captive barnacle geese recorded in 2006, taken from Portugal et al. (Portugal et al., 2007). The behaviour pie charts are superimposed over mass changes of the flight muscle group (see Fig. 3 for details). The dark horizontal bar indicates the moult scores within which wing moult took place. See text for classification into moult scores. The time-budgets taken during moult (signified by the black arrows under August) did not change significantly throughout the moult period, suggesting that the observed significant changes in muscle mass were not a direct result of changes in muscle usage. Muscular changes, particularly mid-moult increases in flight muscle mass (moult score 3) were not accompanied by an increase in locomotor or foraging behaviour.

abductors, is important in jumping (Rubenson et al., 2006) and it is possible that this group is also important for jumping at take off in geese, as well as contributing to running. Thus, the relatively constant *PCSA* of this group probably reflects its use in walking, running and flying. These data demonstrate the importance of studying detailed muscle architecture, because measuring changes in muscle mass alone would be unable to provide such detailed information on the adaptive changes in muscle function.

The only significant increase in fascicle length of any muscle group during moult was recorded in the ankle dorsiflexors. This muscle group is primarily responsible for lifting the foot off the ground to stop it dragging during the swing phase of walking or running and to keep the front of the foot lifted when the heel is striking the ground (Thorpe et al., 1999; Reilly, 2000). It must also play a role in retracting the foot during swimming to enable a rapid return to the propulsive stage of the cycle (Biewener and Corning, 2001). Therefore, an increase in fascicle length of the ankle dorsiflexors may act to increase the velocity with which the foot is lifted and potentially shorten the stance phase that would enable the bird to run faster, which may provide the flightless geese with an increased ability to escape from predators through running and/or swimming.

### Conclusions

It has been possible to conclude that the major changes in leg and breast muscle in moulting captive geese cannot be explained through use or disuse. Despite the birds being unable to fly and thus always reliant on walking, the leg and breast muscles still show adjustments in anticipation of the different forms of locomotion that would be seen in their wild conspecifics. Although the captive birds had never flown and showed no change in activity during moult, their flight muscles atrophied at the start of wing moult before hypertrophying mid moult. Increases in mass, *PCSA* and fascicle length of certain leg muscle groups during moult suggest that when flightless, the leg muscles are functionally adapted to provide greater force and/or manoeuvrability to the birds, to aid escape on foot from predators. Therefore, these muscle changes give potential for increased or decreased performance but do so in an anticipatory rather than a responsive fashion.

### LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | analysis of variance |
| BMA | body mass adjusted |
| GLM | general linear model |
| *l* | fascicle length |
| *L* | fascicle length for a group of muscles |
| *m* | muscle mass |
| *p* | muscle density |
| *PCSA* | physiological cross sectional area |
| θ | angle of pennation of the fibres |

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