ECOPHYSIOLOGICAL ASPECTS OF THE ANNUAL CYCLE OF BARNACLE GEESE, *BRANTA LEUCOPSIS*

by

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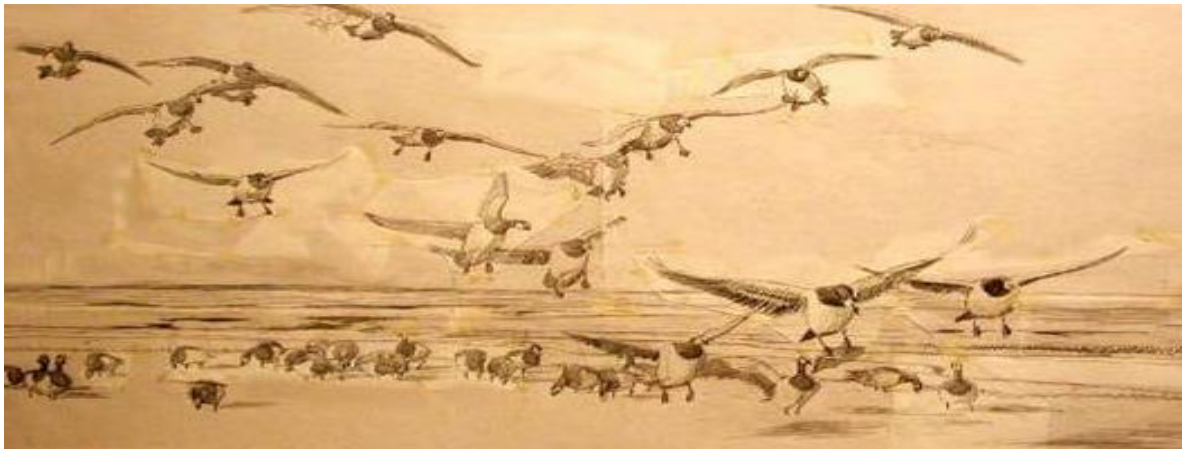
The essence of life is statistical improbability on a colossal scale

- Richard Dawkins

Dedication

This thesis is dedicated to my family, who, from a very young age, supported my enthusiasm for the natural world, and my desire to find out what makes things tick. I will never forget how much time my family have dedicated to my interests, and how encouraging they have been over the years.



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I: GENERAL INTRODUCTION

IA: The annual cycle and the management of time and energy

The timing of annual cycles in birds has evolved to minimise the overlap of energetically demanding events, such as migration, breeding and moult. Such events often place additional nutritional and energetic demands upon the individual over and above the normal demands of general maintenance. Each species has evolved a strategy that is best suited to its own annual requirements and the environmental conditions in which it lives. The importance of the timing of these potentially energetically demanding events in a bird's annual cycle is particularly acute when events such as migration, breeding and moult take place in a relatively short time span, as is the case for many species of bird that breed in the high Arctic. Birds dedicate considerable effort and time to preparing themselves for these energy draining activities (i.e. events that require high energy expenditure), and favourable conditions for deposition of stores are relatively rare, and preparation can only take place during peaks in food availability. Therefore, birds must time the events in their annual cycle to take advantage of foraging opportunities.

In the present study, body mass, body composition, heart rate, body temperature and behaviour were measured year-round in captive and wild barnacle geese, in an attempt to answer questions about aspects of their ecology, behaviour and physiology, and in particular, how these change in response to demanding events, throughout the annual cycle.

1B: Introducing the study species: the barnacle goose - a model bird?

General biology

There are four populations of barnacle geese, *Branta leucopsis*, which are distributed in Western Europe. These are the Greenlandic, Svalbard, Russian and Swedish breeding populations, all of which spend the winter in distinct, separate wintering grounds. The Greenlandic population winters in Ireland and western Scotland, the Svalbard population in northern Britain, and the Russian and Swedish populations winter in Germany and the Netherlands (Owen, 1981; Owen, 1990; Black *et al.*, 1998). Recent censuses estimated there to be around 309,000 individuals for the four barnacle goose populations combined (Owen, 1990).

The Svalbard population occupies discrete breeding and wintering areas, in winter occupying one of the smallest wintering ranges of any goose population in the world, the Solway Firth (Phillips *et al.*, 2003). Numbers on the Solway declined to only 300 in 1948 (Black *et al*., 1998), however, the population responded well to conservation measures, and estimates now for the wintering population on the Solway Firth is in the region of 23,000 individuals.

Barnacle geese tend to mate for life (i.e. once paired, they stay with the same partner until death) and such a strong bond must have a powerful selective advantage. Studies have shown that long-established pairs breed better than newly established pairs (Owen, 1990). If an individual loses its mate during the winter, its chances of breeding successfully in the following summer are only a third of those of mates that

stay together (Owen, 1990). This is because the division of labour between partners is essential to successful breeding, particularly with regards to defending the nest and goslings from predators. Barnacle geese breed in a potentially unpredictable environment and perfect co-operation between mates is required to hatch eggs and rear young successfully, as the goslings need protection from the parents to feed once hatched to prepare for the autumn migration.

Because of their size, barnacle geese are unable to defend their nests against predators such as Arctic foxes *(Alopex lagopus)*, so, therefore, they must try and find a suitable nest site that is inaccessible to such predators (Owen and Ogilvie, 1979). In some parts of Svalbard, the geese breed on cliff ledges, sometimes at heights of over 200 feet. The female takes sole responsibility for the nest, whilst the male offers protection. When the eggs hatch, the parents and their young leave the nest and head for the rearing areas on nearby tundra. At this stage, the female can be exhausted from fasting through incubation, so the male takes full responsibility for guarding the young, while the female feeds for anything up to 20 hours a day (Owen, 1984). The goslings become fully fledged at 7 weeks of age.

Reproduction – capital breeders

Nesting late in the short Arctic summer can have deleterious effects on reproduction, as there is a strong pattern of seasonal decline in reproductive success (Lepage *et al*., 2000). Birds arriving too early in spring, however, may have restricted feeding opportunities owing to snow cover. Therefore, pre-migratory body stores can allow

egg-laying to begin before local foods become available, thereby avoiding any delays in breeding and maximising opportunities for reproductive success (Raveling, 1979; Sandberg and Moore, 1996; Klaassen *et al*., 2006). A true capital breeder relies on body stores brought from the wintering sites or migration areas, to the breeding grounds to produce eggs (Drent and Daan, 1980). Whether geese are exclusive capital breeders (as opposed to income breeders) has been a subject of debate (e.g. Ankney *et al*., 1991; Klaassen *et al*., 2006), as many species of geese will feed on the breeding grounds prior to egg laying, suggesting not all species are strictly capital breeders (e.g. lesser snow geese, *Chen caerulescens caerulescens*, Wypkema and Ankney, 1979). In general, the further north breeding sites are located, the later birds arrive at the sites (Owen and Gullestad, 1984). At these more northerly locations, the summer season is relatively short, and the birds are under severe time constraints. Therefore, to breed successfully, the birds must commence egg-laying soon after arrival. When egg-laying commences within 10 days after arrival on the breeding grounds, the production of eggs must occur mostly from residual body stores. This is because follicle maturation takes about 10-12 days in geese, with an additional 1-2 days for albumen synthesis (Alisauskas and Ankey, 1992). The Svalbard breeding population of barnacle geese do not arrive on their breeding grounds until late May – early June, and on arrival, the females are ready to lay their eggs (Owen and Ogilvie, 1979), which would suggest, for this population at least, that they are capital breeders.

However, during spring migration, the Svalbard breeding barnacle geese take roughly 4-5 weeks to reach their breeding grounds, essentially following the new growth of vegetation northwards tracking the receding snow line (Cope, 2003). Fattening for the

spring migration and reproduction is a three-step process, first on the Solway and then on to Helgeland, a major staging area in Norway (Fig. I.1; Black *et al*., 1991). Here, fat stores expended during the first part of the migration are at least partially replenished. The geese then make a third stop in the southern part of Spitsbergen, the main island in Svalbard, where the birds forage intensively on emerging vegetation, before making the short trip (<30km) to their breeding grounds (Butler *et al*., 1998). Therefore, it can be difficult to determine whether the Svalbard breeding population of barnacle geese are solely capital breeders. What is more likely is the breeding strategy that Klaasen *et al*. (2006) proposed. They suggested that breeding strategies of waterfowl in general are spatially and temporally flexible at both the species and population levels, so that waterfowl may shift towards income breeding in one year and capital breeding in the next, as circumstances dictate.

Replacing flight feathers – simultaneous moult

The replacement of flight feathers occurs annually in the life cycle of most species of birds with only a few exceptions, such as the albatrosses and the greater Magellan goose (*Chloephaga picta*), which may skip moult from year to year (Hohman *et al*., 1992). This though is a trait found only in larger birds. All other birds replace their flight feathers annually, typically in a sequential fashion, i.e. one flight feather at a time, or two flight feathers moulted, symmetrically on each wing. Feathers are composed mostly of proteins, and may constitute up to one third of that contained in the carcass (Murphy and King, 1984; Hohman and Crawford, 1995). Blem (1990) reported that birds have relatively limited capabilities for storage of protein, and are

seemingly inefficient at converting dietary protein into feathers (Murphy and King, 1984). Therefore, many authors have concluded that finding the protein to replace feathers results in moult being energetically costly. Indeed Lindström *et al.* (1993) reported an 85% increase in basal metabolic rate in moulting bluethroats *Luscincia s. svecica*, and a 35 % increase in redpolls *Carduelis f. flammea*, when compared to pre-moult and post-moult values. In most species of birds studied the metabolic rate increases from 5 to 30% above the rate of non-moulting birds (Payne, 1972).

Out of the ten thousand species of birds, only ten orders have evolved the mode of replacing feathers through simultaneous moult (Hohman *et al.*, 1992). Simultaneous moult involves the shedding of all flight feathers at the same time (i.e. simultaneously), rendering the bird flightless (Fig. I.2). Simultaneous moult, and the associated period of flightlessness, only occurs in aquatic and marsh dwelling birds (except the notable exception of female hornbills, when they are blocked into the nest while incubating, Moreau, 1937), as these birds live in an environment where they can feed to some extent and escape predation, even when they cannot fly. Notably, the only passerines that endure a flightless period during wing moult are the aquatic dippers, *Cinclus cinclus*, (Bryant and Tatner, 1988).

Waterfowl are the largest group of birds that undergo simultaneous wing moult, and many species perform annual moult migrations to regular sites for moulting, deemed safe from predators (e.g. Fox and Kahlert, 2005). The duration of the flightless period ranges from approximately 12 days in Eurasian teal, *Anas crecca*, to 42 days in the larger species of swan (Hohman *et al.*, 1992). This period of flightlessness can present

a number of potential problems for moulting waterfowl, as being flightless could restrict the bird's normal capacity to forage and escape predation. Therefore, it is apparent that effective shortening of the flightless period has a selective value (Douthwaite, 1976; Owen and Ogilvie, 1979; DuBowy, 1985; Pehrsson, 1987; Fox and Kahlert, 2005). Studies on wild waterfowl have demonstrated that during this wing moult period, birds lose body mass (Geldenhuys, 1983; Sjöberg, 1986; Van der Jeugd *et al*., 2003), alter their behaviour (Kahlert *et* al., 1996; Adams *et al*., 2000) and significantly increase their rate of metabolism (e.g. Guozhen and Hongfa, 1986).

Barnacle geese undergo their annual wing moult after breeding and just prior to their autumn migration. Based on the mass of feathers and their protein content, Owen and Ogilvie (1979) predicted that the cost of wing moult in barnacle geese would be equivalent to 70 kcal per day over a 38-day period (a total cost of approximately 2675 kcal), which is comparable to about 25% of daily energy expenditure in winter (Ebbinge *et al*, 1975; Owen and Ogilvie, 1979). Birds that are unsuccessful breeders will typically moult early, increasing their time to lay down pre-migratory fat before the end of September. By moulting early, non-successful breeders regain flight earlier than successful breeders and can take advantage of feeding areas that are inaccessible to nesting birds and their families, such as the slopes beneath seabird cliffs (Owen and Ogilvie, 1979). The timing of moult is therefore largely dependent on breeding success. Successful breeders need to ensure that they have sufficient fat to undergo the energetic demands of the moult, which must be completed with sufficient time to lay down the necessary fat reserves for their autumn migration. Nesting females

therefore must replace some of the body reserves lost during egg laying and incubation before they can begin moulting.

Long distance travellers - migration

Using satellite transmitters, Butler *et al.*, (1998) were able track six individual barnacle geese from their breeding area in Ny-Ålesund, Svalbard, to their wintering grounds in Caerlaverock, Scotland (a total of 2500-3000 km, Fig. I.3). The six individuals left Ny-Ålesund around the end of August, and spent about three weeks at the south of Spitsbergen, the largest island in Svalbard. From here, the majority travelled straight to Bear Island (Bjørnøya), while two flew directly to the Norwegian coast without stopping. The four birds that did stop on Bjørnøya spent between four and 12 days there, inhabiting mainly the southern region, before following the Norwegian coast en route to Scotland (Fig. I.3). Through the use of heart rate loggers, Butler *et al.* (1998) established that the geese did not fly continuously between Bjørnøya and Caerlaverock, but stopped periodically, probably while travelling along the Norwegian Coast.

The heart rate data from Butler *et al.* (1998) also made it possible to determine the total flying time during the migratory period. With four of the geese, the total flight duration, from when they left Svalbard to when they arrived in Caerlaverock, was 61.2 ± 2.3 h. The mean duration of the longest, non-stop flight, was 13.1 ± 0.5 h, and the average number of flights longer than one hour was 11.5 ± 0.6 h. The longest individual flight recorded was 14 h, somewhat less than the 30-40 h required to

migrate non-stop from Svalbard to Scotland. However, while the birds tracked did make stops during their migration, it could be argued that from a 'physiological' point of view, for some of the individuals where the stops were brief, these stops were not substantial enough actually to be considered an opportunity to refuel, so the birds are capable of making the journey using their original stores accumulated in the Arctic (Butler *et al.*, 1998). One of the birds appeared to fly for 48 h 45 minutes out of a total of 58 h 51 minutes, around 83% of 2.5 days.

Saving energy - seasonal hypothermia

Butler and Woakes (2001) proposed the possibility that wild migratory birds become hypothermic just before their migration, when food is not scarce, but when the necessity to conserve and/or store energy in the form of fat is of overriding importance. They studied wild barnacle geese, through the use of internally implanted data loggers that recorded abdominal temperature. In total, there was a mean decrease in 24 h abdominal temperature of the geese of 4.4 ^{0}C, over a total duration of 28 days, coinciding with the autumn migratory period. If it assumed that the whole goose experiences the same reduction in body temperature, as was measured for the abdominal cavity, and if the apparent Q_{10} (Heldmaier and Ruf, 1992) is between 2.5 and 3 then, other things remaining equal, the metabolic rate of the geese would be between 34 and 39 % lower at the end of the period of progressive hypothermia than at the beginning (Butler and Woakes, 2001).

Butler and Woakes (2001) predicted what the saving in energy expenditure would be as a result of this hypothermia. They proposed that the average saving in energy expenditure over the 28-day period of hypothermia for a resting 2.0 kg goose would be between 3619 and 4151 kJ. If the energy density of fat is 39 kJ g^{-1}, this amount of energy would equate to between 93 and 106 g of fat. This compares with the 430 g of fat estimated to be used by a 2.3 kg barnacle goose during its migration from Svalbard to Scotland (Butler *et al.*, 1998). This proposed energy saving, however, did not coincide with the deposition of fat prior to migration, but rather with its utilization during the migratory period, and subsequently with its replacement following the migratory flight (Butler and Woakes, 2001).

1C: Tools and Techniques

The measurement of energy expenditure in free-ranging vertebrates

Ecophysiologists and functional ecologists seek to further our understanding of the manner in which organisms operate in their natural environment. The utilisation of energy is one of the key factors that will determine an organism's ability to survive and reproduce (Butler *et al*., 2004). Therefore, information about the energy expenditure of free-ranging animals holds the key to understanding major aspects of their behaviour, in their natural environment (Froget *et al*., 2001; Tolkamp *et al*., 2002). To study energy-expenditure in free-ranging animals, a method is required which can measure energy expenditure at a fine timescale, for long periods of time.

Time-energy budgets (TEB)

Time energy budgets involve collecting data about the time spent in different activities by animals in the field, and then assigning a metabolic cost to each activity, determined either from studies in the laboratory or from allometric equations (e.g. Grémillet *et al*., 1995). The sum of the probability of each activity occurring, multiplied by its energetic cost, gives the total energy expenditure, e.g.:

$$TEE = p_1r_1 + p_2r_2 + p_3r_3 \ldots\ldots\ldots\ldots\ p_nr_n \qquad (1)$$

where *TEE* = totally energy expenditure, p = proportion of time engaged in a particular activity, and r = rate of energy expenditure of that activity. This method has been commonly used due to it relatively low financial cost to the researcher, and general simplicity and accessibility (e.g. Goldstein, 1988). However, the major disadvantage of the TEB method is that it can be extremely time consuming to implement, and it is necessary to assume that behaviour is discrete and that every activity observed can be assigned to one of the categories created by the investigator. More importantly perhaps is their limitation to studying animals that spend a significant portion of their time out of sight, either underwater, underground or in the air. Generally, between TEB studies there is a lack of consensus in methodology, which restricts their accuracy and comparative value. Validations studies have shown that energy expenditure as calculated by TEBs may, when compared with estimates from other techniques, yield values that are too low by as much as 44%, or too high

by as much as 57% (Nagy, 1989). Almost all TEB studies have been restricted to birds, as they are relatively easy to observe in the field and tend to behave predictably.

Doubly labelled water (DLW)

Doubly labelled water is based on the observations of Lifson and McClintock (1966), that oxygen atoms in the metabolically produced carbon dioxide (CO_2) freely equilibrate with the oxygen atoms of water via the action of carbonic anhydrase in the blood. When known amounts of ^{3}H and ^{18}O labeled water are injected into an animal, the ^{18}O water equilibrates with both the CO_2 and water pools, and declines as a function of water influx. The initial dilution of these isotopes after an equilibration period allows determination of the total body water (TBW) volume. The difference between ^{18}O turnover and ^{3}H turnover across the duration of the experiment is a measure of the animal's rate of CO_2 production ($v\dot{C}O_2$), as during metabolism, CO_2 is produced, resulting in a dilution of ^{18}O, while ^{3}H does not change.

The DLW technique relies on a number of assumptions being met and adhered to (see Speakman, 1997). Despite the potential for some problems and inaccuracies, the technique has been widely employed in a range of different species. Validation studies from the laboratory which compare DLW to respirometry, give a good indication of the accuracy of this method. Such studies have shown metabolic rate (MR) to be estimated within 11% (Westerterp *et al*., 1988; Nagy, 1989), though most of these earlier validation studies involved resting animals. Other studies have shown that though DLW can be used to estimate field metabolic rate (FMR), it is possible that

FMR can be overestimated by as much as 36% in aquatic animals (Bevan *et al*., 1995). Similarly, a validation study on barnacle geese (Nolet *et al*., 1992) concluded that though DLW estimates of MR were not significantly different from those obtained by respirometry, the range of individual errors was considerable, and the technique could not, therefore, be used to determine the energy expenditure of individuals.

Advantages of the technique are simplicity of use, and despite some evidence of deleterious effects on breeding birds in terms of nest attendance (Birt-Friesen *et al*., 1989), it is relatively non-invasive. The results of the technique are instantly usable, without the need to do further calibration studies in the laboratory before results can be interpreted. The technique can be used on very small animals, for example, shrew-tenrecs (*Microgale dobonsi*) (Stephenson *et al*., 1994) and long-eared bars (*Plecotus auritus*), which demonstrated no change in behaviour during DLW experiments (Entwistle *et al*., 1994).

A disadvantage of the DLW technique is that the duration of an experiment is limited by the rate of turnover of ^{18}O. Animals must be recaptured and sampled within a specific window of time which allows the ^{18}O level to decline sufficiently to detect a difference from initial levels, but not too far such that the ^{18}O is not different to background levels. This imposes limits on the design of experiments and subject animals used, and there is no guarantee the animal will return to have blood samples taken. Perhaps the most significant disadvantage of the DLW technique however, is that the value for energy expenditure obtained is simply an average for the number of

days the experiment ran for, i.e., the duration between the two sampling points. It is not possible to assign metabolic costs to different activities, thus it is still necessary to construct TEBs, and/or make assumptions about resting metabolic rates (Nagy *et al*., 1984), in order to interpret the energetic data.

Heart-rate method (f_H)

The heart rate method (Butler, 1991; Butler, 1993), which uses fitted devices to transmit or record heart rate (e.g. Woakes *et al*., 1995), has proved to be an increasingly popular and powerful tool when investigating the energy expenditure of free-ranging animals (e.g. Bevan *et* al., 1995; Green *et* al., 2001; Butler *et al*., 2002; Froget *et al*., 2002). This method is based on the relationship between rate of oxygen consumption ($\dot{V}_{O_2}$) and heart rate (f_H), as originally described by Fick's (1902) convection equation for the cardiovascular system of humans:

$$\dot{V}_{O_2} = f_H \times \dot{V}_s (C_a O_2 - C_{\bar{v}} O_2), \qquad (2)$$

where $\dot{V}_s$ is cardiac stroke volume, $C_a O_2$ is the oxygen content of arterial blood and $C_{\bar{v}} O_2$ is the oxygen content of mixed venous blood. If, $\dot{V}_s (C_a O_2 - C_{\bar{v}} O_2)$, the oxygen pulse ($sO_2$ pulse), remains constant or varies systematically, there is a relationship between $\dot{V}_{O_2}$ and f_H, hence making it possible to calculate the former from the latter (Butler, 1993). This relationship has been demonstrated in several species of birds including barnacle geese (Nolet *et al.*, 1992), blue-winged teal (*Anas discors*) (Owen,

1969), black duck (*Anas rubripes*) (Wooley and Owen, 1977), pigeon (*Columba livia*) (Flynn and Gessaman, 1979), American kestrel (*Falco sparverius*) (Gessaman, 1980), marabou stork (*Leptoptilos crumeniferus*) (Bamford and Maloiy, 1980), emu (*Dromaius novahollandiae*) (Grubb *et al*., 1983), tufted duck (*Aythya fuligula*) (Woakes and Butler, 1983) and common eider (*Somateria mollissima*) (Hawkins *et al*., 2000). Relationships have also been demonstrated in a number of mammals including pine marten (*Martes americana*), (Fisher *et al*., 1987), red squirrel (*Scirius vulgaris*) (Pauls, 1980), bottlenose dolphin (*Tursiops truncatus)* (Williams, *et al*., 1993) and Californian sea-lion (*Zalophus californianus*) (Butler *et al*., 1992).

The heart rate method for estimating FMR has become better developed and more widely used in recent years, as it is facilitated by advances in technology for measuring and recording heart rate in the field through the use of miniaturized heart rate data loggers (HRDLs, e.g. Green *et al*., 2005). This technique has been used to monitor continuously the rate of energy expenditure in several bird species, including macaroni, *Eudyptes chrysolophus*, king, *Aptenodytes patagonicus,* and gentoo, *Pygoscelis papua,* penguins, and black-browed albatross, *Diomedea melanophyrys* (Green *et al*., 2005; Froget *et al.*, 2002; Bevan *et al*., 2002; Bevan *et al*., 1995).

The heart rate method relies on accurate calibrations between $\dot{V}_{O_2}$ and f_H. The ideal approach would be to calibrate this relationship in each animal that is to be used in the field, however, the disturbance associated with calibration, the difficultly of obtaining animals, and potential interference with selected individuals usually makes this impracticable (Green *et al*., 2001; Green and Frappell, 2007). Generally, therefore,

this relationship is established in the laboratory and then applied to free-ranging animals, taking into account the associated inter and intra-individual variability (e.g. Green *et* al., 2001; Froget *et al*., 2001). This latter point is essential, as a wide range of errors demonstrates that f_H cannot be used to estimate the MR of individual animals, if each animal is not individually calibrated.

Some inconsistencies have been detected in the relationship between f_H and $\dot{V}_{O_2}$, which is something the current project will address. The relationship has been found to vary between different seasons (Holter *et al*., 1976), dates (Gessaman, 1980), social scenarios (Flynn and Gessaman, 1979, and modes of locomotion (Hawkins *et al*., 2000). The principle advantage of the f_H method, however, is that it can provide estimates of MR at a far greater temporal resolution than other techniques, the resolution being limited only by the calibration procedure and method of recording heart rate (Green *et al*., 2001). The data logger's record heart rate as a series and so, in conjunction with behavioural data, individual activities can be assigned an energy cost, as long as stroke volume does not change with activity type. Again, recent developments in technology have meant that automated recording devices such as time-depth recorders (Bevan *et al*., 1997), speedometers (Boyd *et al*., 1999), salt water switches (Bevan *et al*., 1995) and satellite transmitters (Butler *et al*., 1998) can record behavioural data without the need for continuous observations. However, externally mounted devices can impose additional energetic costs by increasing drag when swimming or flying, which in turn can effect foraging and breeding success (e.g. Carborne *et al*., 1996). Therefore, to account for this problem, HRDLs are implanted into the abdominal cavity of the animal.

Heart rate data loggers

The HRDLs (Fig. I.4) were originally developed by Woakes and Butler (1975) at The University of Birmingham, to allow long term recording of heart rate and body temperature in free-ranging animals. The miniature data logging systems were based on solid-state memory devices, designed to enable heart rate and body temperature to be recorded over long periods of time with good time resolutions (Woakes *et al.*, 1992). The loggers were designed to be implanted so they could survive on the subject species for some time, and so the ECG electrodes could be placed subcutaneously and provide a good ECG signal without the problem of movement, changes in skin insulation and physical abuse (Fig. I.5; Woakes *et al.*, 1992). The loggers were developed to be the smallest possible size, and to operate on a single lithium cell of 3.6V. The data logger consists of three main elements; the electrocardiogram (ECG) detection circuit, digital control, and memory. In addition, an interface unit is required to allow data and programming variables to be transferred between loggers and a computer base unit.

The unencapsulated HRDLs (Fig. I.4a) are approximately 55 mm x 25 mm x 7 mm in size and weigh 25 g. Before they are implanted they are encapsulated in a waterproof layer of paraffin wax and encapsulated in silicon rubber to provide waterproofing and biocompatibility (Fig. I.4b). The temperature sensor of the encapsulated data logger is calibrated by immersion in water baths of known temperature.

Critical analysis of the techniques

The utility of each technique for measuring energy expenditure in vertebrates will depend, to a large extent, on the question being asked. Butler *et al*. (2004) suggested that f_H will be a superior approach for larger animals (>1 kg), and where an energetic cost is desired for different activities. DLW would be a better approach for smaller animals (> 1 kg). In the present, as the aim is to assign an energetic cost to certain events such as moult, the f_H is by far the most suitable technique.

Techniques for estimating body composition

Body fat stores are frequently related to individual fitness, and therefore, techniques that measure body composition accurately may allow biologists to assess the relationship between physical condition and survival, productivity, and behaviour (Green, 2001). The body composition of an animal can be quantified accurately using whole body homogenization followed by chemical extraction (Walsberg, 1988). However, this approach is destructive and only allows one measurement. Many different techniques have estimated body composition using indirect, non-invasive techniques such as morphometric measurements and qualitative visual assessments (Wirsing *et al*, 2002). These methods though, in the past, have suffered from a lack of validation and are often inaccurate, imprecise and therefore incapable of quantifying an animal's fat reserves (Walsberg, 1988; Green, 2001; Wirsing *et al*, 2002), but with

suitable validation they could be a useful tool in predicting the body composition of animals.

Many of the techniques have been used to assess body composition in birds (Castro, 1990; Scott *et al*, 1991 and 1996; Moe *et al,* 2002), with varying degrees of success. To investigate the interaction of body composition with other physiological variables, throughout the annual cycle, in the same individual birds, an accurate non-destructive technique is required.

Total body electrical conductivity (TOBEC)

Total body electrical conductivity (TOBEC®) is an indirect method of assessing body composition that has been developed over the last thirty years, mainly for use in the biomedical and the meat processing industries. With this technique, the subject is placed within a chamber surrounded by a conductive coil, producing a measure of total body conductivity (Scott *et al.*, 2001). TOBEC relies on the principle that the electrical conductivity of an organism is proportional to its fat-free mass (Wirsing *et al.*, 2002). The output signal from TOBEC will give an estimate of lean body mass that can be converted into fat mass (Walsberg, 1988), by subtracting the lean mass from the total body mass (Scott *et al.*, 2001).

The potential application of TOBEC within the field of animal biology, particularly ecology and physiology, was not fully appreciated until Walsberg (1988) published a critical evaluation of the technique and its use on small birds and mammals. Since then, TOBEC has been used on a broad range of animals including mammals (Wirsing *et al.*, 2002), birds (Castro, 1990; Scott *et al.*, 1991 and 1996), birds eggs (Williams *et al.*, 1997), fish (Gillooly and Bayliss, 1999) and reptiles (Angilleta, 1999).

A number of factors can influence TOBEC readings and consequently its accuracy (Scott *et al.*, 1996). With the TOBEC instrument, the most uniform section of the magnetic field occurs in the centre of the sampling area (Walsberg, 1988; Scott *et al.*, 2001), so it is vital to position the animal within the coil in a manner that is easy to repeat. Because positioning of the subject is so important when using TOBEC, each time a reading is taken, the animal must remain still, and in the same position within the chamber for all recordings. With birds, various methods have been devised to achieve the desired 'stillness' that is required to gain accurate readings from TOBEC, including rubber bands (Skagen *et al.*, 1993), Velcro jackets (Scott *et al.*, 1991) and nylon mesh stockings (Walsberg, 1988). Anaesthesia has been used during TOBEC measurements (Gillooly and Bayliss, 1999; Witter and Goldsmith, 1997; Gillooly and Bayliss, 1999), to ensure the animal is positioned uniformly in the chamber, to reduce movement and possibly to reduce stress on the animal (Scott *et al.*, 2001). However, anaesthesia is not necessarily suitable for use in conjunction with TOBEC in warm-blooded creatures, as the associated drop in body temperature alters the TOBEC reading (Tobin and Finegood, 1995).

Scott *et al.* (2001) reviewed all validation studies (up to 1999) that used TOBEC as a technique for measuring body composition in live wild animals – 23 in total. Of the validation studies reviewed, 14 endorsed the TOBEC technique as effective, while nine concluded that TOBEC was no more effective that other simpler, cheaper alternatives such as morphometrics or fat scoring.

Ultrasound

Ultrasound has been used and employed extensively for a broad spectrum of applications. It has been an effective aid in military and civil navigation, and a useful diagnostic tool in human and veterinary medicine. Ultrasound works on the principle that a high frequency sound emitted from a source will travel through a medium until it hits a reflector, and then return as an echo to the source. Time differences between sound emission and the returning echo, and the intensity of the echo may be used to obtain information about distance and the properties of the reflector (Starck *et al.*, 2001).

Ultrasound is a relatively simple method for body composition analysis (Baldassare *et al.*, 1980). All that is required is a detailed knowledge of the anatomy of the studied animal, and experience at interpreting the images (Starck and Burann, 1998; Dietz *et al.*, 1999). When considering non-invasive techniques of body composition, ultrasound is particularly useful because it allows morphometric measurements of structures *in situ*, and requires only manual restraint of the animal, so in comparison

to TOBEC, for example, it is a relatively non-stressful method. One problem with using ultrasound on wildfowl, for example, is their plumage, the dense layer of feathers being a real obstacle to the use of ultrasonography for body composition analysis. However, Farhat and Chavez (1999) found that by using a multipurpose ultrasound gel, they could sufficiently part the feathers to expose a small area of breast skin of mallards without causing any long term damage to the feathers or the bird.

Isotope Dilution Method

Water is not evenly distributed in body tissues, and it is this assumption that forms the basis for the isotope dilution method. Fat contains substantially less water than lean tissue and this difference means that the fatter an organism becomes, the lower the water content as a percentage of its body mass (Speakman *et al.*, 2001). The first attempts to use isotopes in body condition studies came as a result of the discoveries in the 1930s of stable isotopes of oxygen and hydrogen. Von Hevesy and Hofer (1934) used oxygen and hydrogen isotopes to 'label' the water directly, and the isotope dilution method grew out of these initial studies (Speakman *et al.*, 2001).

The isotope dilution technique is based on a similar principle to that of a mark/recapture scheme frequently used in ecology. There are 'n' molecules of water in the body, and a certain number of 'marked' water molecules are introduced into the body in the form of an isotope. A sample of body water is then taken after a suitable time period, to examine the extent to which the marked water molecules have been

diluted in the total population of water molecules (Speakman *et al.*, 2001). This is achieved by measuring the isotopic enrichment in the sample (the 'marked' molecules). This marking is done by replacing one of the hydrogen molecules with a heavy isotope of hydrogen – deuterium ^{2}H or tritium ^{3}H, or the oxygen molecule with a heavy isotope of oxygen – ^{17}O or ^{18}O. As it possible to count the number of 'marked' water molecules, an estimate can be calculated from the known molecular mass of the marked water molecules.

Morphological Indicators

The main advantage of using morphological indicators is their simplicity and cheapness. Previously, many question marks have hung over their effectiveness and ability to predict body composition, leading Hayes and Shonkwiler (2001) to suggest that the method should be abandoned entirely. However, they were referring to studies that have utilized the technique without any sort of validation, either before, or after the study.

Studying morphological measures involves using the external measures of the animal (overall size), for example, body mass, wing chord, foot length, bill depth etc, as an indicator of body condition, and from this, develop body condition indices (Moe *et al*, 2002; Viggers *et al.*, 1998; Larsson and Forslund, 1991; Johnson *et al.*, 1985). These body condition indices are then thought to reflect (or represent) variation in diverse aspects of organismal quality, such as health, nutritional status, or body fat.

They have been used, to varying degrees of success, on numerous animal species in previous studies, particularly those that involve a field based element, or those which include species that are endangered or are of conservation concern. These include kittiwakes (*Rissa tridactyla*) (Moe *et al.*,2002), redshanks (*Tringa totanus*) (Scott *et al.*, 1996), Hawaiian geese (*Branta sandvicensis*) (Zillich and Black, 2002), barnacle geese (Larsson and Forslund, 1991; Black *et al.*, 1998), mute swan (*Cygnus olors*) (Sears, 1998), Antarctic fur seals (*Arctocephalus gazelle*) (Arnould**,** 1995), red squirrels (Wirsing *et al.*, 2002) sperm whales (*Physeter macrocephalus*) (Evans *et al.*, 2003) and even wolf spiders (Lycosidae) (Jakob *et al.*, 1996).

Biologists have used two basic approaches for estimating body condition from external morphology. These two approaches are based on the construction of ratio variables, e.g. body mass divided by length, and the generation of residual variables (e.g. residuals from the regression of mass on length).

Wirsing *et al.* (2002) applied these principles to red squirrels, yellow-bellied marmosets, *Petaums australis*, and snowshoe hares, *Lepus americanus*, to estimate body condition. They measured a total of nine morphological features, including skull length, chest circumference, hind-foot length and total body length, before using the "gold standard" fat extraction method (i.e. destructive sampling and chemical fat extraction) on the animals, for validation. They then employed stepwise regression to generate a predictive model of total body water and lean body mass. Total body mass (TBM) values were divided by the best linear metric of structural size raised to the first, second, and third power, to include in the model. They ascertained what

morphological feature was found to be the strongest correlate (SC) of lean body mass, and then selected it as the best metric of structural size (i.e. TBM/SC, TBM/SC^2, TBM/SC^3). Additional indexes of condition were added into the model, including those derived from the residuals from the line generated by regressing TBM against a further two measured morphological features. By applying the correct statistical analysis, Wirsing *et al.* (2002) found that most of the morphometric variables included in their study were closely related to total body water and lean body mass, suggesting that a variety of measurements may serve as viable ways to estimate the two values.

Abdominal Profile Index

In common with morphological indicators, abdominal profile indexes are perhaps most appropriate when studying either wild populations of birds, or endangered species. Westneat *et al.* (1986) described how certain bird species become extremely distressed when handled, and handling can actually cause a temporary negative effect on overall condition. Therefore, abdominal profile indices can be extremely useful. They were originally developed by Owen, in 1981, and the index is thought to reflect the amount of accumulated abdominal fat. Since abdominal fat is a good indicator of overall body fat (Thomas and Mainguy, 1983), Owen (1981) argued that the abdominal profile index (or API) gives a useful estimate of the overall body mass of individual geese. The method allows repeated assessments of individually marked

birds, so that variation in ‘fatness’ can be tracked on a temporal and spatial scale (Krementz and Pendleton, 1990; Zillich and Black, 2002). The usefulness of the API has been shown in many aspects of waterfowl ecology, including the profitability of foraging within a season (Owen, 1981), in different social groups and classes (Black and Owen, 1989), between years (Owen and Black, 1989) between habitats (Owen, 1984) and in relation to specific energetic requirements (Loonen *et al.*, 1991).

The problem with APIs is that it is a subjective method of assessment, not a direct measurement of mass. Zillich and Black (2002) attempted to validate the API using captive Hawaiian geese (or nene). Through weighing the geese, and using morphological indicators (as well as principle component analysis), they were able to demonstrate that the API can be a useful tool to monitor mass variation.

Critical analysis of techniques

When studying body composition in larger animals, certain considerations have to be given when deciding on a suitable method of non-invasive body composition analysis. TOBEC and ultrasound require the subject to remain still before any kind of reading can be taken, and most studies that have applied this method have been focusing on smaller animals, such as passerine birds and mice (Scott *et al.*, 1991 and 1996; Wirsing *et al.*, 2002), which were sedated before any readings were taken. It is also not known the effects that TOBEC could potentially have on any implanted logging devices (and vice versa). Another feature of these studies is that the readings only needed to be taken once a year. Therefore, any work that would require more

frequent, repeated readings in larger animals could become problematic. Typically with larger animals, isotope dilution method and morphological indicators are most frequently used (Rumpler *et al.*, 1987; Arnould, 1995; Jakob *et* al., 1996; Delong and Gessaman, 2001). These two methods do not involve any sedation, or require the subject to remain still for any long periods of time.

1D: Summary and research objectives

The principle aim of the present study and the chapters in which these objectives are addressed, are as follows:

1. Record the annual cycle in body mass of captive barnacle geese, to investigate whether mass changes over the annual cycle, and if so, whether these are associated with certain events in the annual cycle (Chapter II).
2. Record the behaviour of the captive geese at set points in the year to ascertain if, despite being in captivity, their behaviour during wing moult changes in a similar way to the behaviour changes associated with wing moult in wild birds (Chapter II).
3. Investigate the muscular changes that may occur in moulting geese, particularly to ascertain if, despite being in captivity, the bird's primary locomotor muscles follow the same temporal pattern of atrophy and hypertrophy as are found in wild moulting waterfowl (Chapter III).

4. Calibrate a non-invasive technique for measuring year round body composition in captive geese, to investigate whether captive birds follow a similar pattern of fat deposition to that of their wild counterparts, and also to relate changes in fat content to their overall energetics (Chapter IV and V).
5. Calibrate the $f_H / \dot{V}_{O_2}$ relationship at six points in the annual cycle in captive geese, to investigate the possible correlations between changes in body mass and body composition, and activity of the cardiovascular system (Chapter VI).
6. Investigate year round abdominal body temperature in captive geese (Chapter VII).
7. Where possible, make comparisons between data recorded in captive geese, to wild birds (Chapter VII).

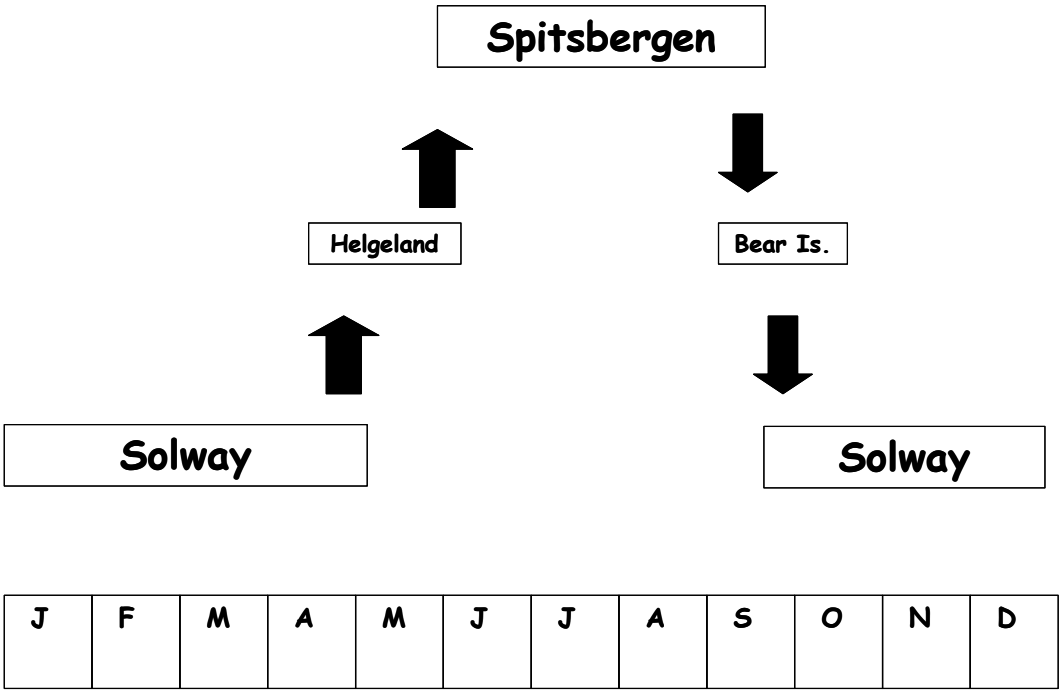
A general aim of this study is to ascertain the degree to which the physiological, anatomical and behavioural changes that occur in the annual cycle in barnacle geese are innate, i.e., largely independent of environmental cues. The captive birds are maintained under conditions that are quite different to those experienced by the wild birds. The wild geese experience constant daylight during the summer in Svalbard, undertake long distance migratory flights, etc, while the captive geese are sedentary and experience a rhythmic light/dark cycle, even in the summer months. The term innate in this study is taken to mean 'largely independent of environmental cues or stimuli'.

Animal welfare

Since I have studied the physiological responses to events within the annual cycle in the geese, it has been essential to ensure that the experimental procedures have caused as little stress to the animals as possible. Animals that did not become accustomed very rapidly to the experimental conditions were removed from any trials. In particular, any bird that did not become accustomed to the respirometer box was removed from the study. This only happened in one case, the remainder of the birds becoming fully accustomed to the experimental set up. Post surgery (HRDL implantations), birds were monitored closely and no bird had lost a significant amount of body mass when they were reweighed 5-7 days after surgery. Heart rate and body temperature data from the HRDLs showed that recovery from surgery was a two step process, with heart rate initially being heightened on gaining consciousness from the anaesthetic. Between 3-4 hours post surgery, heart rate then decreased, before decreasing a further 5-6 hours later and returning to pre-surgery levels within 24 hours of the surgery.

All regulated procedures were performed by British Home Office licensed personnel in possession of a Personal License, and working under the auspices of a corresponding Project License, as set out in the Animals (Scientific Procedures) Act 1986.

(a)



(b)

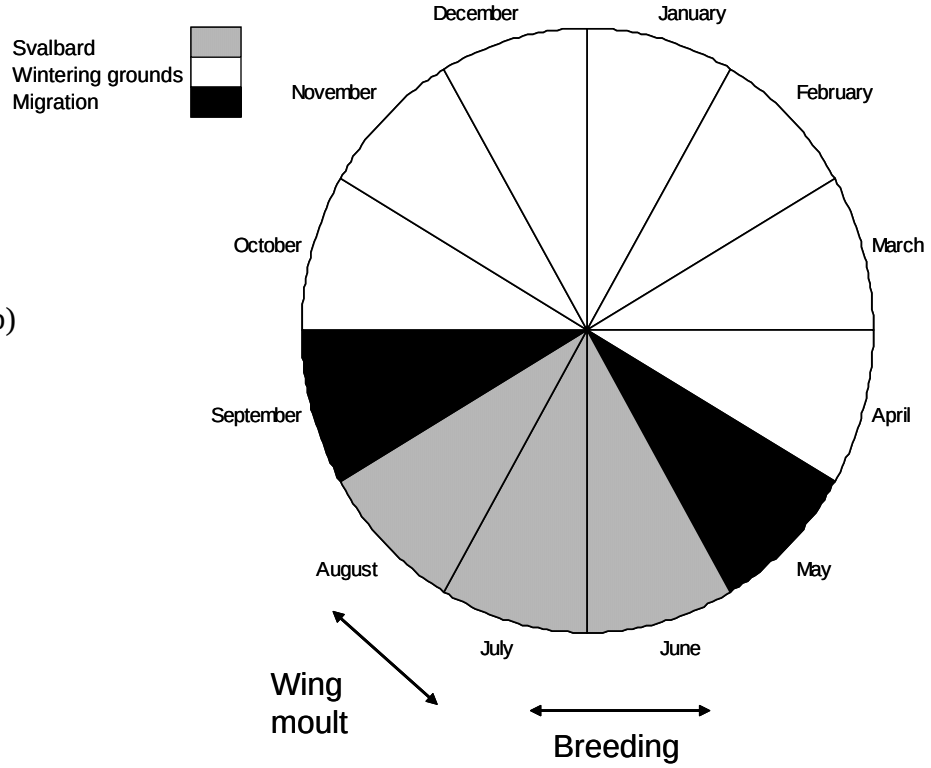


Figure I.1: (a) Organisation of the annual cycle of the Svalbard breeding population of barnacle geese, and (b), indicating when and where moult and breeding take place.

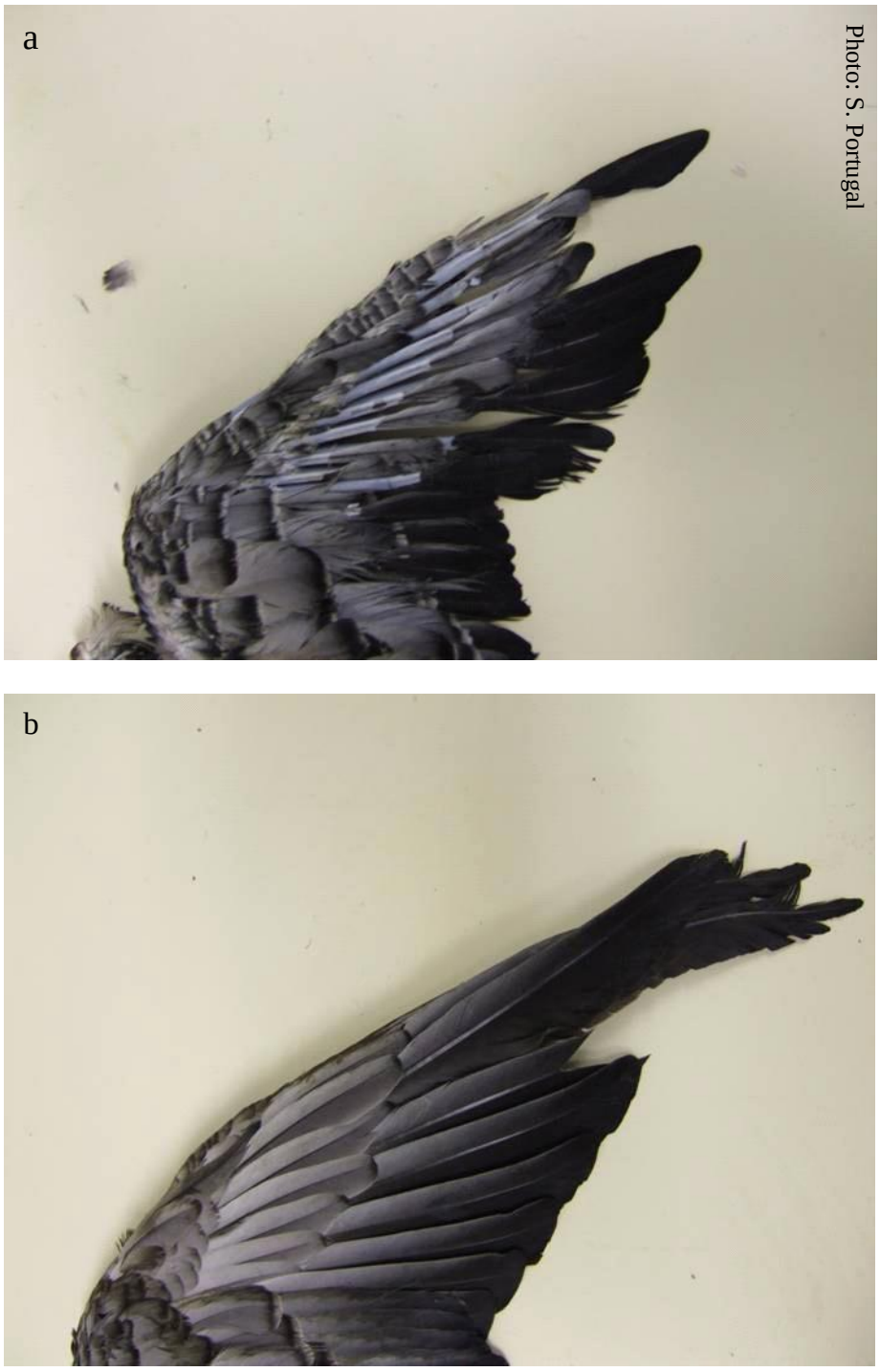


Figure I.2: A captive barnacle goose (a) during, and (b), post, wing moult.

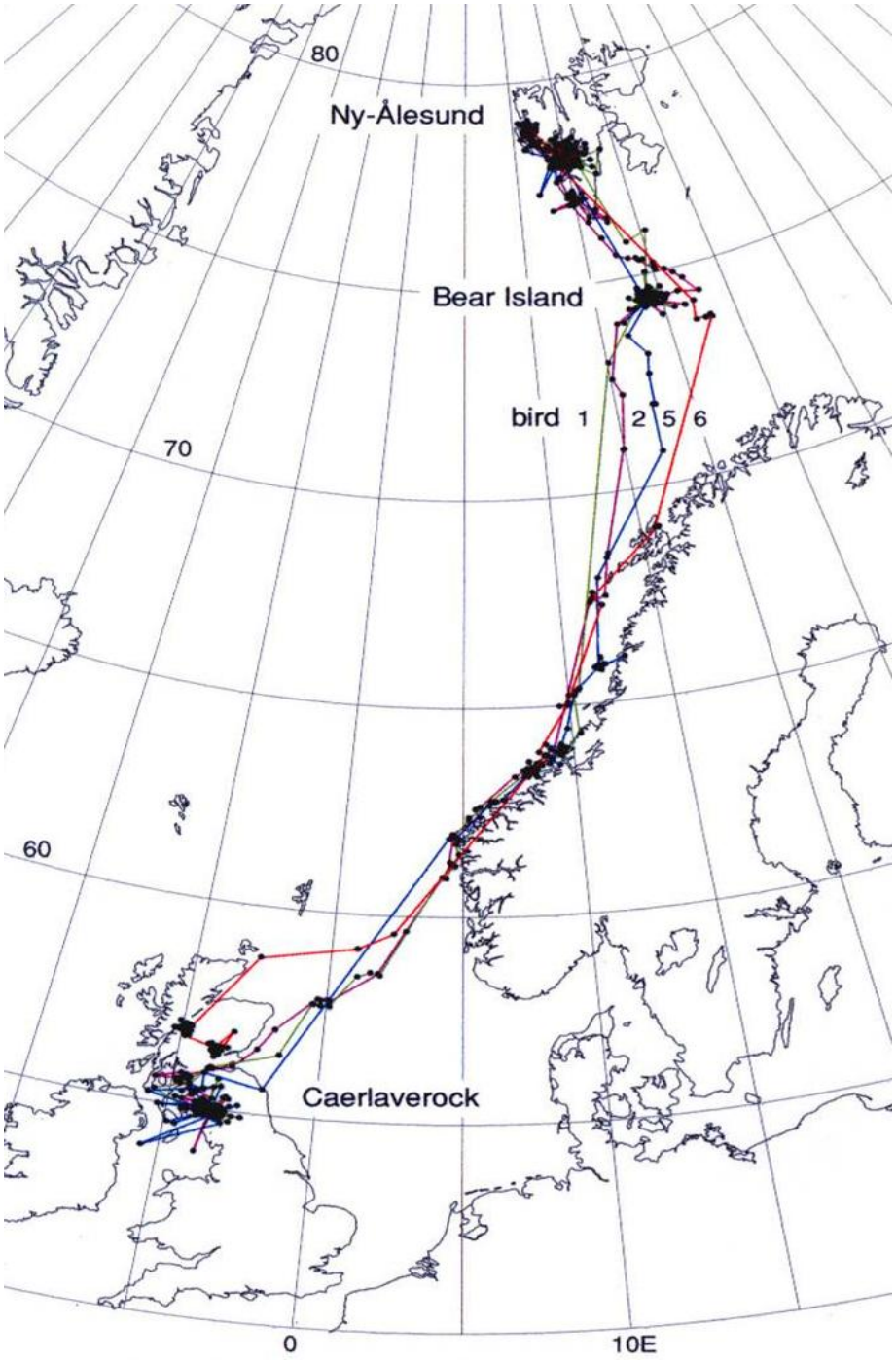
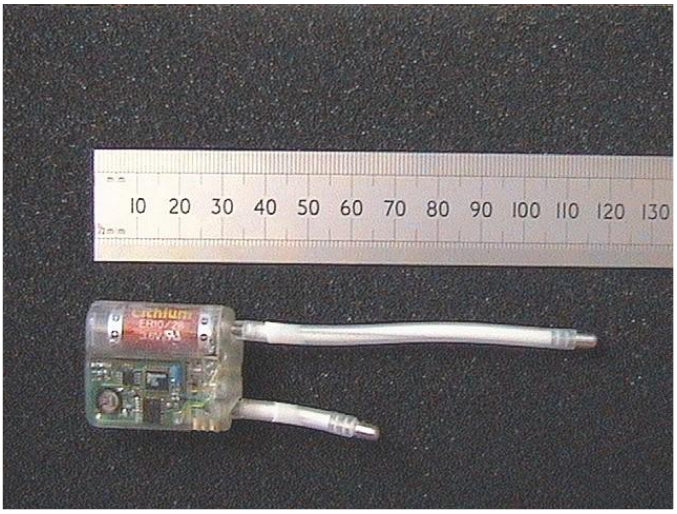
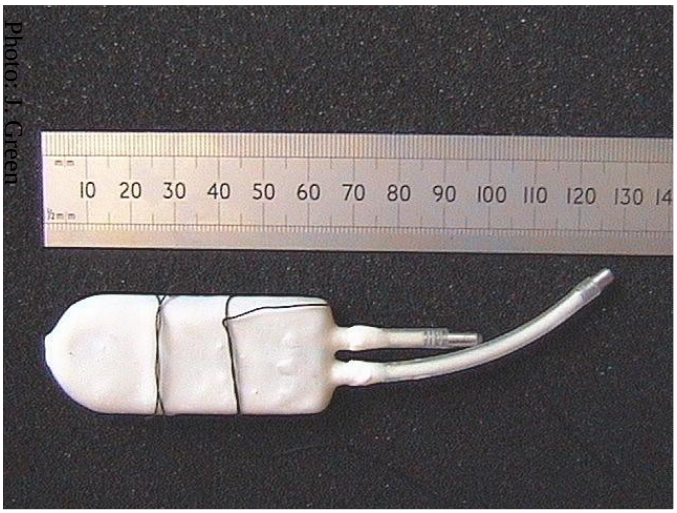


Figure I.3: Autumn migration routes of four barnacle geese fitted with satellite transmitters (taken from Butler *et al*., 1998).

(a)

Photo: J. Green





(b)

Photo: J. Green

Figure I.4: An un-encapsulated heart rate data logger (a), and (b) encapsulated in silicone prior to implantation.

Photo: A. Fahlman

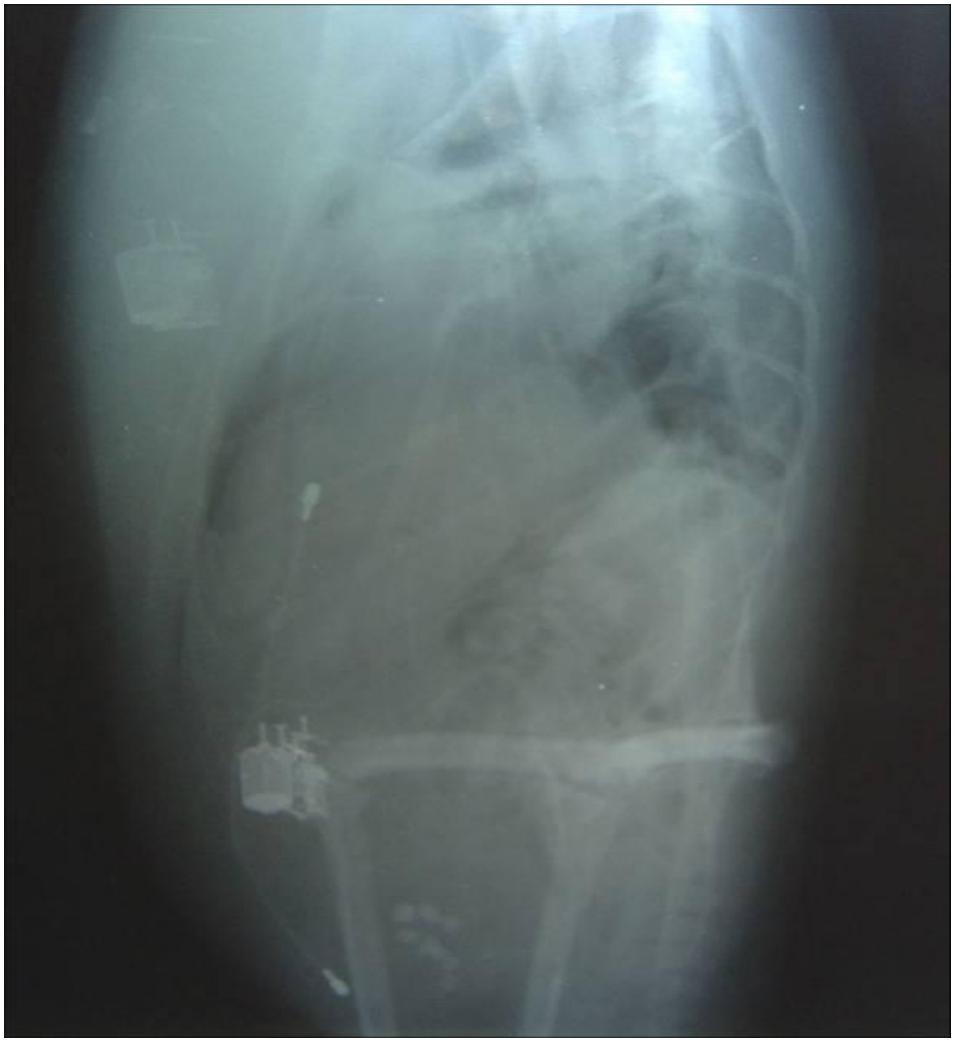


Figure I.5: X-ray of a king penguin (*Aptenodytes patagonica*), detailing the internal positioning of a heart rate data logger. Also shown in this x-ray are temperature sensors (top left) fitted to measure stomach temperature.

II. ANNUAL CHANGES IN BODY MASS AND RESTING METABOLISM IN CAPTIVE BARNACLE GEESE (*BRANTA LEUCOPSIS*): THE IMPORTANCE OF WING MOULT

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*Many different physiological changes have been observed in wild waterfowl during the flightless stage of wing moult, including a loss of body mass. We aimed to determine whether captive barnacle geese (*Branta leucopsis*) would show the characteristic decrease in body mass during their wing moult, even though they had unlimited and unrestricted access to food. Fourteen captive geese were weighed at 1 – 2 week intervals for two complete years. During the flightless period of the moult, body mass decreased by approximately 25 % from the pre-moult value. To understand the basis of this change, the rate of oxygen consumption was measured during daytime and nighttime at six points in the second year, and at three points (before, during and after wing moult) behavioural observations were made. Measurements of the rate of oxygen consumption taken during moult showed an 80 % increase above that of the nonmoulting periods of the year. We propose that metabolism was increased during moult because of the cost of feather synthesis. Although food was available, the captive birds chose not*

to forage and instead increased the proportion of time spent resting. It is likely that this behaviour in response to wing moult is a strategy to avoid predation in the wild. Thus, the endogenous nature of this behaviour has potential survival value for wild birds of this species .We conclude that the increase in metabolism led to the use of endogenous energy reserves because the birds reduced rather than increased their food intake rates and as a result, the barnacle geese lost body mass during wing moult.

SJP developed the methodology, conducted the data collection, analysed the data and wrote the manuscript. PJB and JAG discussed and developed methodology, data analysis and conclusions, and aided in the writing of the manuscript.

Introduction

Timing of the annual moult is an important component of energy allocation in birds (Hohman *et* al., 1992; Gates *et al*., 1993). Waterfowl are one of 10 orders of birds that undergo a post-breeding simultaneous flight feather moult, rendering them flightless for a period of approximately 15-45 days (Woolfenden, 1967, Hohman *et al*., 1992). Barnacle geese, *Branta leucopsis*, undergo a simultaneous wing moult just prior to their autumn migration, lasting approximately 32 days (Owen and Ogilvie, 1979). In the wild, this period of flightlessness could present a number of problems for the geese, such as restricting their normal capacities to forage and escape predation. Therefore it is apparent that shortening of the flightless period has a selective value (Douthwaite, 1976; Owen and Ogilvie, 1979; DuBowy, 1985; Pehrsson, 1987; Fox and Kahlert, 2005).

Mass loss during wing moult has been documented in a number of waterfowl species in the wild, including the Russian breeding population of barnacle geese (Van der Jeugd *et al*., 2003), Barrow's goldeneye *Bucephala islandica* (Van de Wetering and Cooke. 2000), greylag geese *Anser anser* (Fox and Kahlert, 2005), mallard *Anas platyrhynchos* (Pehrsson, 1987), Eurasian teal *Anas crecca* (Sjöberg, 1986) and South African shelduck *Tadorna cana* (Geldenhuys, 1983).

A loss in body mass during moulting might be adaptively advantageous, since a lighter bird may be able to fly again sooner than a heavier bird, and therefore be able

to escape predators or move sooner to a more suitable habitat (Geldenhuys, 1983; DuBowy, 1985; Sjöberg, 1986). However, Hanson (1962), Ankney (1979) and Dolnik and Gavrilov (1979) suggested that wing moult is nutritionally stressful and energetically demanding and increases in metabolism of up to 40% have been recorded in moulting waterfowl (e.g. Guozhen and Hongfa, 1986). Energetic costs of moult can include nutrient demands for feather components, increased amino acid metabolism, changes in water balance, an increase in blood volume and enhanced heat loss (King, 1980; Lovvorn and Barzen, 1988).

As well as these physiological changes, many species of waterfowl also display a marked difference in behaviour during the flightless moult period (Owen and Ogilvie, 1979; Murphy, 1996; Adams *et al*., 2000; Van de Wetering and Cooke, 2000). Waterfowl often become inactive, spending longer periods resting or hauled out of the water, devote less time to foraging and general maintenance, and may switch to nocturnal feeding (Thompson, 1992; Adams *et al*., 2000; Kahlert *et al*., 1996). Panek and Majewski (1990) proposed that these changes in behaviour are a result of the increased risk of predation as a result of being flightless, and this behavioural change results in a loss of feeding opportunities, and consequently a 18% drop in body mass.

Relatively little is known about the effects of captivity on wing moult in waterfowl (e.g. Hanson, 1962). By having birds in captivity and removing predation pressure and restrictions on foraging, it is possible to establish how inherent these moult rhythms and associated behaviours are and to investigate their possible causes. Thus, we tested the following hypotheses: (1) Captive barnacle geese will lose mass during

the wing moult, despite having unrestricted access to food and no predation pressure (2) Resting metabolic rate (measured as the rate of oxygen consumption during rest) will increase during the flightless period of the moult as a consequence of the cost of regrowing flight feathers (3) Captive barnacle geese will increase time dedicated to resting and maintenance behaviour during moult, while decreasing time dedicated to foraging and locomotion (4) Heavier birds will lose proportionally more mass during moult, to minimise the duration of the flightless period, through the allocation of fat stores to feather regrowth.

Materials and Methods

Birds

A captive population of 14 barnacle geese obtained as 3-week old goslings was maintained under natural light in a large outdoor aviary at the University of Birmingham. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK) which has held a self-sustaining captive population of this species since 1982. While the origin of this population is unknown, the geese have therefore not migrated for at least 25 years. 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 food and water were available **ad libitum.**

Weighing

Throughout a 2 year period (2004, 2005), the geese were weighed at one or two week intervals to the nearest 5 g. Birds were hooded to reduce stress and placed in a darkened plastic box for weighing. Handling was kept to a minimum.

Moult Score

The stage of wing moult was determined during the weighing sessions, using a 6-point classification moult score system developed for use with waterfowl and alcids (e.g. Geldenhuys, 1982; Ankney, 1984; Bridge, 2004) (0) pre-wing moult (1) primaries and secondaries remain, new primary pin visible (2) primaries missing and most secondaries remain (3) all primaries and secondaries missing (4) new primaries emerged just beyond primary coverts (5) primaries visible well beyond primary coverts and secondaries visible beyond secondary coverts (6) post wing-moult.

Time-activity budgets

The activity budgets of the captive Barnacle Geese were recorded during 2005 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 hrs GMT), afternoon (12:00-1700 hrs GMT) and evening (17:00-22:00 hrs 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 (i.e. no torrential rain when the geese would

shelter under vegetation). An individual goose was selected and watched for a total period of 5 min, with activities being 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.

Seventeen separate behaviours were recorded during the study and pooled into six general categories (Austin, 1987; Adams *et al.*, 2000): foraging (feeding and pausing), resting (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).

Resting rate of oxygen consumption measurements

Resting rate of oxygen consumption ($\dot{V}_{O_2}$) was measured overnight in darkness between the hours of 23:00 and 07:00, and during the day in the light between the hours of 11:00 and 14:00. Birds were placed inside a Perspex box (74 cm high x 58 cm long x 47 cm wide) and $\dot{V}_{O_2}$ measured using open circuit respirometry (Withers, 2001). Air temperature within the chamber was 19-21 ^{o}C, which is within the thermoneutral zone for barnacle geese (Calder and King, 1974). Food (not water) was withheld from the birds for 8 h before they were placed in the respirometer box. Resting rate was calculated from the lowest value when averaged over 5 min periods

(Withers, 2001). Thus, those data collected during the night are equivalent to basal rates of oxygen consumption, or basal metabolic rate – BMR (McNab, 1997; Frappell and Butler, 2004). Day resting rate of oxygen consumption was recorded to ascertain if it would follow the same pattern throughout the year as that of night resting. The data were collected during the second year of the study (2005), in February, May, July, August, September and November, and the same six birds were sampled in each session.

Respirometry equipment

Two respirometry systems were used simultaneously to record resting $\dot{V}_{O_2}$, in order to minimise the duration of the sampling sessions. Information regarding the set-up and equipment for system 1 are described elsewhere in detail (Green *et al.*, 2001, Chapter VI) and for system 2 by Wilson *et al.* (2006). The extent to which each respirometry system leaked was determined by pumping oxygen-free nitrogen gas (BOC gas, UK) into the chamber at a known rate (Fedak *et al.*, 1981). The calculated values of gas exchange were adjusted to compensate for the loss of chamber gas.

The rate of oxygen consumption was calculated using the equations of Depocas and Hart (1957), as modified by Withers (1977) and Koteja (1996). As carbon dioxide was not measured in system 1, for these experiments equation 3a (Withers, 1977) was used to calculate $\dot{V}_{O_2}$ (Ward *et al.*, 2002), where the respiratory

exchange ratio (RER) was assumed to be the mean value measured in the birds in system 2. This procedure would introduce an error of less than 1% into the calculated $\dot{V}_{O_2}$ (Koteja, 1996), given the measured variation in RER. Equation 3b from Withers (1977) was used for system 2. Data from both systems were pooled.

Statistical analysis

Repeated measures ANOVA were performed to compare the mean values for each weighing session, and on measurements of resting $\dot{V}_{O_2}$. Post-hoc Bonferonni corrected paired t-tests were used to compare resting $\dot{V}_{O_2}$ between sampling sessions ($P < 0.003$). ANCOVA was used to compare rate of mass change during the wing moult period between the 2 years, and to investigate the relationship between body mass and $\dot{V}_{O_2}$.

Linear regression was used to examine the influence of body condition on rate of body mass change during the moult. For this, mass was adjusted for structural size (size-adjusted body mass = body mass (g)/tarsus length (cm), Van de Wetering and Cooke, 2000).

Percentage data for each behavioural category from each sampling session was arcsin transformed and a 2-way ANOVA (time of day and month) with post-hoc Tukey HSD tests ($P < 0.05$) was performed to determine whether there were differences in the mean proportion of time dedicated to each category of behaviour between the 3 different times of year, and between morning, afternoon and evening observations.

All tests were considered significant at $P < 0.05$. Values given are means ± SEM.

Results

Annual cycles in body mass and moult

Mean body mass of the 14 captive barnacle geese (Fig. II.1) changed significantly throughout the annual cycle for both years of the study ($P < 0.0001$). As they had reached sexual maturity, the birds were significantly heavier in year 2 than year 1 ($P < 0.0001$) (Owen and Ogilvie, 1979).

Peaks in body mass were observed in January 2005 (2143 ± 89 g), and early July (1951 ± 62 g, 2034 ± 75 g for 2004 and 2005 respectively) followed by a significant drop in body mass of approximately 430 g (25%) during the moult periods in both years ($P < 0.0001$). In 2005 the first flight feathers were dropped during the last week of June, and by July 14th, all bar-one of the birds was classified as moult stage 3 (all primaries and secondaries missing) or higher. The flightless period was estimated to be 38 days. The lowest body mass was recorded towards the end of the wing moult (moult score 5), in mid-August (1528 ± 41 g, 1596 ± 53 g for 2004 and 2005 respectively). There was no significant difference between the 2 years in mean body mass at this time (paired *t*-test, $P = 0.09$), although body mass prior to moult was significantly different between the 2 years (paired *t*-test, $P < 0.001$). Rate of mass change during the wing moult was not significantly different between the 2 years (ANCOVA, $P = 0.850$).

Following the completion of moult (moult score 6), mass increased significantly ($P < 0.001$) over the following 7-8 weeks before reaching a plateau in mid-October. Body mass remained stable throughout the winter months until an increase in December and January, when the highest mass of year 2 was recorded. From the middle of January, mass declined to reach a level similar to that in the autumn (1820 ± 12 g and 1850 ± 32 g respectively for 2004 and 2005), from which it began to increase in May/June.

There was a significant relationship between decrease in body mass during moult and size-adjusted initial body mass (Fig. II.2a). After adjusting for body size, heavier birds lost body mass at a proportionately greater rate. There was also a significant relationship between % time spent resting during wing moult, and body mass lost during moult, calculated as a % of the initial pre-moult body mass (Fig. II.2b). Moreover, a significant relationship was found between % time spent resting during moult, and size-adjusted initial body mass (Fig. II.2c).

Resting rate of oxygen consumption

There was significant variation throughout the year of both day and night resting $\dot{V}_{O_2}$ (Fig. II.3, $P < 0.001$ and $P = 0.004$, respectively). Maximum night and day resting $\dot{V}_{O_2}$ were observed during the moult in a period of mass change, and averaged 27.2 ± 1.4 ml min^{-1} and 32.7 ± 2.0 ml min^{-1} respectively. The minimum nighttime value (14.91 ± 1.2 ml min^{-1}) was recorded in November, a period of stability when body mass did not change significantly. Minimum daytime value was observed in May (22.5. ± 3.0 ml min^{-1}). Oxygen uptake measurements taken during moulting periods

(July – August) were significantly higher than those measured during the pre and post moult periods. Mass specific night-time $\dot{V}_{O_2}$ followed a similar pattern, with values during the moult period of 16.2 ± 1.2 ml min^{-1} kg^{-1}, compared to, for example, 8.9 ± 0.3 ml min^{-1} kg^{-1} for May. There was no significant relationship between body mass and $\dot{V}_{O_2}$.

Behaviour

Two-way ANOVA revealed no significant difference in time budgeting between morning, afternoon and evening observations within each sampling period. During August (i.e. wing moult), the geese spent 64 ± 1.9 % of their time resting, which was significantly greater than the 45 ± 2.4 % and 43 ± 1.6 % of their time spent resting in June and November respectively (Fig. II.4). Foraging dropped during wing moult, from 23 ± 2.0 % in June and 24 ± 1.7 % in November to 7 ± 0.6 % in August ($P < 0.001$). Maintenance behaviour increased significantly during wing moult ($P < 0.001$) from 12 ± 1.4 % and 9 ± 1.1% in June and November respectively, to 20 ± 1.6 % when the birds were flightless.

Discussion

Body mass and behavioural changes during wing moult

Despite having constant access to food, the captive barnacle geese lost approximately 25% of their body mass during the wing moult in both years of the study. A similar degree of mass loss was recorded in wild non-breeding waterfowl – such as male greylag geese moulting on Saltholm, Denmark (Fox *et al*., 1998). Sjöberg (1986) found that teals lost between 10-20% of their body mass during the flightless period, a similar figure to that noted in red-billed teal and South-African shelduck (Douthwaite, 1976; Geldenhuys, 1983). Sjöberg (1986) noted that many species of wild waterfowl moult in extremely favourable habitats and can therefore compensate for the energetically demanding wing moult period by increasing their daily food intake. As such, he concluded that any observed mass loss must be an adaptation for decreasing the flightless period by enabling the bird to fly on partially regrown flight feathers. However, atrophy of flight muscles in moulting waterfowl (e.g. Ankney, 1979; Marden, 1987), and a lack of consistency in mass loss during wing moult between populations of the same species (Fox and Kahlert, 2005), suggests this is unlikely to be broadly applicable.

Panek and Majewski (1990) proposed that the cause of mass loss during wing moult in wild waterfowl was the change in behaviour and loss of foraging opportunities, brought about by an increased risk of predation. Therefore birds were not able to compensate for the increased energetic demands during moult by increasing their food intake. They noted that mallards with only 1-3 primary flight remiges missing still continued to exhibit what they described as ‘secretive behaviour’, and still continued to lose body mass, indicating that the birds were aware of their vulnerability to predation whilst flightless (Pehrsson, 1987; Adams *et* al., 2000). In this study, the

captive barnacle geese showed a marked difference in behaviour during the flightless period of the wing moult, increasing the time they spent in maintenance and resting and decreasing the time spent foraging (Fig. II.4).

Kahlert *et al*. (1996) found that only flightless wild greylag geese would respond to grey herons *Ardea cineria* and helicopters overhead by returning to water for safety, whereas fully flighted birds would not respond. In addition these moulting geese spent on average 19 minutes resting on the water after a 'spook' event, potentially losing valuable feeding time (Kahlert, 2006). During wing moult, the greylag geese spent just 8.9 hours foraging in comparison to 16.2 hours when fully flighted (Fox and Kahlert, 1999). Furthermore, these birds did not compensate for this reduction in foraging time by increasing peck rate (Fox and Kahlert, 1999).

Studies conducted on other wild moulting waterfowl have shown varying changes in resting levels and feeding effort during the flightless period. Austin (1987) reported a 10% increase in time spent resting during the flightless stage in lesser scaup *Aythya affinis*, while mottled ducks *Anas fulvigula* spent only 9% of their time feeding during wing moult in comparison to 65% of their time before and after, a trait also observed in harlequin ducks *Histrionicus histrionicus*, black ducks *Anas rubripes* and canvasbacks *Aythya valisineria* (Paulus, 1984; Bowman, 1987; Thompson, 1992). Other species exhibited different compensatory behaviours. For example redheads *Aythya Americana*, red-crested pochards *Netta rufina* and greylag geese fed primarily at night during wing moult (Bailey, 1981; van Impe, 1985 Kahlert *et al*., 1996).

Despite having constant access to food and no obvious predators, the captive barnacle geese in the present study still changed their behaviour during the flightless period of wing moult and did not increase their food intake rate. These findings suggest that increased resting during a potentially vulnerable stage of the bird's annual cycle is an innate behaviour.

Rate of mass loss and body size

In the present study heavier geese lost mass faster than lighter geese; a similar observation was noted in the Russian breeding population of barnacle geese and Barrow's Goldeneye (Van der Jeugd *et al*., 2003; Van de Wetering and Cooke, 2000). This may explain the peak in body mass prior to wing moult. If, as hypothesised by Owen and Ogilvie (1979), Sjöberg (1986) and Douthwaite (1976), mass loss during the flightless period is an adaptation to regain flight sooner, gaining mass prior to this period would appear to be a waste of energy. If, however, being in good condition before the onset of wing moult enables a bird to achieve higher remigial growth rates and thus shorten the flightless period compared to birds in poorer condition, there is an obvious advantage to gaining mass before wing moult. Using endogenous reserves (mainly fat) during wing moult can be a strategy that allows birds to spend less time feeding and to occupy safer habitats that reduce the risk of predation (Thompson, 1992; Van de Wetering and Cooke, 2000). This will enable birds in better condition to reduce activity more so than those in poorer condition and possibly reduce the chance of being predated upon. Comparing two Russian breeding populations of barnacle geese, Van der Jeugd *et al.* (2003) found birds in one population to be 200 g heavier at

the onset of wing moult. During the flightless period, however, body mass in these heavier birds declined three times more rapidly in comparison to that of the other population, and the flightless period was shorter.

Rate of oxygen consumption measurements

There was a distinct annual cycle in resting $\dot{V}_{O_2}$ in the captive geese used in the present study (Fig. II.3). The night resting $\dot{V}_{O_2}$, which is equivalent to basal $\dot{V}_{O_2}$ (a proxy for basal metabolic rate – BMR) was approximately 80% higher during the wing moult when compared to the post moult period in November. Ward *et al.* (2002) sampled night resting rates of oxygen consumption in early September, and recorded a mean night resting $\dot{V}_{O_2}$ of 25.3 ml min^{-1}, compared to values from the present study of 25.3 ml min^{-1} for late August (post moult), and 22.0 ml min^{-1} for mid-September. Nolet *et al.* (1992) took measurements of rate of oxygen consumption from captive barnacle geese during the late winter, and reported a mean night resting $\dot{V}_{O_2}$ of 16.9 ml min^{-1}, compared to values from the present study of 19.8 ml min^{-1} for early February and 14.9 ml min^{-1} for late November (17.3 ml min^{-1} winter mean). These comparisons suggest that there is an annual cycle in BMR in captive (and potentially wild) barnacle geese. This conclusion is supported by seasonal variations in night resting heart rate in macaroni penguins, *Eudyptes chrysolophus*, (Green *et al*., 2005), wild great cormorants, *Phalacrocorax carbo*, (White *et al*., unpub. data), and eider ducks, *Somateria mollissima* (Guillemette *et al*., 2007).

The observed increase in BMR during wing moult is greater than that reported for most species (Payne, 1972). For example Lindström *et al.* (1993) reported a 35 % increase in basal metabolic rate in captive moulting redpolls *Carduelis f. flammea*, when compared to pre-moult and post-moult values. However, the majority of studies have been on passerines that have a sequential moult, often lasting a number of months as opposed to waterfowl with their simultaneous wing moult, and this may explain the higher values recorded in the captive barnacle geese. In contrast Guozhen and Hongfa (1986) reported increases in $\dot{V}_{O_2}$ of only 25% and 35% for Eurasian teal and European shoveler, *Anas clypeata*, respectively. This discrepancy may be due in part to the timing of metabolic measurements with respect to the duration of the moult. Penguins also have a rapid moult during which they are unable to forage and rely on endogenous reserves. Green *et al*. (2004) found that the metabolic rate of macaroni penguins increases, then decreases to non-moulting levels during this moult fast, with a peak approximately 40% above non-moulting levels. In the present study rate of oxygen consumption measurements were taken in the middle of the wing-moult period (Fig. II.3), but this may not have been the case in other studies.

Are data from the present study applicable to wild geese?

No published study has previously recorded the year round mass of barnacle geese, wild or captive, although other studies (Owen and Ogilvie, 1979; Tombre *et al.*, 1996; Phillips *et al.*, 2003) have weighed wild barnacle geese at various times of the year.

Tombre *et al.* (1996) captured female geese in Ny-Ålesund, Svalbard, within three days of their arrival from their spring staging posts in Norway. Masses recorded were 2099 ± 55 g and 2219 ± 44 g for 1993 and 1994 respectively (n = 13 and 11 respectively) and these are heavier than masses taken from the captive geese in the present study around the same time (e.g. 1827 ± 37 g 5th May 2005, Fig. II.1). Phillips *et al.* (2003) caught birds at their wintering grounds on the Solway Firth between December 1999 and January 2004 and recorded a mean body mass of 2000 g (n = 20, no SEM provided), which is similar to the winter masses in the captive geese during the second year of the present study (e.g. 2105 ± 53 g, 12th December 2005). Mean body mass of moulting adult birds' in Svalbard was 1788 ± 8 g and 1586 ± 7.8 g for non-breeding males and females respectively (Owen and Ogilvie, 1979), compared to a combined mean of 1596 ± 53 g for the captive geese during moult. Van der Jeugd *et al.* (2003) recorded body masses of barnacle geese during wing moult of 2002 ± 169 g and 1769 ± 177 g for males and females respectively, although they noted that approximately 20% of the birds caught were non-breeders and had completed wing moult.

Thus, despite their not migrating or breeding, and having constant access to food, captive barnacle geese show a distinct annual cycle in body mass that is generally similar to measurements recorded at various points in the year from wild populations of barnacle geese.

Summary

Data presented here provide evidence that captive barnacle geese follow a distinct annual cycle in BMR and body mass. During wing moult, the geese show a significant drop in body mass, as a result of reducing time spent foraging and the observed increase in BMR associated with the costs of moulting. Despite having constant access to food, the captive birds did not increase time dedicated to foraging to compensate for the increase in metabolism. Although food was available, the captive birds chose not to forage and instead increased the proportion of time spent resting. It is likely that this behaviour in response to wing moult is a strategy to avoid predation in the wild. As the captive barnacle geese exhibit such behaviour, it suggests this behavioural response to being flightless in this species at least is innate.

Acknowledgements

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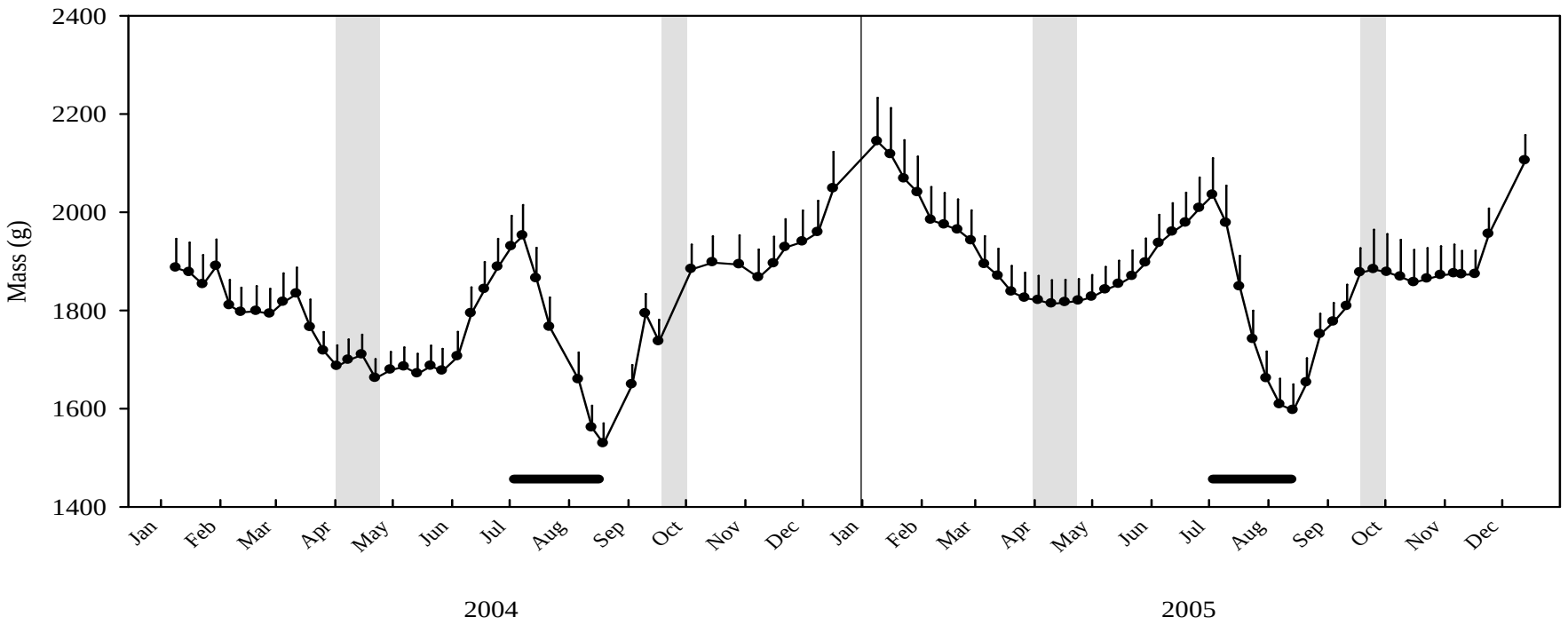
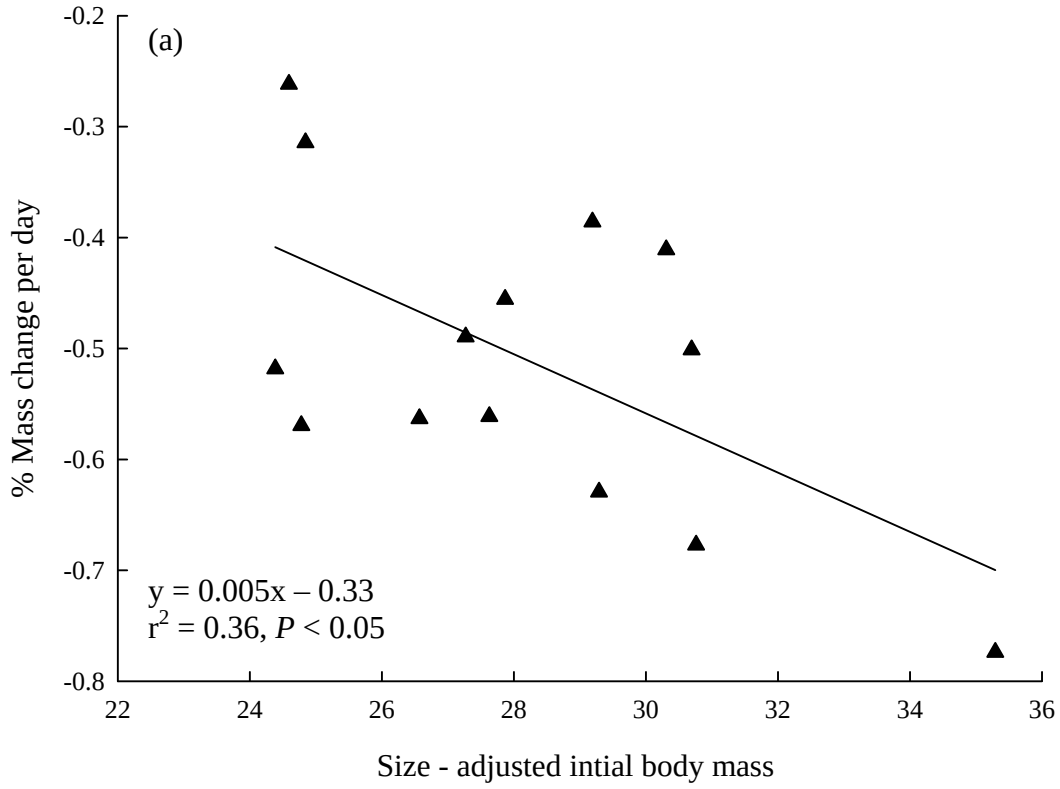
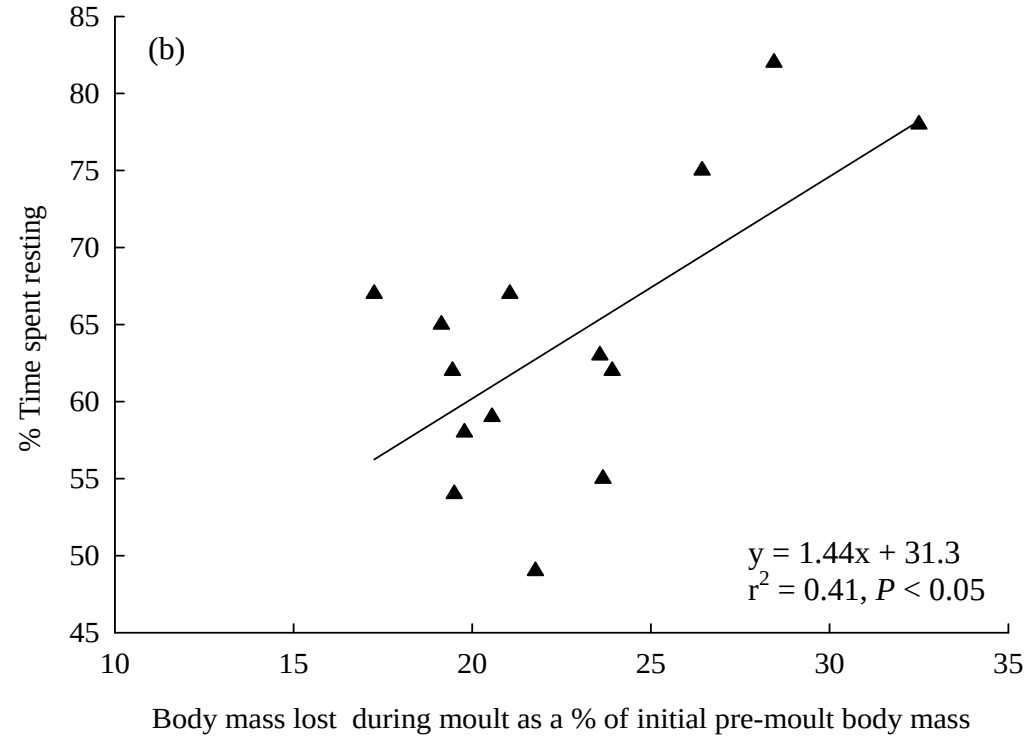


Figure II.1: Year round mean (± SEM) weekly body mass for 14 captive barnacle geese for 2004 and 2005. Horizontal black bars indicate approximate period of wing moult. Shaded areas relate to migratory periods in wild barnacle geese. Mass changed significantly throughout the year for both years sampled (repeated measures ANOVA $P < 0.0001$ for years 1 and 2 respectively).


(a)
% Mass change per day
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
22
24
26
28
30
32
34
36
y = 0.005x – 0.33
r2 = 0.36, P < 0.05
Size - adjusted intial body mass


(b)
% Time spent resting
85
80
75
70
65
60
55
50
45
10
15
20
25
30
35
y = 1.44x + 31.3
r2 = 0.41, P < 0.05
Body mass lost during moult as a % of initial pre-moult body mass

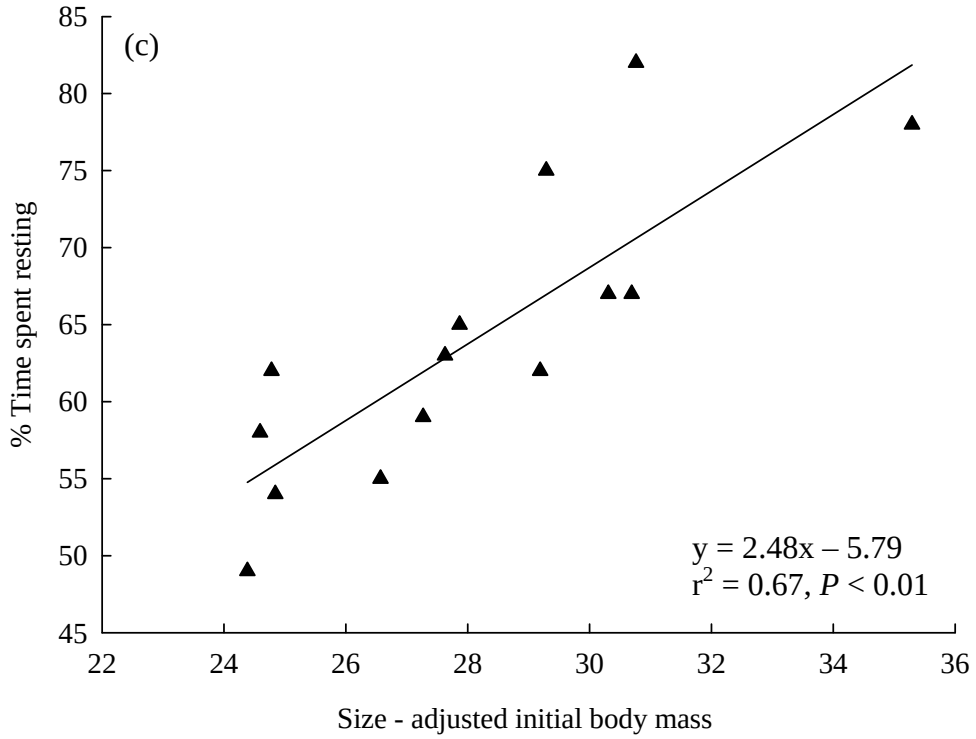


Figure II.2: For 14 captive barnacle geese, the relationship between (a) mass change and size-adjusted initial body mass during wing moult, (b) % time spent resting during wing moult and body mass lost as a % of initial pre-moult body mass, and (c), % time spent resting and size-adjusted initial body mass. All three regressions were significant.

Size-adjusted body mass is calculated as body mass (g) divided by length of the tarsus (cm).

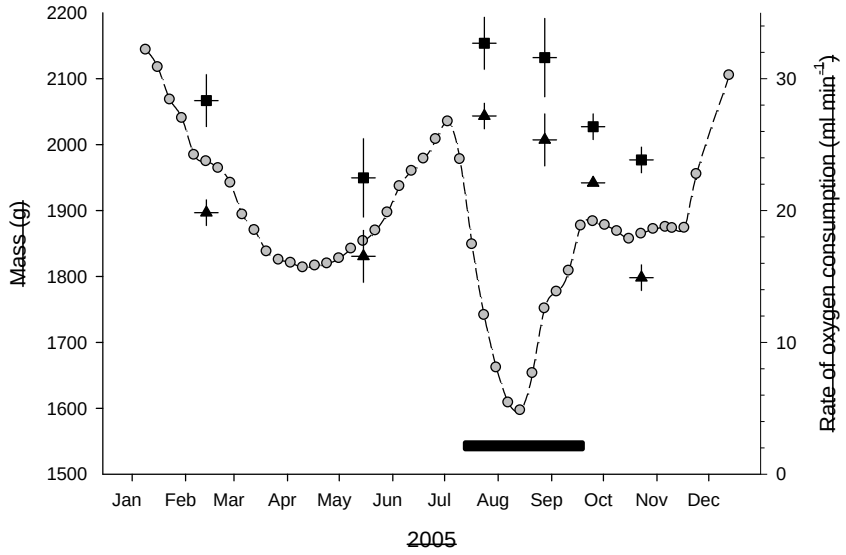
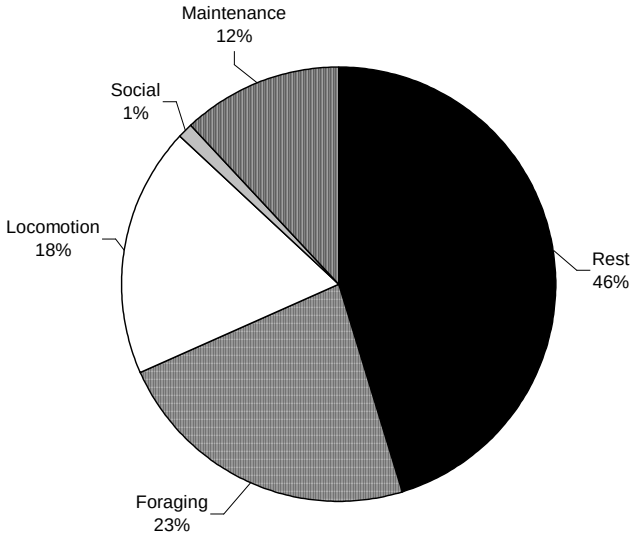


Figure II.3: Year round average weekly masses for 16 barnacle geese (grey circles) with rates of oxygen uptake measurements (n = 6 ± SEM) at six points of the year. Triangles represent night resting values and squares day resting values. X error bars represent duration of sampling period. Both day and night resting rates varied significantly throughout the year (repeated measures ANOVA, $P < 0.001$ and $P < 0.004$ respectively) and followed a similar pattern. Black bar indicates the wing moult period.

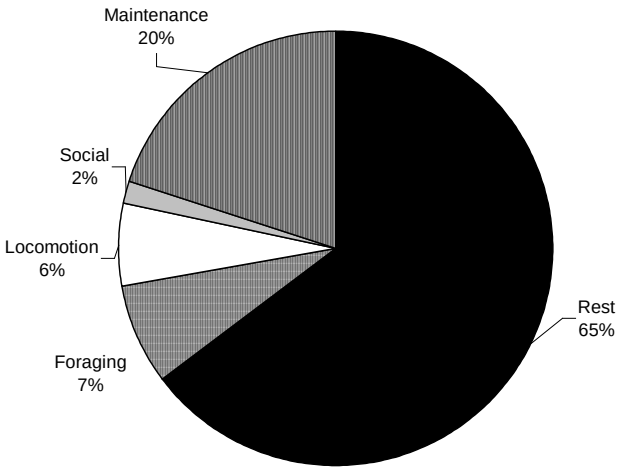
Figure II.4: Time activity budgets (as %) for 16 captive Barnacle Geese, for pre - (a, June), during (b, August) and post - (c, November) wing moult. The time dedicated to alert behaviour was less than 1% and is not shown here.

(a)



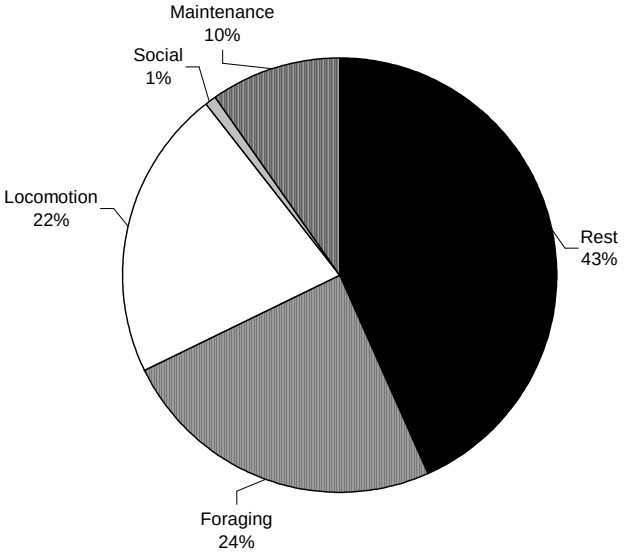
Pre-wing moult

(b)



During wing moult

(c)



Post-wing moult

III. TESTING THE USE\DISUSE HYPOTHESIS: PECTORAL AND LEG MUSCLE CHANGES IN CAPTIVE BARNACLE GEESE *BRANTA LEUCOPSIS* DURING WING MOULT

Steven J. Portugal, Susannah K. S. Thorpe, Julia P. Myatt and Patrick J. Butler

Most species of waterfowl undergo an annual simultaneous wing feather moult, during which they are flightless for a period of approximately 20-30 days. Previous studies on wild moulting waterfowl have demonstrated that flight and leg muscles go through periods of atrophy and hypertrophy, thought to be in response to the change in usage of the locomotor muscles. To investigate the innate nature of these muscular changes, 40 captive barnacle geese were culled at set points before, during and after wing moult, to investigate, primarily, the changes in mass of the flight and leg muscles. Muscles were dissected and grouped together based on function, and physiological cross sectional area and fascicle length calculated to ascertain which muscle groups were responsible for the major force production in the legs. At the onset of moult, flight muscles atrophied and leg muscles hypertrophied by 30% and 13% respectively. At peak wing moult, flight muscles hypertrophied significantly by 25%, without any increase in locomotor activity. At this stage, leg muscles hypertrophied significantly by 28%. Overall however, there was no significant change in the total mass of the locomotor muscles during the study period. Physiological cross sectional area of the major force producing muscles of the leg increased

significantly during early moult when the leg muscles hypertrophied, and then decreased as pectoral muscle hypertrophied at the time when flight would have been resumed in wild birds. By removing the natural movements and activity of wild animals, it has been possible to conclude that the major changes in leg and breast muscle in moulting captive geese cannot be explained through use/disuse. Exercise patterns and food availability may affect the magnitude of these changes, but in the absence of any photoperiod cues, these muscular changes must be controlled through an endogenous process.

SJP developed the methodology and planning, conducted the data collection, preparation, analysed the data and wrote the manuscript. SKST developed the methodology, dissection protocols and muscle identification and aided in the writing of the manuscript. JPM assisted with all dissections. PJB discussed data analysis and conclusions, and aided in the writing of the manuscript.

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Introduction

The use/disuse hypothesis contends that high-resistance exercise will cause hypertrophy of skeletal muscle, while failure to exercise a muscle group for prolonged periods will lead to atrophy (Alexander and Goldspink, 1977). Atrophy of muscle involves a decrease in protein synthesis resulting in a loss of muscle mass and a loss of muscle strength, with even short periods without weight bearing having significant effect on the muscle (Boonyarom and Inui, 2006). While little work has been carried out on hypertrophy/atrophy in birds, studies on healthy human subjects show that atrophy of the leg begins shortly after being bedridden, and Kawashima *et al*. (2004) demonstrated that within five days of bed rest, the adductor, knee extensor and knee flexor leg muscle groups had undergone a 5% reduction in function. Conversely, during hypertrophy, the largest increase in muscle size and strength occur in the first 5-7 days of increased muscle use (Narici *et al*., 1989).

Most species of waterfowl undergo a simultaneous wing feather moult, typically after breeding, which renders them flightless for a period of approximately 28 days (Hohman *et al*., 1992). Studies on wild waterfowl have shown that during this wing moult period, 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 rate of metabolism (e.g. Guozhen and Hongfa, 1986).

Another 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 (Ankney, 1979; Ankney, 1984; Gaunt *et al.*, 1990; Fox and Kahlert, 2005). This has been interpreted as a direct response to a change in use and activity of the muscles, i.e., use or disuse (e.g. Fox and Kahlert, 2005). In the case of moulting waterfowl, it therefore follows that flight muscles atrophy when the birds become flightless and cease all flight activity. This leaves the birds reliant on running, walking or swimming to forage and escape predation, and the leg muscles consequently hypertrophy in response to the increased use (Hohman *et al*., 1992). Towards the end of wing moult wing muscles again hypertrophy (Ankney, 1979). In some birds such as grebes, this hypertrophy coincides with increased wing flapping behaviour which may serve to build up their flight muscles in preparation for the resumption of flight (Piersma, 1988; Jehl, 1997). In waterfowl, however, hypertrophy in the flight muscles towards the end of wing moult (e.g. Ankney, 1979) is not normally accompanied by an increase in exercise or activity (Ankney, 1983), suggesting that alternative mechanisms may be involved. This is supported by a study

of captive (but of wild origin) red knots, *Calidris canutus* (Dietz *et al*., 1999). Despite not being capable of forward flapping flight, the variation in breast muscle mass throughout the year resembled the pattern found in the wild birds, whereas variation in stomach mass differed significantly from that found in the wild free-living red knots. This suggests that changes in muscle and stomach masses may occur by different mechanisms within an animal (Dietz *et al*., 1999).

Previous studies on atrophy and hypertrophy during wing moult have focused on changes in absolute mass of flight muscles and total leg musculature. However, atrophy and hypertrophy occur to differing degrees, and at different speeds according to the function and fibre type profile of each muscle (Alexander and Goldspink, 1977). Furthermore, it is muscle physiological cross-sectional area (PCSA) and muscle fascicle length, rather than muscle mass, which are the best predictors of function (Wickiewicz *et al*., 1983). An increased PCSA reflects an increase in force generating potential, while an increase in fascicle length allows the generation of force over a wide range of motion and increases the velocity of shortening of the muscle (Thorpe *et al*., 1999). An increase in the PCSA and fascicle length of leg muscles could enhance the ability of flightless moulting waterfowl to employ rapid running or swimming to escape from potential predators. A more detailed analysis of muscle dimensions 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,* has shown that, despite having constant access to food and protection from predators, they respond in a similar way both physiologically and behaviourally to wing moult as their wild conspecifics (Portugal *et al*., 2007). In that study, the geese lost approximately 25% of their body mass during the flightless phase and their resting metabolic rate increased by 80%. Behaviourally the birds responded to wing moult by significantly increasing the time dedicated to resting and decreasing the time spent involved in locomotion and foraging, when compared to pre and post moult values (Portugal *et al*., 2007).

The aim of the present study was to see if, despite being bred and raised in captivity and unable to fly (since flight feathers are clipped), captive barnacle geese show cycles of hypertrophy and atrophy consistent with the use/disuse hypothesis. We tested the following hypotheses (i) flight muscle would atrophy at the start of wing moult (ii) breast muscle would hypertrophy towards the end of wing moult in preparation for the resumption of flight, without changes in activity or exercise, (iii) total leg muscle mass would increase mid-moult, despite a decrease in activity (iv) PCSA of the major force producing leg muscles (ankle plantarflexors, knee extensors, hamstrings and adductors) will increase mid-moult allowing the birds to exert the greater forces necessary to power fast running and/or swimming, to escape predators while flightless (v) fibre length in the hamstrings and adductors will increase mid-moult to allow force to be produced over a greater range of motion, with quicker velocity of shortening in these muscle groups, resulting in an increased manoeuvrability of the leg.

Materials and Methods

Birds

A captive population of 40 barnacle geese obtained as 3-week old goslings was maintained under natural light in large outdoor aviaries at the University of Birmingham. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK) which has held a self-sustaining captive population of this species since 1982. 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.

Different groups of four birds were sampled every fortnight from the 10th July (one week prior to the commencement of wing moult) to the 3rd November (when wing moult had been fully completed for two months) 2006, with 2 extra sampling periods at the end of July and beginning of August during peak wing. Each bird was anesthetized 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) into the intertarsal vein, to avoid damaging the muscles required for analysis. Final body mass was measured (± 5 g) before the carcasses were double-bagged and frozen at -20^{o}C until the dissections were performed.

Moult Score

A moult score was used to determine stage of moult 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 and (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 2 major flight muscles and 29 major leg muscles (see table III.1) were removed from the birds, muscles were identified based on Howell (1938) and 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 and taken out individually and weighed on electronic scales to the nearest 0.01 g.

Muscle belly length (e.g. Thorpe *et al.*, 1999) was obtained by measuring the distance from the most proximal fibres to the most distal fibres. External tendons were removed. In order to reveal the muscle fascicles (fascicle being a bundle of fibres visible to the naked eye, reflecting the number of sarcomeres in series, Thorpe, 1997), a cut was made along the length of the muscle belly, at 90^{o} to the internal tendon (e.g. Thorpe *et al*,, 1999 Payne *et al*., 2005). 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 fibres lay in the plane of the cut and muscle fascicle lengths were measured (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. Where fascicle length was not uniform, a number of measurements were taken and a mean value used. Measurements of fascicle length were taken from randomly distributed areas and depths within the muscle belly.

Muscle analysis

As shown in Portugal *et al.* (2007), body mass of captive barnacle geese changes substantially throughout the annual cycle, and there are potential difficulties with comparing muscle dimensions between geese of varying mass and within each goose as body mass changes throughout the moult phase. Therefore the data require normalization, and the geometric scaling approach was used to achieve this. In geometrically similar animals, mass should scale directly to body mass, lengths to $(\text{body mass})^{1/3}$ and areas to $(\text{body mass})^{2/3}$ (Alexander *et al*., 1981). Muscle mass, PCSA and muscle fascicle length of the pectoralis were plotted against body mass, and power trend lines were fitted to the data to determine whether the above geometric relationships held true for the geese (see Fig. III.1a, III.1b and III.1c for details).

To allow for intra specific comparisons to be made, major leg and flight muscles were grouped into functional categories (Alexander *et al*., 1981; Vanden-Berge and Zweers, 1993), the composition of which is detailed in Table III.1. Raw data was normalised by scaling each muscle mass to a constant body mass of 2 kg. This figure (2 kg) was used as it was the mean annual body mass for 40 captive barnacle geese in Portugal *et al.* (2007), and is the average body mass for an adult barnacle goose, sexes combined, quoted in Cramp and Simmons (1977). Subsequently, as geometric relationships held true (Fig. 1.1), fascicle length was scaled by (body mass 2 kg)$^{1/3}$ and PCSA by (body mass 2 kg)$^{2/3}$. Muscle mass and PCSA of the flight and leg muscle groups were doubled (e.g. Jehl, 1997) to account for the side that was not dissected (assuming the muscles are symmetrical).

The physiological cross sectional area (reflecting the number of sarcomeres in parallel, Thorpe, 1997) of the muscle can be obtained from

$$A = m/(p.l) \qquad (1)$$

where m = muscle mass, p = density [1.06 kg m^{-3} (Mėndez and Keys, 1960)] and l is fascicle length. The proportion of muscle force transmitted can depend on the angle of pennation of the fibres (such that PCSA = $mlpl \times \cos\theta$, where θ is the angle of pennation of the fibres with respect to the line of pull of the muscle). Pennation angle was not included in our estimates of PCSA. Smith *et al*. (2006) measured pennation angles in the hindlimb muscles of the ostrich, *Struthio camelus,* and found all angles

to be close to 30^0. The cosine of 30 is close to one and would thus have little effect on the estimations of PCSA.

Masses and PCSAs were calculated as group totals, which are the sum of constituent muscles in each functional group (Thorpe *et al*., 1999). As some muscles form a greater percentage of the total mass of a group than others, muscle group fascicle length 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:

$$L = \sum m_j / \sum (m_j / l_j^{-1}) \qquad (2)$$

where L is the fascicle length for a group of muscles of which the jth member has a mass m_j and fascicles of length l_j (Alexander *et al*., 1981). Force (N) was estimated by multiplying PCSA by the maximum isometric stress of vertebrate skeletal muscle (0.3 MPa, see Medler, 2002).

Behaviour

Behavioural data were taken from Portugal *et al*. (2007), which also details the methodology for the behavioural observations. 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 to the changes in muscle mass. However, it was possible 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. Data included under the 'locomotor' category in Portugal *et al*. (2007) are presented in greater detail here.

Significant differences in muscle mass, PCSA and fascicle length were assessed using ANOVA with post-hoc Fisher's LSD tests applied to distinguish which sampling periods were significantly different. Repeated measures ANOVA was used to test for significance differences in time spent per day wing flapping, before, during and after moult. All tests are considered significant at $P < 0.05$. Values given are means ± SEM.

Results

Body mass changed significantly over the duration of wing moult (Fig. III.2a, ANOVA, $P < 0.01$), with body mass at moult score 3 being significantly lower than all other moult stages. Between moult scores 1-3, the geese lost, on average, 600 g. However, the net mass of the total locomotor muscles (flight and leg groups combined) did not change significantly over the study period (Fig. III.2b, ANOVA, $P = 0.6$). Total leg muscle mass though (Fig. III.2c) did increase significantly from 159 g to 183 g between moult score 2 and 3 (equivalent to 13 %), before decreasing significantly to 130 g at moult score 4. Over the same period, total flight muscle mass (Fig. III.2c) decreased significantly between moult score 1 and 2, losing 85 g (33 %), before increasing significantly again at moult score 3 (see next section).

Flight muscles

As stated above, the mass of the flight group changed significantly throughout the period of study (Fig. III.3a, ANOVA, $P < 0.01$). Fisher's LSD tests showed flight muscle mass at moult score 2 to be significantly lower than those at all other moult stages (1, 3-5). The increase in flight muscle mass from moult stage 2 onwards was not accompanied by an increase in wing-flapping behaviour (Fig. III.4, repeated measures ANOVA, $P < 0.91$) in the captive geese the previous year (Portugal *et al.*, 2007). There was no significant change in the physiological cross sectional area or fascicle length of the flight muscle group across the 5 moult scores (Fig. III.3b, c).

Behavioural data from the same flock of geese recorded the year prior to the present study (taken from Portugal *et al*., 2007), suggests that the observed significant changes in muscle mass were not a direct result of changes in muscle usage, as muscular changes, particularly increase in flight muscle mass from moult score 3 onwards, were not accompanied by an increase in locomotor behaviour (Fig. 5) .The daily time energy budgets recorded during moult did not change significantly throughout that period (Portugal *et al*., 2007).

Leg muscles

Throughout the period of the study, the mass of all leg muscle groups changed significantly (ANOVA, $P < 0.01$), except for the ankle dorsiflexors. Generally, all the leg muscle group masses followed a similar pattern of muscle mass loss and gain (Fig. III.6a); group muscle mass increased between moult scores 1-3, and then decreased

significantly between moult scores 3 and 4. Fisher's LSD tests showed muscle group masses at moult score 4 to be significantly lower than those masses at moult score 3 in all but one (ankle dorsiflexors) of the muscle groups. Leg muscle groups atrophied by 2% (ankle dorsiflexors), 30% (ankle plantarflexors), 40% (deep extensors and flexors), 38% (gluteal group,) 48% (knee extensors) and 35 % (hamstrings), respectively, between moult scores 3 and 4.

The PCSA of the ankle plantarflexors, knee extensors, hamstrings, flexors and extensor groups all changed significantly throughout the study period (i.e. before, during and after moult). Like muscle mass, those muscle groups where PCSA changed significantly followed a similar pattern (Fig. III.6b), with little or no change in PCSA between moult scores 1 and 3, but a significant decrease between moult scores 3 and 4. Fisher's LSD tests also showed that PCSA's at moult score 4 were significantly lower than pre-moult values (i.e. moult score 1) in all leg muscle groups except for the gluteals where little change was observed. Between moult score 3 and 4, each muscle group underwent a significant reduction in PCSA of 49% (ankle dorsiflexors), 29% (ankle plantarflexors), 44% (deep extensors and flexors), 24% (knee extensors) and 40% (hamstrings). Overall, total PCSA for the leg muscles decreased significantly from 103.9 cm^2 at moult score 3 (when the bird's are least capable of flying) to 72.0 cm^2 at moult score 4, coinciding with the end of the flightless phase of wing moult. Force (Table III.2) followed a similar pattern to that of PCSA for the leg muscle groups.

Of the leg muscle groups, fascicle length changed significantly in the ankle dorsiflexor group only (ANOVA, $P < 0.05$), increasing significantly between moult stages 1 and 2, and stages 3 and 4. All other leg muscle groups showed a non-significant trend of increasing fascicle length between moult scores, 1, 2 and 3 before decreasing at moult stage 4 (Fig. III.6c).

Discussion

Overall changes in flight and leg muscle mass

Despite the geese never having flown, these results show that the captive barnacle geese still experienced significant changes in their flight and leg muscle groups throughout the moult process, as predicted in Hypothesis 1. The approximate 30% atrophy of the flight muscles found in the present study is consistent with that reported from wild waterbirds, with values ranging from 17% in mallards *Anas platyrhynchos* (Young and Boag, 1982) to 33% for black-necked grebe *Podiceps nigricollis* (Jehl, 1997) (for full review of muscle atrophy values see Piersma, 1988 and 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). It would appear therefore that captive conditions do not affect the level of atrophy in barnacle geese flight muscles at the onset of wing moult. This is supported by results for captive red knots, which experienced no atrophy of their flight muscles after 8 months in captivity (Dietz *et al*., 1999). The lack of atrophy is despite the birds being restricted to a few minutes hovering flight per day, compared to more than 2 hours of flight in free-living red knots during the non-breeding season, when flight muscle is hypertrophied prior to migration (Piersma *et al*., 1993).

As predicted in our hypotheses, following the major flight muscle hypertrophy during mid-moult (moult score 2), flight muscle mass returned close to non-moult values by moult score 4, despite no significant increase in wing flapping behaviour. These results agree with comparable data for wild blue-winged teals *Anas discors* (Brown and Saunders, 1998) and brant geese (Ankney, 1984). The increase of 25% in flight muscle mass mid moult in the captive geese again is also similar to previously reported values for lesser snow geese *Anser c. caerulescens*, (Ankney, 1979), which experience a 50% increase in the pectoral muscle mass during mid wing moult with no observed increase in wing flapping behaviour.

While there has been much discussion about the mechanisms responsible for mid-moult hypertrophy of flight muscles (Ankney, 1979; Hohman *et al*., 1992, Fox and Kahlert, 2005), the response of the leg muscles to the flightless stage of wing moult in waterbirds has generally been assumed to be a responsive mechanism to the increased use as a result of the flightless period, i.e., use/disuse (e.g.. Piersma, 1988; Fox and

Kahlert, 2005). In the captive barnacle geese the total mass of the leg muscles increased by 13% between moult stages 2 and 3, before decreasing 28% between moult stages 3 and 4 (an 18% drop in comparison to initial total leg muscle mass at moult stage 1). These changes in mass of the leg muscles were in the opposite direction to those of the pectoral muscles. Wild moulting greylag geese followed a similar pattern of leg muscle changes during moult, with a period of hypertrophy (36%) followed by atrophy (11%). In wild dabbling ducks and geese, increases in leg muscle mass during moult of 16%, 41%, 27% and 30% have been reported for mallard, giant Canada geese *Branta canadensis maxima*, snow geese and gadwall *Anas strepera*, respectively (Young and Boag, 1982; Rosser and George, 1985; Ankney, 1979; Hay, 1974).

The sudden atrophy of the leg muscles of captive barnacle geese from moult score 3 onwards supports the proposal of Brown and Saunders (1998) that leg muscles would need to atrophy before the re-commencement of flight, otherwise the leg muscle mass could hinder flight. Brown and Saunders (1998) also suggested there would be an optimum cross over point mid-moult where leg muscles can assist with take off to reduce the requirement of the recovering pectoral muscle. This would support the conclusion from our previous study on captive geese (Portugal *et al.*, 2007), where body mass loss occurs potentially to enable flight on partially atrophied flight muscles, rather than being an adaptation to enable flight earlier on partially regrown flight feathers. However, data presented in the current study provide evidence that the flight muscles, following atrophy, have returned to their original mass by moult score 4, when the flight feathers have grown sufficiently to enable flight. It is possible

though that slightly increased leg muscle mass may assist with take off, once flight is regained. Ankney (1984) noted that in moulting brant geese, the pectoral muscles were not back to 'normal' mass until, or after, the birds were fully capable of flight.

Within the two locomotor muscle groups, there were significant changes in muscle mass during the study period. However, overall locomotor muscle mass did not change significantly; the changes in muscle mass were compensatory. Compensatory leg and flight muscle changes were also observed in Canada, snow and greylag geese, black duck *Anas rubripes*, lesser scaup *Aythya affinis* and mallard (Ankney, 1984; Ankney, 1979; Fox and Kahlert, 2005; Reinecke *et al.*, 1982; Austin and Fredrickson, 1987; Young and Boag, 1982). Hansen (1962) concluded that these muscle changes were a result of an energy deficit and nutritional stress, and during moult, protein was being reallocated. Pectoral mass in the geese from moult score 3 onwards, however, is increasing at a time when body mass is decreasing, and in most moulting waterfowl, hypertrophy of the flight muscles coincides with growing feathers, suggesting there is no constraint on supply of protein (e.g. Fox *et al*., 2008). Nevertheless, a reallocation of protein would certainly be of benefit as an energy saving mechanism, although not necessarily resulting from a protein deficit as suggested by Hansen (1962).

Muscle architecture, PCSA and fascicle length

Despite significant changes in mass of the flight muscle group, there was no significant change in PCSA or fascicle length. This implies that, when the effect of body mass is removed, flight ability is not significantly different during moult

compared to non-moult. Therefore, as soon as the flight feathers are sufficiently re-grown, the flight muscles are fully capable of flight.

The changes observed in the PCSA of the leg muscle groups suggest the leg architecture is modified to increase force production to assist with quicker running, to aid escape when moulting birds are flightless. The results presented here suggest that the PCSA (and mass) of the leg muscles did not significantly increase prior to flightlessness, as hypothesised, although they did reduce significantly once the ability to fly had been regained at moult stage 4. In theory, both moult score 1 and 5 should represent non-moult values for mass, PCSA and fascicle length. However, for both mass and PCSA, values for moult score 5 were significantly lower than those for moult score 1 for all groups except the gluteals. This brings into question the point in the annual cycle at which muscle dimensions can be considered to be at normal values (see also Fox and Kahlert, 2005). Evidence presented here and in previous studies on captive barnacle geese (e.g. Butler and Woakes, 2002; Portugal *et al*., 2007) suggests that many aspects of the physiology and behaviour of barnacle geese are largely independent of specific environmental cues, and must in part be reliant on entrained endogenous generated rhythms to structure their annual cycle, to some extent. Ankney (1984) commented that many species of geese are effectively behaviourally flightless during the breeding season, since they are incubating eggs or rearing goslings. Furthermore, in wild snow geese, hypertrophy of the leg muscles starts prior to the wing moult and its associated flightless phase. Therefore, it is possible that in the captive barnacle geese, leg muscle hypertrophy and increased PCSA of the major force producing leg muscles, 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). Similarly, the pectoral muscles may also have become atrophied during the breeding season, prior to wing moult. Therefore, it is likely that moult score 5 (October-November), not 1, should be considered 'normal' for the captive geese. If this is correct, all leg muscle groups do show an increased capacity for powering leg dominated locomotion when flightless, be it behaviourally flightless when guarding goslings or actually flightless during wing moult. If we assume moult score 5 to be the normal state for the geese, comparing the percentage reduction in PCSA between moult scores 3 and 5 could give an indication of the increases experienced prior to the flightlessness. Physiological cross-sectional area of the ankle dorsiflexors, knee extensors, hamstrings and adductors would have potentially increased to the greatest extent prior to the flightless phase. The biggest decrease in PCSA at moult score 4 is in the adductor group. Changes in PCSA observed in this study, particularly at moult stage 4 when PCSA in all of the leg muscle groups has decreased, also contradict expectations arising from the use/disuse hypothesis. This is because although captive barnacle geese exhibit increased resting during peak wing moult, foraging and locomotion levels increase once they have regrown their flight feathers (Portugal *et al*., 2007), and as Narici *et al*. (1989) have demonstrated, the largest increase in muscle size and strength occur in the first 5-7 days of increased muscle use.

Geese take off by running along land or water, or jumping to aid take-off. The only muscle group to have maintained a constant PCSA throughout moult was the gluteal group, which is responsible for rotating, flexing and adducting the hip. In guineafowl,

Numida meleagris, the iliotibialis lateralis, part of the gluteal group, is important in jumping (Rubenson *et al*., 2006). It is possible that this group is also important for jumping in geese, in take off. This may imply that the PCSA of this group remains constant throughout the wing moult period to aid with take off once flight is resumed.

Overall, the ankledorsiflexors, hamstrings and adductors had the longest fascicle length of all leg muscles, suggesting that these muscle groups require the ability to generate force over a wide range of motion and/or contract with an increased velocity of shortening. However, since muscle power is proportional to muscle volume, muscles that show limited force producing potential due to long fascicle lengths are still capable of high power production (Williams *et al*., 2007). In barnacle geese, these adaptations are likely to enhance the ability of the bird to exert force throughout a swimming foot cycle and rapidly retract the lower limb and foot during walking or running during the swing phase (Reilly, 2000; Biewener and Corning, 2001). Interestingly, the only significant increase in fascicle length was recorded in the ankle dorsiflexors. This muscle group is primarily responsible for lifting the foot off the ground to stop it dragging along the floor 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). Kinematic studies of walking quail, *Coturnix japonica,* have shown that maximum extension of the ankle occurs at foot down (Reilly, 2000). Therefore, an increase in fascicle length of the ankle dorsiflexors could increase the velocity with which the foot is lifted, and potentially shorten the stance

phase, enabling the bird to run faster. The ability to exert force over a wider range of movement may also facilitate increased speed during running and swimming. Combined with an increase in the PCSA of the muscle, these changes could provide the flightless geese with an increased ability to escape from predators through running and/or swimming.

Summary

By removing the natural movements and activity of wild animals, it has been possible to conclude that the major changes in leg and breast muscle in moulting captive geese cannot be explained through use/disuse. Despite the birds being unable to fly and thus always reliant on walking, the leg and breast muscles still showed compensatory adjustments, in anticipation of the different forms of locomotion that will be required to enhance fitness in the wild. Increases in 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 manoeuvrability to the birds, to aid escape on foot from predators. Therefore, these muscle changes give potential for increased or decreased work, but do so in an anticipatory rather than a responsive fashion.

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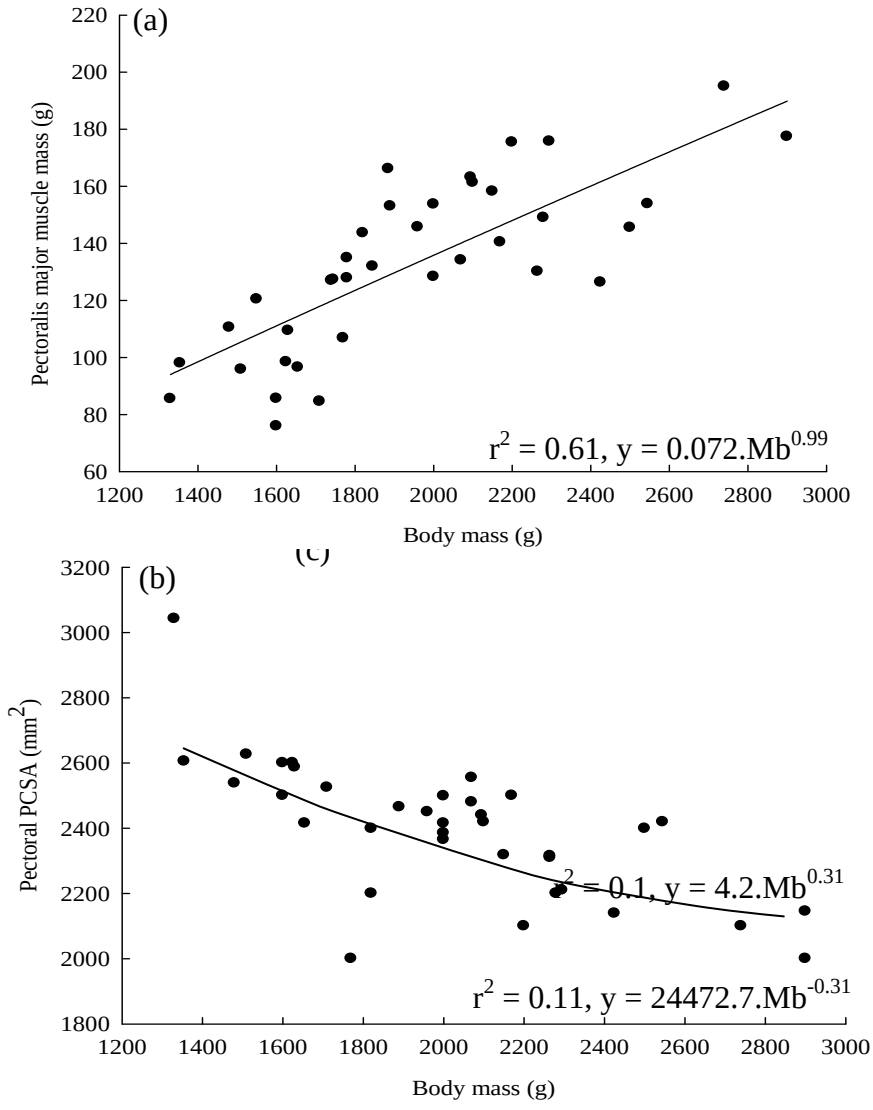

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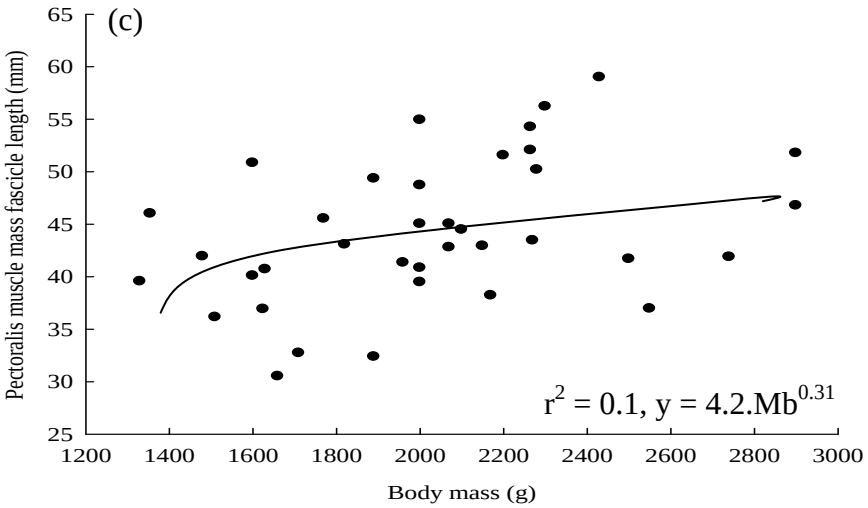

Figure III.1: Scatter diagram to show the relationship between body mass and (a) pectoralis muscle mass, (b) PCSA and (c) fascicle length, in 37 captive barnacle geese sampled pre, during and post moult. Power trend lines have been fitted to the data and their equations are provided on the plots. All r^2 values were significant ($P < 0.05$).

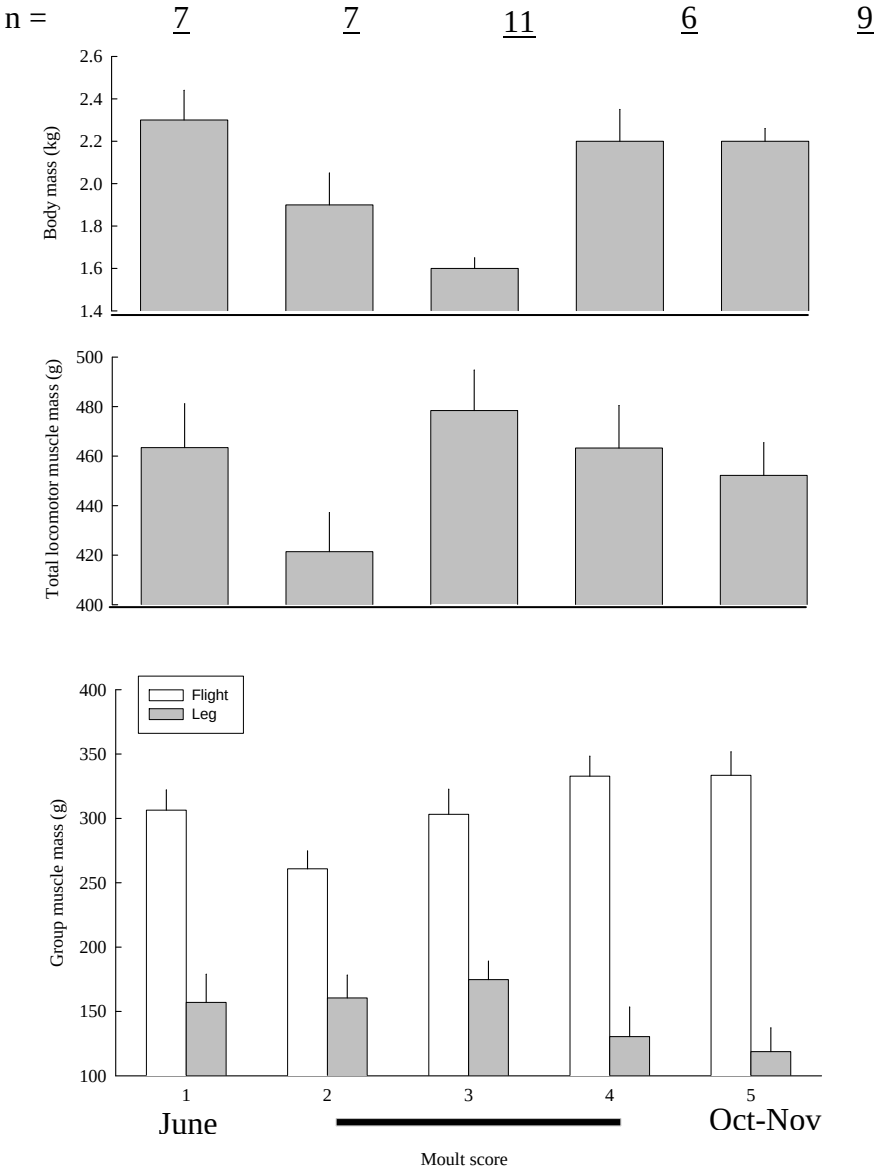

Figure III.2: Changes (± SEM) in body mass (a) and total mass of the major locomotor muscle groups (b) of 40 captive barnacle geese, sampled before, during and after wing moult. Moult score (see Methods) is used as an index of moult stage, approximate dates are included. Changes in muscle mass of the flight and leg muscle groups are detailed in plot (c). The black bar represents approximate wing moult period (Jul-Aug). Overall locomotor muscle mass did not change significantly over the duration of the study period (ANOVA, $P = 0.6$).

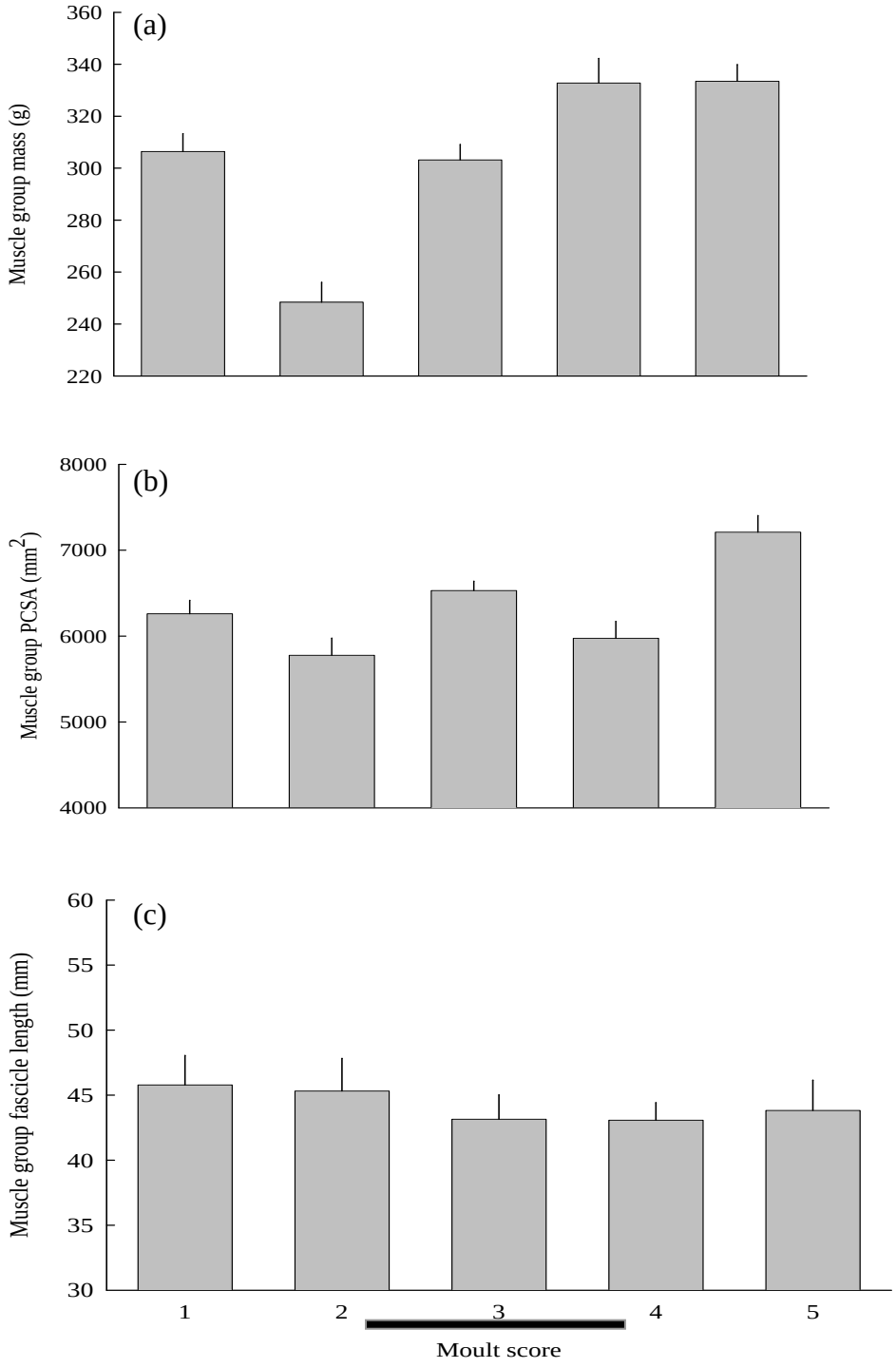

Figure III.3: Mean (± SEM) values of (a) muscle mass, (b) PCSA and (c) fascicle length of the flight muscle group in 40 captive barnacle geese sampled before, during and after wing moult. Moult score is used as an index of moult stage. Flight group muscle mass changed significantly throughout the study period (ANOVA, $P > 0.01$), whereas physiological cross sectional area (PCSA) and fascicle length did not.

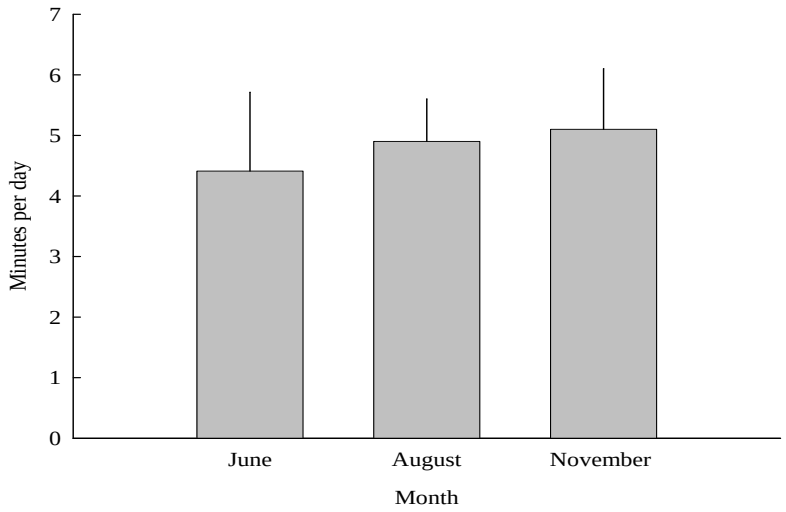

Figure III.4: Mean (± SEM) time spent per day wing flapping for a flock of 16 captive barnacle geese, sampled pre (June), during (August) and post (November) wing moult. There was no significant difference between the three sampling periods (repeated measures ANOVA $P = 0.91$).

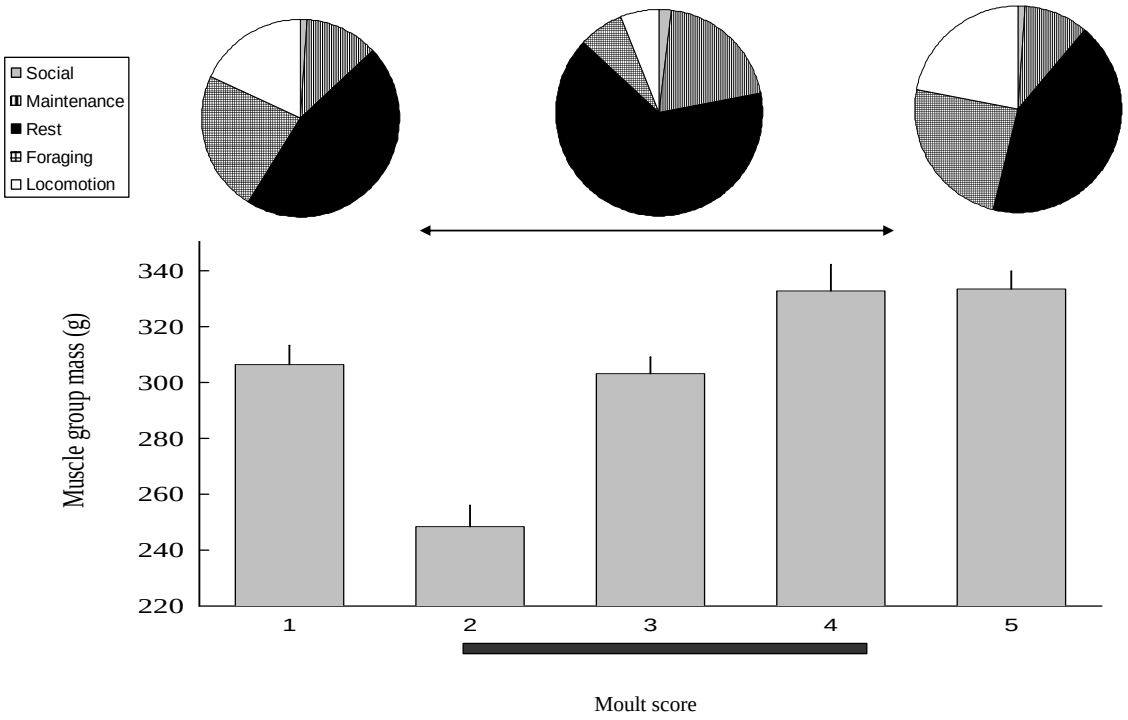

Figure III.5: Behavioural data from 14 captive barnacle geese recorded in 2006, taken from Portugal *et al* (2007). The behaviour pie charts are superimposed over mass changes of the flight muscle group during wing moult. The black bar indicates the moult scores within which wing moult took place. The time energy budgets taken during moult (signified by the black arrow) did not change significantly throughout the moult period (Portugal *et al.*, 2007), suggesting that the observed significant changes in muscle mass were not a direct result of changes in muscle usage, as muscular changes, particularly mid-moult increase in flight muscle mass (moult score 3), were not accompanied by an increase in locomotor or foraging behaviour.

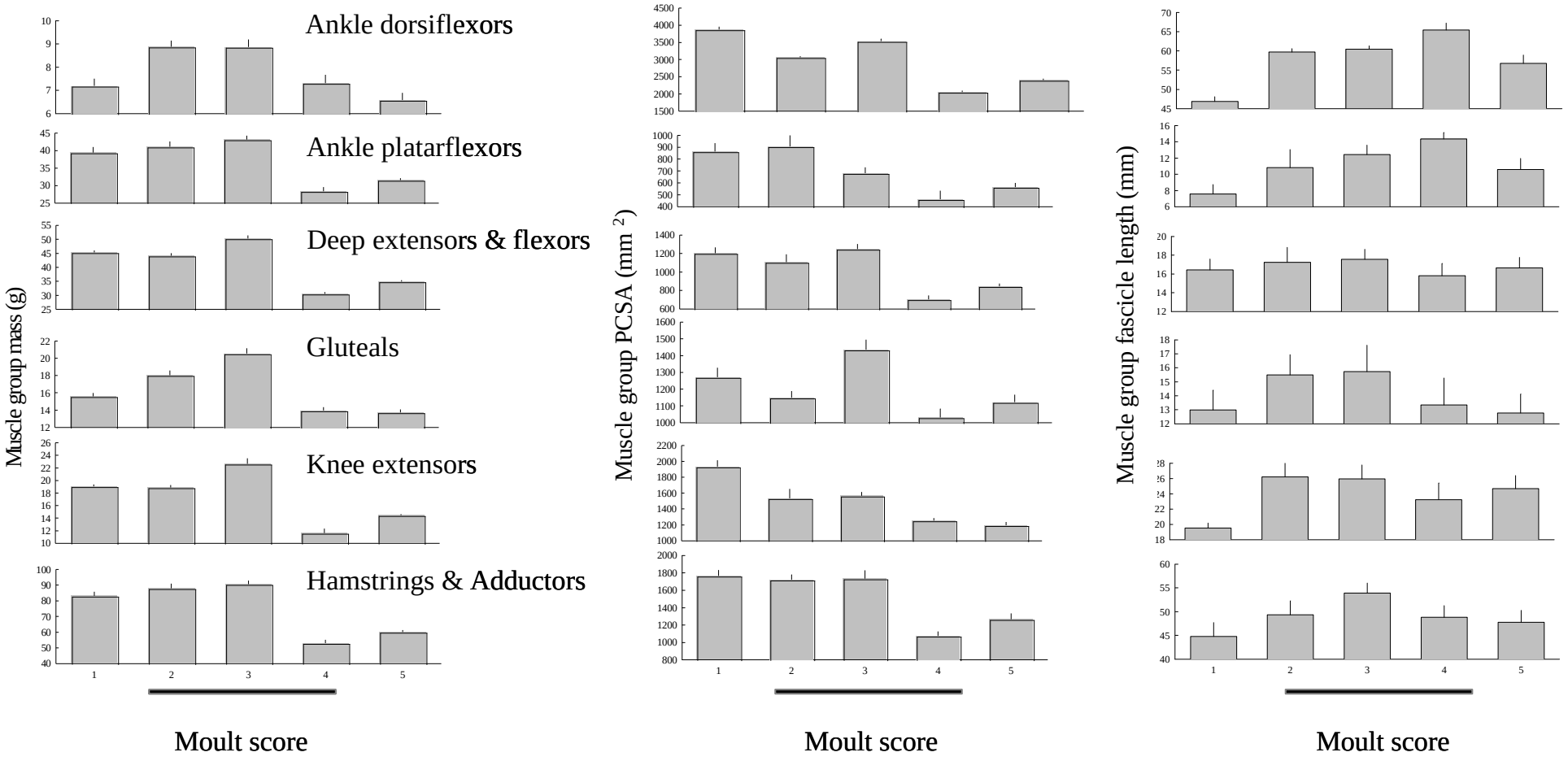
(a)
(b)
(c)
Ankle dorsiflexors
Ankle platarflexors
Deep extensors & flexors
Gluteals
Knee extensors
Hamstrings & Adductors
Muscle group mass (g)
Muscle group PCSA (mm 2)
Muscle group fascicle length (mm)
Moult score
Moult score
Moult score

Figure III.6: Mean (± SEM) mass (a), physiological cross sectional area (PCSA, b) and fascicle length (c) of the major leg muscle groups in captive barnacle geese, sampled before, during and after wing moult. Moult score (see Methods) is used as an index of moult stage. The black bar represents approximate wing moult period. By the end of moult score 4, the birds have regained the ability to fly.

Muscle group	**Muscles within group**
Flight	*pectoralis, supracoracoideus (*pectoralis minor*)*
Ankle dorsiflexors	*tibialis anterior*
Ankle plantarflexors	*gastrocnemius medialis and lateralis*
Deep ankle flexors and extensors	*deep flexors 1-8 and deep extensors 1-3*
Gluteal group	*iliotibialis lateralis (*tensor fasciae latae), *iliotrochantericus cranialis (*iliacus), *iliotrochantericus caudalis*
Knee extensors	*femorotibialis (*vasti*), femerotibialis lateralis*
Hamstrings and adductors	*iliotibialis cranialis (*sartorious*), pubo-ischio-femoralis (*adductors*), caudofemoralis (*piriformis*), ambiens, iliofibularis (*biceps femoralis*), flexor crusis lateralis (*semitendinosus*), flexor crusis medialis (*semimembranosus*), ischiofemoralis*

Table III.1: Muscle composition of the major flight and leg muscle groups. Muscle names and functions are based on Howell (1938) and Vanden-Berge and Zweers (1993). Alternate/common names are in brackets. Note muscles are grouped according to their primary function.

Muscle group/Moult score	1	2	3	4	5
Flight	1020	814	1030	1161	1081
Ankle dorsiflexors	158	335	221	188	179
Ankle plantarflexors	181	184	164	155	168
Deep ankle flexors And extensors	220	263	162	161	190
Gluteal group	156	241	57	69	85
Knee extensors	149	153	132	121	125
Hamstrings and adductors	392	449	321	187	235

Table III.2: Mean calculated Force (N) of the flight and leg muscle groups for each moult score (see Methods).

IV. ESTIMATING FAT CONTENT IN CAPTIVE BARNACLE GEESE (*BRANTA LEUCOPSIS*): VALIDATION OF HYDROGEN ISOTOPE DILUTION TECHNIQUE AND MORPHOLOGICAL INDICATORS

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Total body fat (TBF) of nine captive barnacle geese (*Branta leucopsis*) was predicted using two non-invasive techniques; isotope dilution and morphological indicators. Actual TBF and total body water (TBW) were determined via chemical analysis. Deuterium oxide (D_2O) was used to estimate TBW (TBW D_2O), and from this, TBF calculated using both the regression (D_2O REG) and the Pace and Rathbun (D_2O P/R) approaches. 11 morphometric measurements were taken from the birds and a principal component analysis performed to construct various predictive condition indices. D_2O using the plateau approach underestimated TBW by approximately 20%, giving values significantly different from those of chemical analysis and desiccation (TBW DES). However, TBW D_2O and TBW DES were highly correlated once the sexes had been separated, so TBW D_2O could be adjusted accordingly before TBF was calculated. TBF values predicted by D_2O P/R, principal component 2 and body length did not differ significantly from values obtained by direct chemical analysis ($P < 0.05$), whereas principal component 1 and D_2O REG were poor predictors of body fat (Table IV.1). Results showed TBF estimated by D_2O P/R and body length to be significantly correlated with actual TBF, and it was concluded that if the relationship

between TBW D_2O and TBW DES remains constant, D_2O P/R could be used to provide repeated measurements of TBF. Although body length predicted TBF accurately, it was excluded, as it was difficult to envisage how it would reflect temporal changes in fat deposition. Overall it is likely that D_2O P/R would only be sufficiently accurate to study population means of the captive barnacle geese, and allow for broad scale seasonal comparisons.

SJP developed the methodology, conducted the data collection and preparation, analysed the data and wrote the manuscript. PAR, JRS and RAM helped develop isotope sampling protocol, procedure and analysis, JAW and MMM provided equipment and assistance with homogenate preparation, RMB provided equipment and assistance with fat extraction, and CRW provided logistical support with all practical aspects of the work. PJB and JAG discussed methodology, data analysis and conclusions, and aided in the writing of the manuscript.

* a version of this chapter has been combined with Chapter V to form a manuscript, which is currently in the final stages of submission to the Journal of Avian Biology.

Introduction

Fat deposits are the primary energy reserves in birds (Walsberg, 1988; Blem, 1990), and fat storage is important for avian reproduction (e.g. Walsberg, 1983), migration (e.g. Biebach *et al*., 1986) and over-winter survival (e.g. Blem, 1990). Thus, seasonal patterns of fat deposition and catabolism identify periods of energy surplus and limitation when foraging behaviour is adjusted to meet those constraints (Blem, 1976; Drobney, 1980). Fat content has been shown to be vital in particular for migrating birds, with regards to departure times, stop-over durations and successful reproduction on arrival at the breeding grounds (e.g. Prop *et al*., 2003).

Traditionally, fat is measured using chemical extraction of carcasses, typically via a Soxhlet apparatus (Sawicka-Kapusta, 1975; Blem, 1990; Reynolds and Kunz, 2001). However, the destructive nature of this technique clearly precludes its application to rare and endangered animals, and limits the study of within individual temporal variations in fat deposition. This has lead to the development of non-destructive techniques (see Speakman, 2001, for review). These techniques include isotopic water dilution (e.g. Mata *et al*., 2005), gas dilutions (e.g. Henen, 1997), ultrasound (e.g. Dietz *et al*., 1999), bioelectrical impedance (e.g. Farley and Robbins, 1994), total body electrical conductivity (TOBEC®) (e.g. Castro *et al*., 1990), and morphological indicators (e.g. Conway *et al*., 1994).

The isotope dilution method is one of the most frequently used non-destructive techniques for determining body composition (Speakman *et al.*, 2001). Water is not evenly distributed in body tissues, and proteinaceous tissue contains substantially

more water than fat (Speakman *et al*., 2001). Isotope dilution predicts the total body water content by dilution of a stable isotope, and then estimates the total fat content based on the inverse relationship between body fat and body water (Robbins, 1993; Speakman *et* al., 2001; Mata *et al*., 2005). Two approaches are typically employed to predict total body fat (TBF) from total body water (TBW). The Pace and Rathbun (1945) approach assumes that the water (*w)* content of the lean body mass (LBM) is constant between species, allowing predictions of TBF to be estimated once TBW is obtained via isotopic dilution. Sheng and Huggins (1979) noted however that this assumption about LBM might be inaccurate. The second approach accounts for this possible error in LBM by deriving a species-specific relationship between TBF and TBW content, obtained from direct fat extraction and desiccation (the removal of water) respectively (Robbins, 1983). Once TBW is determined through isotopic dilution, TBF is then estimated from the calibration relationship (e.g. Reilly and Fedak, 1990). This technique though does rely on there being a significant relationship between TBW and TBF, which is not always the case (Winstanley *et al*., 1998).

Morphological indicators typically involve the development of a condition index based on morphometric measurements taken from the animal (Hayes and Shonkwiler, 2001; Hwang *et al*., 2005). External measurements of animals are relatively simple and if one of these measurements, a combination of measurements, or a body condition index is highly correlated with TBF, morphological measurements can be used to predict TBF and thus how fat stores change over time. Three commonly used condition indices are ratio index (e.g. Jakob *et al*., 1996), slope adjusted ratio index

(Le Cren, 1951) and residual index, the morphological technique to be employed in the current study (e.g. Schulte-Hostedde *et al*., 2001). Residual index involves the regression of body mass on overall body size (typically derived from a principal component analysis, e.g. Wiklund, 1995), and the residuals of this regression can be used as an index of condition (Dobson, 1992; Guinet *et al*., 1998; Schulte-Hostedde *et al*., 2001; Hayes and Shonkwiler, 2001). However, not all studies validate the relationship between condition index (residuals) and TBF, and it has been demonstrated previously that each species has a different condition index that best predicts its TBF (Simpson *et* al., 1992; Krebs *et al*., 1993; Weatherhead and Brown, 1996; Winstanley *et al*., 1998).

Captive barnacle geese (*Branta leucopsis)* have a pronounced annual cycle in body mass, with changes in body mass temporally associated with critical events in their annual cycle (Portugal *et al*., 2007). For example, captive barnacle geese lose approximately 25% of their body mass during the flightless phase of their annual wing feather moult (Portugal *et al*., 2007), something also observed in wild moulting waterfowl species (Hohman *et al*., 1992). It has been proposed that the majority of mass loss during wing moult in waterfowl is a direct result of fat catabolism (Hohman *et al*., 1993a; Portugal *et al*., 2007) so, to track temporal changes in fat catabolism and deposition, a non-destructive technique of estimating the fat content of the birds is required. Therefore, the current study calibrated two non-destructive body composition techniques, by comparing the amount of total fat extracted from captive barnacle geese through chemical analysis, to fat content predicted by the isotope dilution method and morphological indicators.

Materials and Methods

Birds

A captive population of nine barnacle geese obtained as 3-week old goslings was maintained under natural light in a large outdoor aviary at the University of Birmingham. The birds were nine years of age at the start of the study. 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.

For each goose, food was withheld for 7 hours prior to deuterium oxide (D_2O, Sigma-Aldrich, 99.98%) administration and the sampling of blood for D_2O analysis. Immediately following collection of post-equilibration blood sample, each bird was anesthetized with 5% isoflurane gas mixed with a 4:1 ratio of air and oxygen, and then injected with a lethal dose of pentobarbitone (140 mg/kg). Final body mass was measured (± 5 g) and feathers were plucked. The carcasses were then double-bagged and frozen at -20^{o}C until compositional analysis was performed. The birds were all killed in November, a period of stable body mass in the annual cycle of captive barnacle geese (Portugal *et al.*, 2007). Ideally, the techniques would be calibrated at various points in the year when body mass was different, but as only nine geese were available, the sample sizes would have been too small.

Isotope administration and analysis

D_2O dosage was calculated using equation 12.1 from Speakman (1997);

$$\text{dosage} = \{0.65(\text{body mass (g)} \times \text{DIE}\}/\text{IE} \quad (1)$$

where the constant, 0.65, is the approximate proportion of the body comprised of water, DIE is the desirable initial enrichment (ppm) and IE is the injectate enrichment (ppm). Birds were weighed to the nearest 5 g. Blood samples were taken from either the brachial or intertarsal vein for determination of background enrichment of D_2O, immediately prior to D_2O administration. A disposable 1 ml syringe (Terumo) fitted with a 24-gauge stainless steel hypodermic needle was used for administration of the D_2O, via an intra-peritoneal injection into the lower abdomen. D_2O was injected slowly and the needle left in for five seconds, to avoid any injectate seeping out (Speakman *et al*., 2001). The actual amount of D_2O injected was accurately determined by weighing the syringe to the nearest 0.0001 g before and after injection. Subsequent blood samples were collected 90 min after administration of the D_2O, based on pilot data showing that 90 min was an appropriate length of time for the isotope to equilibrate (Fig. IV.1). Blood samples were drawn up in 50 μL non-heperinised glass capillaries (Vitrex, Cambridge, UK) and immediately shaken to the centre of the capillary, which was flame sealed with a butane gas torch (Radio Spares, Corby, UK) and then wax sealed. Capillaries were then stored in an air-tight container. Information regarding isotope analysis is described elsewhere in detail (Speakman and Krol, 2005).

Calculation of ^2H dilution space

Isotope dilution space was calculated by the plateau method (Halliday and Miller, 1977), using equation 4 of Speakman *et al*. (2001);

$$N_{mol} = \frac{M_{mol}(E_{in} - E_p)}{(E_p - E_b)} \qquad (2)$$

where N_{mol} is the molar quantity of water present in the body, E_{in} is the enrichment of the material introduced into the animal, M_{mol} the molecular weight of the D_2O, E_b is the background enrichment of this material in the animal, and E_p the enrichment measured after the 'dispersal' process is completed.

Assuming the constancy of the water content of LBM (Pace and Rathbun, 1945, hereafter referred to as D_2O P/R), estimates of body composition could be obtained as follows:

$$\%LBM = \frac{\%TBW}{w}, \qquad (3)$$

$$\% \text{ fat} = 100 - \% \text{ LBM}, \qquad (4)$$

where TBF, TBW and LBM were expressed as a percentage of fresh body mass. Here, *w* is the mean fractional water content of LBM for the nine geese (0.731 ± 0.013, range 0.68-0.78). This value is within the range for mammal and bird species (see

Mata *et al*., 2005, for references). TBW determined via D_2O dilution (hereafter referred to as TBW D_2O) was corrected using the relationship between TBW D_2O and TBW obtained through desiccation (hereafter referred to as TBW DES), before TBF was determined.

For the regression approach (e.g. Robbins, 1993, hereafter referred to as D_2O REG), TBF derived by chemical analysis was regressed against TBW DES, and this relationship was then used to estimate TBF based on TBW D_2O (Farley and Robbins, 1994; Hildebrand *et al*., 1998). Again, TBW D_2O was corrected using the relationship between TBW DES and TBW D_2O before using it to determine TBF.

Carcass analysis and fat extraction

To measure body composition, frozen carcasses were thawed and reweighed (<1% change in mass), before being sliced into segments and homogenised in a Hobart E 4522 meat grinder (excluding feathers). Each bird was passed through the grinder four times (2 times through a wide bore blade and 2 times through a small bore blade), and was hand mixed between each round (e.g. Baduini *et al*., 2001). Eight preweighed aliquots of each homogenate (6 ± 2.3 g) were dried in an oven at 60^{o}C until the mass of the homogenate remained constant (~72 h, Kerr, 1982). The TBW of each bird was then calculated by multiplying the proportion of water evaporated from each homogenate by the wet body mass of each bird after injection (e.g. Shaffer *et al*., 2006).

Dried homogenates were transferred to pre-weighed cellulose, single-thickness 22 x 80 mm extraction thimbles (Whatman, UK). Thimbles were inserted in a Soxhlet apparatus and fat was extracted for 7 hours with petroleum ether. After extraction, thimbles were dried at 60^{o}C for 14-16 hours and extracted lipids were weighed to determine lipid content (% mass) for each aliquot. The lipid content of each dried carcass was determined from the mean of eight aliquots. If the standard deviation of the mean exceeded 2%, additional aliquots were extracted until the SD was less than 2%.

Morphological measurements

In total 11 different measurements were taken from each goose, based on those described in Wiklund (1995) but see also Proctor (1993) for definitions and diagrams of body parts. All measurements were made to the nearest mm, using callipers, a ruler or a tape measure and all measurements were taken four times in succession, to calculate a mean and a repeatability estimate.

Parts measured were: *tibiotarsus* from the joint with the femur to the joint with the tarsometatarsus; *foot span*, the distance on the ventral side between the tip of the hind toe and the tip of the middle toe, not including claws; *middle toe* (digit 3) from the joint with tarsometatarsus (visible when the toe was bent slightly downward) to the posterior end of the middle toe (digit 3) not including the claw (the toe was bent downward and gently stretched and measured on the dorsal side); *head width*, measured across the dorsal surface between the two eyes; *bill length*, measured from

the base of the maxilla to the tip of the premaxilla; *bill depth*, depth of the bill from top to bottom at the nasal aperture; *neck circumference*, measured at the base of the neck around fused thoracic vertebrae; *tail length*, from the pygostyle to the tip of the central feathers; *'wing'* measured from the chest flank at the joint between the wing and body (glenoid fossa), and the radiale/ulnare region; *'primary'* measured from the radiale/ulnare region to the tip of the 9th primary; *body length*, from the tip of the beak to the tip of the central feathers of the tail.

Statistical analysis

Measured values are reported as means ± SEM and ± SEE (Zar, 1984) when regressions are used to predict TBF. Two tailed paired *t*-tests were used to compare differences between TBF predicted by each technique and total fat extracted, and standard % errors calculated. Correlations between TBF estimates and chemical determinations were assessed by linear regression.

A principal component analysis was conducted on the body size measurements (e.g. Wiklund, 1995). The first two principal components (PC1 and PC2) were regressed against body mass and total extracted fat content, and also against each individual measured body part. Each individual body part measurement was also regressed against body mass. The residuals of the PC1 – body mass regression were used as an index of condition. To determine whether these residuals reflected TBF, the estimates of condition were regressed against TBF and TBW. A GLM ANOVA model was used

to investigate the difference in the relationship between the sexes with TBW, TBF and body mass. A level of significance $P < 0.05$ was used in all tests.

Results

Chemical analysis

Mean body mass of the nine captive barnacle geese was 2751 ± 143 g (Table IV.1). The mean body mass for the male geese was 3019 ± 160 g compared to females, which had a mean body mass of 2416 ± 113 g. Mean TBW DES for the nine captive barnacle geese was 1684 ± 103 g (Table IV.1), equivalent to 61 ± 1.1% of body mass. For female geese mean TBW DES was 1465 ± 34 g (61 ± 1.1%) and for male geese, 1859 ± 143 g (62 ± 1.2%). The difference in TBW DES between males and females was significant (t-test, $P = 0.04$), although not so when TBW DES is treated as a % of body mass. There was a significant relationship between TBW DES and body mass (Fig. IV.2a) for male geese. Mean total fat extracted from the birds was 445 ± 35 g, equivalent to 16 ± 0.9% of body mass. Mean TBF for male birds was 498 ± 52 g (16 ± 1.2%) and female birds, 379. ± 21 g (16 ± 0.5%). There was no significant difference between males and females in TBF (t-test, $P = 0.09$), and % TBF ($P = 0.6$), and no significant relationship was found between TBF extracted and body mass.

TBW regressed against total fat showed there to be a non-significant trend ($P = 0.059$, Fig. IV.3) between the two. Percent water was not consistently related to percent fat, and there was no significant relationship between these two variables.

Comparison of dilution spaces and total body water (TBW)

Mean body water pool estimated by D_2O (Table IV.1) was 1110 ± 71 g (or 40 ± 2.4%). For male birds, mean TBW D_2O was 1144 ± 87 g (or 36 ± 0.7%) and for female geese, 1082 ± 65 g (or 47 ± 2.1%). On average, TBW D_2O underestimated TBW by 574 g (or 20.8%) when compared to TBW DES. Overall, TBW D_2O was significantly lower than TBW DES (*t*-test, $P < 0.001$). There was a significant relationship between TBW DES and TBW D_2O, within each sex (females, $r^2 = 0.89$, $P < 0.05$, males, $r^2 = 0.95$, $P < 0.05$; Fig. IV.4), but the regression lines for the two sexes were significantly different (GLM ANOVA $P < 0.001$). Because TBW DES and TBW D_2O were significantly related, TBW D_2O could then be corrected for this underestimation using the equations shown in Fig. IV.4.

TBW D_2O had a significant relationship with body mass, once the sexes were separated ($r^2 = 0.98$, $P < 0.001$ for both sexes, Fig. IV.2b), highlighting the difference between TBW DES and TBW D_2O. The regression lines between the two sexes were again significantly different (GLM ANOVA $P < 0.001$). There was no significant relationship between TBW D_2O and TBF.

Morphometrics

Out of the 11 morphological measurements taken, tibiotarsus and primary had the highest repeatability values (Table IV.3). None of the body part measurements was significantly correlated with body mass. There was no significant difference in repeatabilities values for bone characters and feather characters. There were strong positive correlations between body length, middle toe length and primary length, and also between primary length and middle toe length.

From the principal component analysis, the variation in size explained by PC1 was 76.2%. All morphological measurements loaded positively on PC1 but factor loadings for PC2 were not in a consistent direction (Fig. IV.5). Out of the body parts measured, primary length, body length and wing contributed most to the size-vector (i.e. had the highest scores on PC1). By adding the second principal component, the degree of explanation of variation in size and shape increased by 12%; a cumulative value of 88.8%.

Neither PC1 nor PC2 had a significant relationship with body mass, and thus it was not appropriate to use this regression and its subsequent residuals to construct a condition index to predict TBF. Therefore PC1 and PC2 were both regressed against total fat content extracted. PC1 (but not PC2) had a significant relationship with TBF (Fig. IV.6a and b), although % TBF, for either of the sexes, did not. Of the univariate measurements, body length (Fig. IV.6c) and primary length had a significant

relationship with total fat content ($P < 0.01$ and $P = 0.05$ respectively), and the regression between body length and total fat extracted was used to predict TBF.

Fat comparisons

Fat content for each bird was calculated using each predictive technique and compared to total fat extracted, and data are presented in Table IV.1. *t*-tests demonstrated that the D_2O REG technique and PC1 gave significantly different values of TBF compared to those values obtained from fat extraction. D_2O P/R, body length and PC2 values of estimated TBF were not significantly different from total fat derived from extraction.

Based on % error (Table IV.2), D_2O P/R, body length and PC2 predicted total fat content most accurately, with mean algebraic errors of +2.6%, -0.008% and -0.65% respectively (Table IV.2). However, the % error range for PC2 (-39.80% - + 59.36%) was far greater than for D_2O P/R (-13.99% - +27.2%), while body length had the smallest range of % errors (-11.9% - +13.6%). TBF extracted was significantly correlated with TBF predicted by D_2O P/R ($P = 0.007$) and body length ($P < 0.001$) (Table IV.1, Fig. IV.7a and 7b).

Discussion

Chemical Analysis

Total fat content of the captive barnacle geese (16.1% of body mass), measured through Soxhlet extraction, was consistent with values reported for other wildfowl species at various points in the annual cycle (Table IV.4). TBW DES (61.2%) was also consistent with values reported for other waterbirds (Table 12.1, Speakman, 1997). Therefore we can assume that the chemical analysis of the geese is reliable, and gives accurate values of TBF and TBW (although see Reilly and Fedak, 1990, for potential errors in body composition values from chemical analysis).

TBW and body composition estimates by isotope dilution

Background variation of D_2O in the geese was low, which is not surprising given that all birds were maintained in a single aviary and were feeding on a constant diet with the same source of water (see Tatner, 1990). Figure IV.1 indicates that the isotope mixed fully with the body water pool of the birds. D_2O underestimated TBW by approximately 20%, and the values obtained for TBW D_2O were significantly different from those obtained by TBW DES. Previous studies have shown the dilution of isotopic water in an animal's body water pool slightly overestimates TBW, but usually predicts true body water from desiccation within 2-5% (Sheng and Huggins, 1979; Reilly and Fedak, 1990; Farley and Robbins, 1994). Mata *et al* (2006) reported an average difference in TBW of 6%, and Johnson and Farrell (1988), 9%. Therefore

the 20% underestimation of TBW by D_2O in the present study is a significant deviation from what is normally reported from studies that have used isotopes to predict TBW, and it is unclear what the cause of this may be.

Schulte-Hostedde *et al*. (2001) found with a range of small mammals, that percent water was not consistently related to percent fat, which would question the general utility of hydrogen isotopes as a predictor of fat content. As there was also no significant relationship between percent water and percent fat in the captive geese in the present study, this may be the most likely cause of the underestimation of TBW by D_2O. Dosage of the isotope was based on equation 12.1 of Speakman (1997), which assumed a TBW equivalent to 65%, and data from this study show mean TBW of the geese to be 61%. Therefore for future work, the dosage could be decreased accordingly, although it is unlikely that this would correct the significant underestimation of TBW by D_2O.

As D_2O substantially underestimated the body water pool, its use would seriously overestimate TBF content. However, the highly significant relationship between TBW D_2O and TBW DES meant it was possible to correct TBW D_2O using the equation derived from the regression relationship between the two. Of note were the significantly different relationships for male and female geese between TBW D_2O and various body components such as body mass. Such a difference was not seen when comparing TBW DES with body mass. The relationship between TBW D_2O and TBW DES is also significantly different for male and female birds, as is the relationship between TBF extracted and TBF predicted by D_2O P/R. Therefore when

correcting TBW D_2O and when estimating TBF, different regression equations are required for each sex, something not reported in the literature prior to the present study.

Once the two separate equations for each sex were applied and TBW D_2O corrected, TBF estimated using the D_2O P/R approach was a good predictor of TBF (Table IV.1, Fig. IV.6a). Previous studies have alluded to the absence of any absolute value for the water content of LBM as being a problem for conversion of body water estimates derived from isotope dilution into estimates of TBF (Sheng and Huggins, 1979; Speakman *et al*., 2001), and thus being critical of the Pace and Rathbun (1945) approach (e.g. Sheng and Huggins, 1979; Reilly and Fedak, 1990). In the present study, chemical analysis of the captive birds meant that the correct value for water content of the LBM (see Methods) could be inserted into the calculation, thus eliminating this source of error.

Reilly and Fedak (1990) commented that "Pace and Rathbun (1945) may have flaws in principle but could produce acceptable results in practice". They argued though that regressions between TBW DES and various body components was a far better approach than that of Pace and Rathbun (1945) for predicting TBF, and in theory if direct relationships between TBW and TBF can be derived, there would be potentially less margin for error and fewer assumptions (Reilly and Fedak, 1990). This approach has worked successfully for a number of species (e.g. Winstanley *et al*., 1998), but the relationship between TBW DES and TBF would need to be significant to then use TBW as a predictor of TBF. In the present study, using the D_2O REG approach was

not a good predictor of body fat (Table IV.1), even after correcting TBW D_2O. This is likely to be because of the non-significant relationship between TBW DES and TBF through extraction. In fact, D_2O REG was one of only two techniques that predicted values of TBF that were significantly different to the values obtained through chemical analysis.

It is uncertain if maintaining the birds on a high quality *ad libitum* diet has an effect on the body composition of the birds, and on the distribution of fat and water around the body. For example, Cramp (1977) reported an average body mass of 1827 g and 1619 g for male and female wild barnacle geese respectively, while Kear (2005) reported a body mass range for the birds of 1700 – 2400 g. In comparison, average body mass in the captive birds in the current study was 3019 g for male, and 2416 g for female birds. Clearly, these captive barnacle geese where exceptionally heavy birds, even in comparison to a peak body mass of 2143 ± 89 g recorded previously in captive barnacle geese (Portugal *et al*., 2007). As fat contains substantially less water than lean tissue, the fatter an organism becomes, the lower the water content as a percentage of its total body water (Speakman *et al*., 2001). Based on TBW D_2O alone, the almost obese nature of the birds could explain the low TBW estimates by the D_2O. However, despite the heavier body mass, the relative percentages of fat and water from chemical analysis are consistent with those published in the literature, suggesting that the unusually heavy nature of the geese was not a factor in the D_2O underestimating the TBW.

Predicting total fat content using morphometric measurements

The typical approach when using avian morphometrics to estimate body condition is to use residuals from a regression of mass on a body size measurement or PCA. As there was no significant relationship between body mass and PC1 or PC2, it was not appropriate to use a PC1 or PC2 body mass regression and subsequent residuals to construct a condition index that could then be used to predict TBF (e.g. Schulte-Hostedde *et al*., 2001). Therefore, PC1 and PC2 were regressed directly against total fat extracted to see if they could be used as predictors of TBF. PC1 is typically an index of structural size and was not a good indicator of TBF (Table IV.1). PC2 is typically an indicator of condition, and was a reasonable predictor of TBF, particularly compared to PC1. However, the correlation value between fat estimated using the total fat extracted – PC2 regression and actual TBF was very low (Table IV.1).

Although PC1 (as a measure of structural size) was a poor predictor of TBF, out of the univariate morphological measurements, there was a significant relationship between body length and TBF extracted (Fig IV.5), and TBF predicted using the equation from this relationship was highly correlated with actual TBF extracted (Fig. IV.6b, Table IV.1). It is unusual for one single body measurement to be so effective at predicting TBF, particularly one that encompasses a mixture of bone and feather characteristics (i.e. from the tip of the bill to the end of the tail). It does raise the question of whether body length was good at predicting a TBF for that particular time of year, rather than body length being a good method for predicting temporal changes in TBF. It is

unlikely that body length will change over time (except possibly during tail feather moult), but fat stores almost certainly will. It is difficult therefore to envisage how body length would reflect these changes in TBF.

Summary

Winstanley *et al*. (1998) stated "the method chosen and the acceptability of results is a balance between the accuracy of TBF required against the need to leave the subject alive after the measurement". With regards to future work and the purpose of the current study, it is essential the animals remain alive as the long-term goal is to measure annual cycles in fat deposition in captive barnacle geese. Therefore the advantage of the D_2O P/R approach is that it will allow repeated measurements of body composition on the same animals.

It is more than likely that the D_2O P/R approach will generally only be sufficiently accurate to study 'population means' of the captive barnacle geese, and allow for broad scale comparisons. Assuming the water content of LBM is constant, and the relationship between TBW D_2O and TBW DES is consistent, the calibration relationship can be used to correct TBW estimated from D_2O, and then TBF predicted using the Pace and Rathbun (1945) approach, for year round estimates of fat content.

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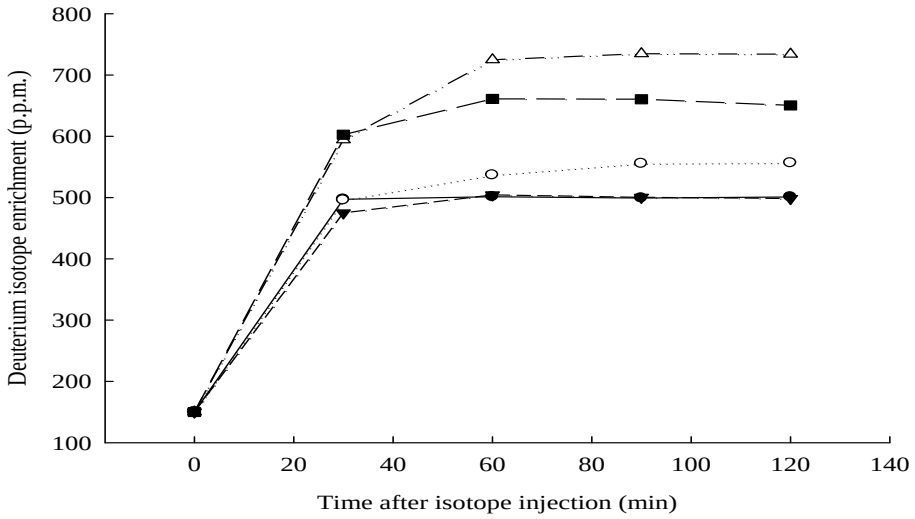


Figure IV.1: Pattern of isotope enrichment following dosing with deuterium oxide, in five captive barnacle geese.

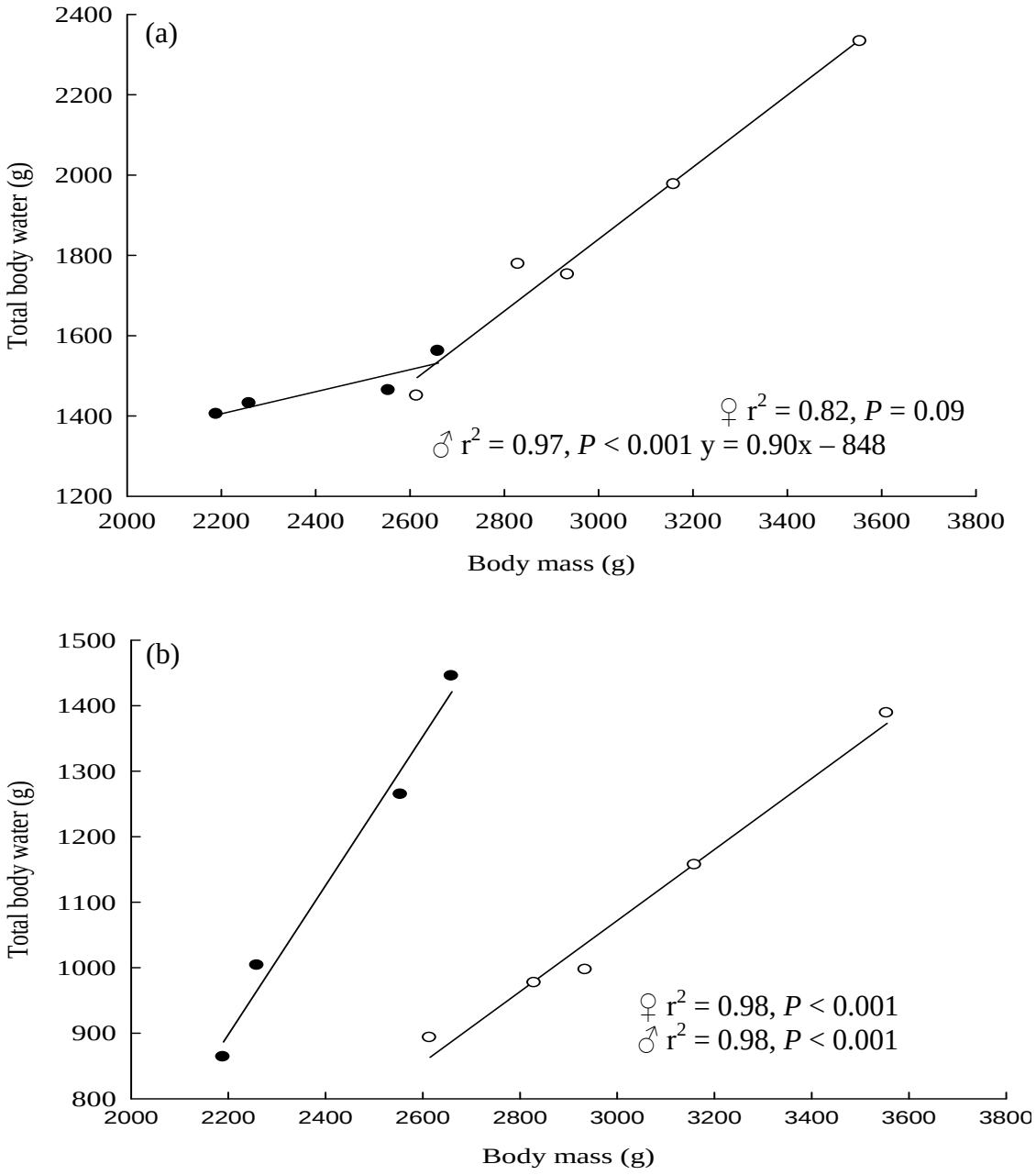


Figure IV.2: Relationships between body mass and a) total body water (TBW) obtained through desiccation, and b) TBW estimated from D_2O isotope dilution (y = 1.14x-1604 for females, y=0.54x-555 for males). Closed circles are females (♀, n=4) and open circles are males (♂, n=5).

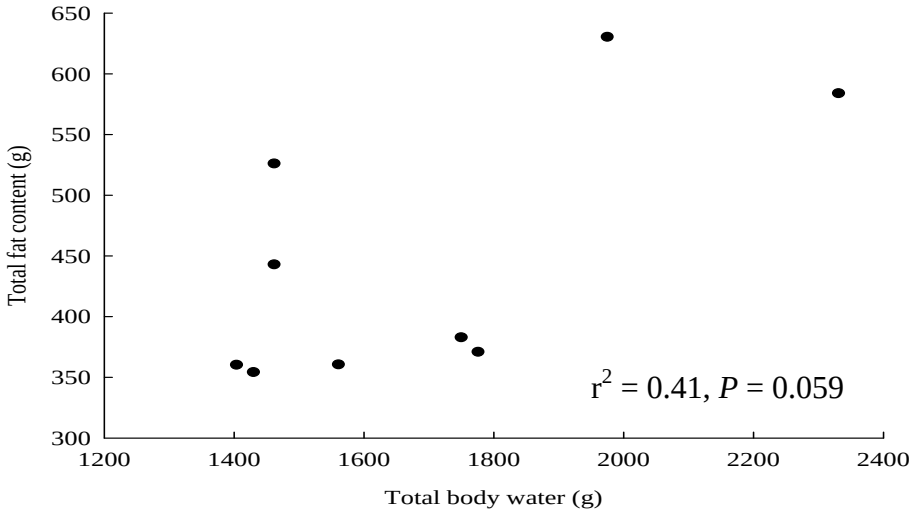


Figure IV.3: Relationship between total fat content obtained through extraction and total body water obtained through desiccation, for nine captive barnacle geese.

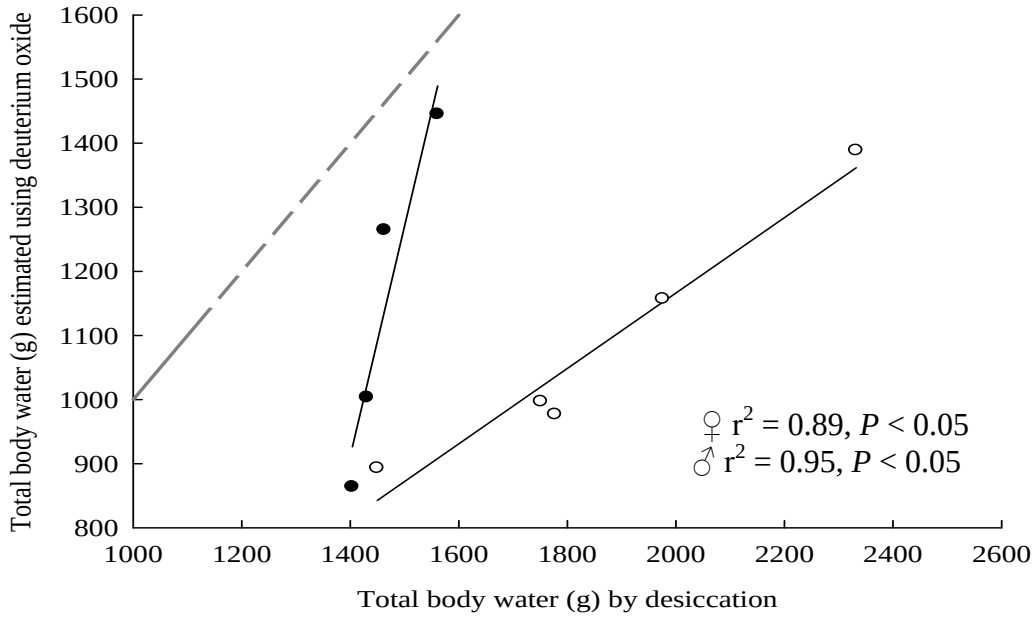


Figure IV.4: Total body water (TBW) estimated via D_2O isotope dilution regressed against TBW obtained by desiccation, for nine captive barnacle geese. Closed circles are females (♀, n = 4, y = 3.58x + 4105) and open circles are males (♂, n = 5, y = 0.58x + 9.9). As both regressions were significant, it was possible to correct TBW estimated by the D_2O dilution with TBW obtained via desiccation. Dashed line represents the line of equality.

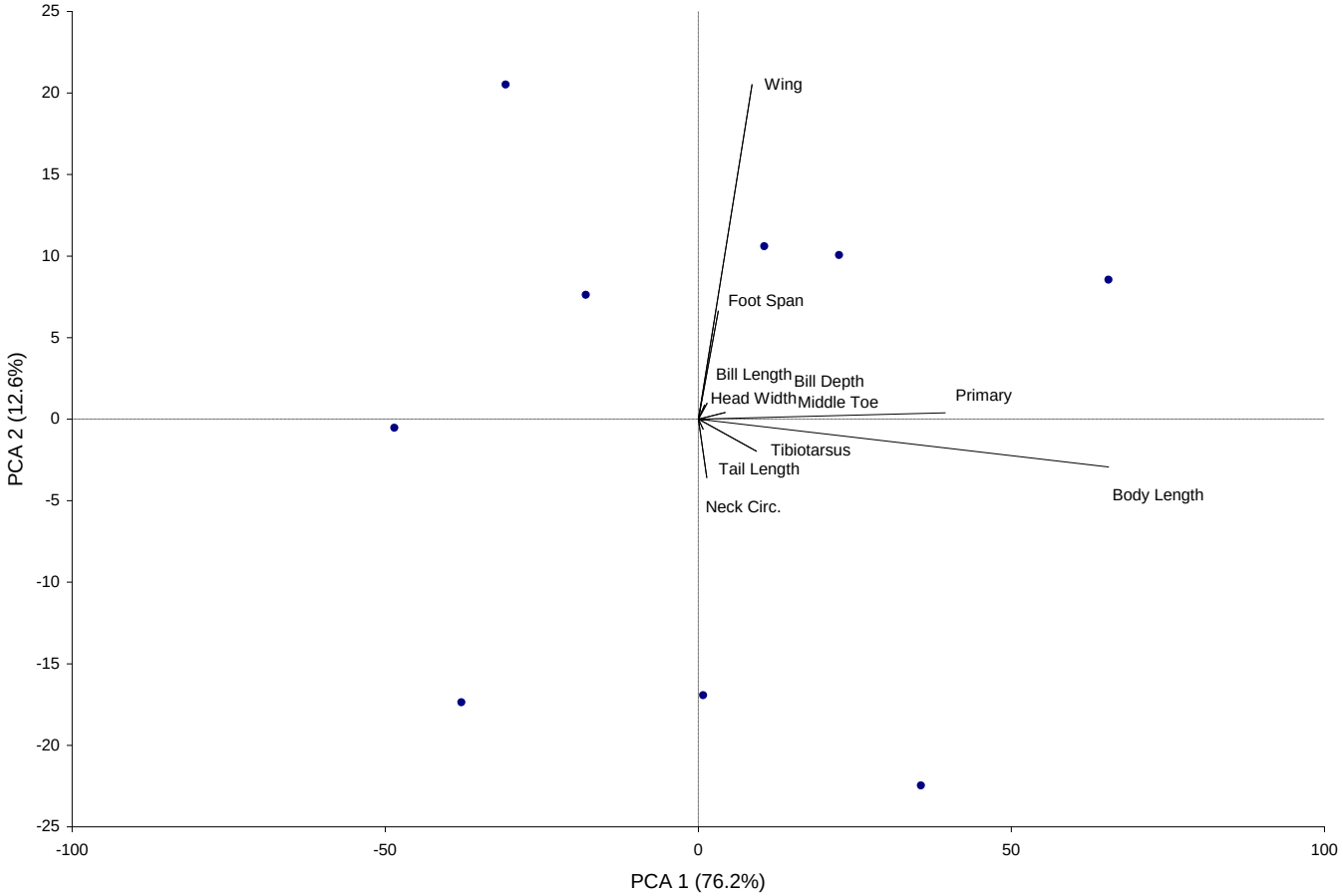
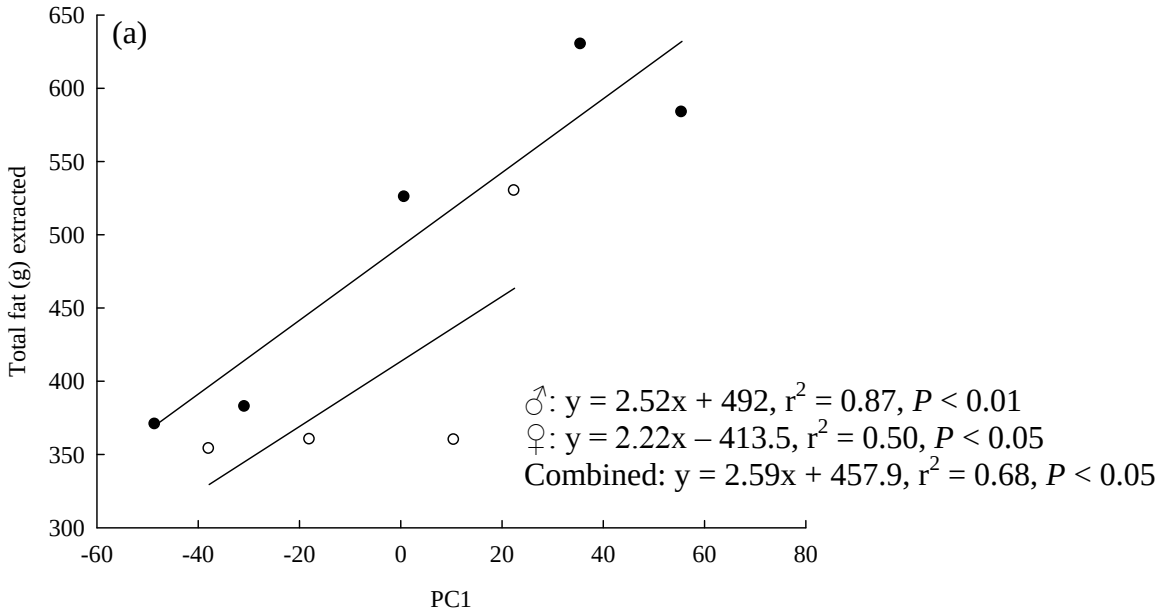
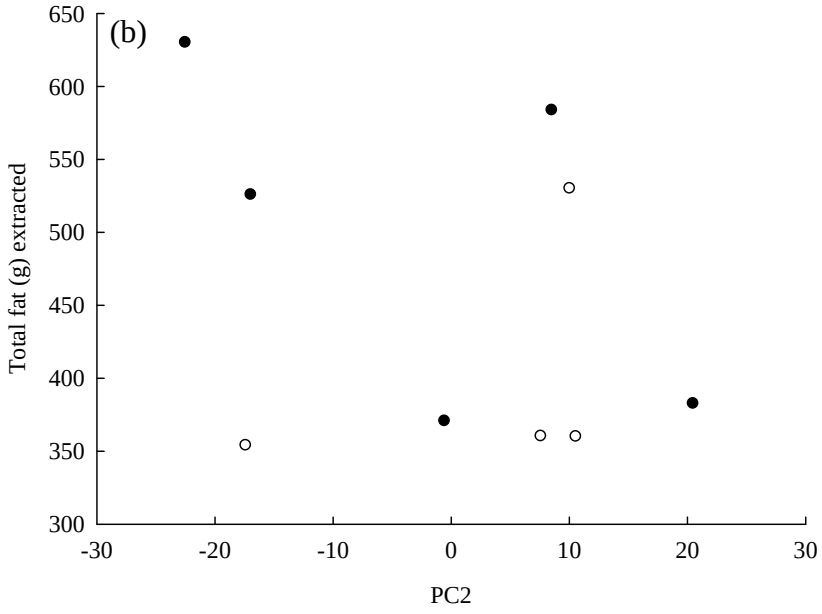


Figure IV. 5: Principal component plot based on 11 morphological measurements, taken from nine captive barnacle geese.


(a)
Total fat (g) extracted
650
600
550
500
450
400
350
300
-60
-40
-20
0
20
40
60
80
PC1
♂: y = 2.52x + 492, r2 = 0.87, P < 0.01
♀: y = 2.22x – 413.5, r2 = 0.50, P < 0.05
Combined: y = 2.59x + 457.9, r2 = 0.68, P < 0.05


(b)
Total fat (g) extracted
650
600
550
500
450
400
350
300
-30
-20
-10
0
10
20
30
PC2

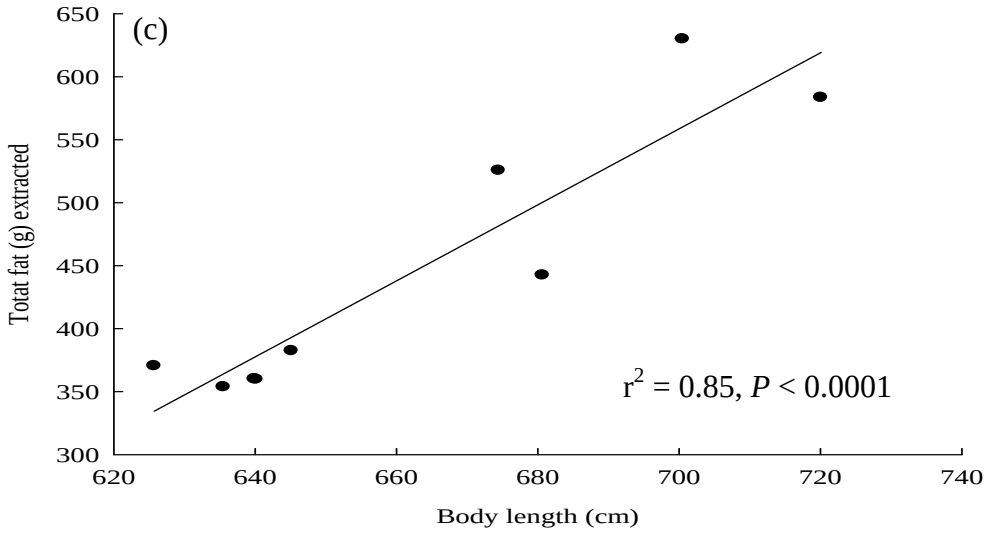


Figure IV.6: Relationship between a) PC1 b) PC2 c) body length (y = 3.02x - 1553) and total body fat, for nine captive barnacle geese. For a) and b), closed circles are females (♀, n=4) and open circles are males (♂, n=5).

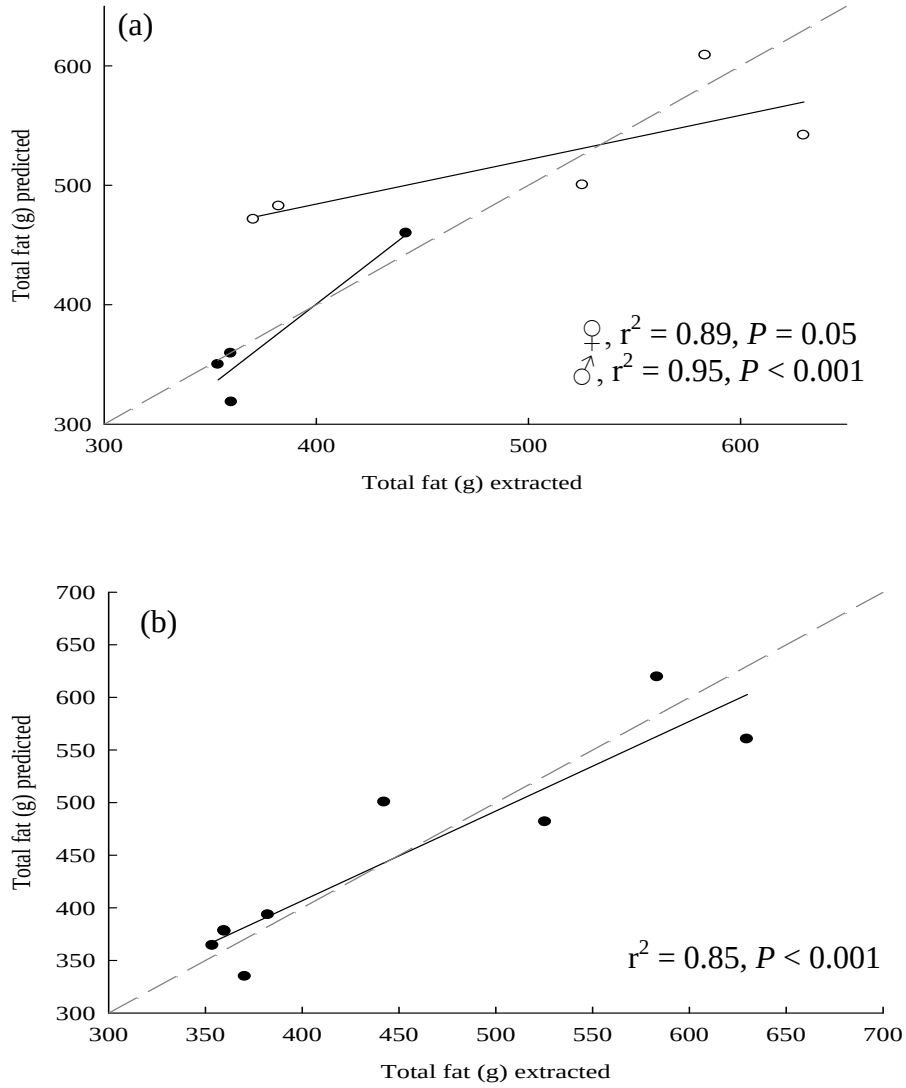


Figure IV.7: Predicted fat content regressed against actual total fat content for 9 captive barnacle geese. Predicted total fat was estimated from a) D_2O isotope dilution, and calculated using the Pace and Rathbun (1945) approach. Total body water (TBW) from the D_2O dilution was calibrated for each sex (see Fig. IV.2) against actual TBW obtained via desiccation, before total fat content was calculated. Closed circles are females (♀, n=4, y = 1.62x + 104) and open circles are males (♂, n=5, y = 0.25x + 1180), b) body length, and the relationship between body length and total fat content extracted (y = 3.017x - 1553, see Fig. IV.5c). Dashed lines represent line of equality.

Bird	Sex	Body Mass	TBW DES	TBW D_2O	Total Fat Ext.	D_2O REG[#]	D_2O P/R[#]	Body Length[#]
1	F	2190	1405	863	359.85	284.93 ± 40.29	359.22 ± 16.31	378.18 ± 18.08
2	F	2555	1463	1264	442.53	374.45 ± 36.55	459.83 ± 35.59	500.33 ± 17.02
3	F	2260	1431	1003	353.78	316.18 ± 38.55	349.80 ± 22.75	364.12 ± 19.45
4	F	2660	1562	1445	360.12	414.83 ± 31.60	318.38 ± 17.38	377.34 ± 18.15
5	M	2830	1777	977	370.53	310.22 ± 30.58	471.30 ± 3.13	334.55 ± 22.77
6	M	3555	2332	1388	583.45	402.20 ± 70.73	608.72 ± 5.07	619.28 ± 31.00
7	M	3160	1976	1157	629.89	350.44 ± 41.13	541.80 ± 3.29	560.21 ± 23.24
8	M	2935	1751	997	382.48	314.69 ± 29.90	482.37 ± 4.66	393.15 ± 16.81
9	M	2615	1463	893	525.61	291.48 ± 36.55	500.18 ± 3.04	481.61 ± 15.73
Mean ± SEM/SEE		2751 ± 143	1684 ± 103	1109 ± 71	445 ± 36	340 ± 39	457 ± 12	445 ± 20
***t*-test**						P = 0.01	P = 0.82	P = 0.99
Corr.						P = 0.491	P = 0.007*	P = <0.001*

Table IV.1: Summary of total fat content through extraction of 9 captive barnacle geese, compared to fat estimated using the isotope dilution technique and morphometric measurements. All values presented are in g. 'D_2O REG' (column 7) refers to total fat content estimated from the relationship between total body water obtained through desiccation (TBW DES) with total body fat (TBF) through extraction. 'D_2O P/R' (column 8) refers to the Pace and Rathbun (1945) approach for predicting total fat content estimated from TBW derived from isotope dilution (TBW D_2O). *P* values for '*t*-test' correspond to results from paired t-test comparisons between estimates of total fat content for each non-destructive technique, and actual total fat extracted. *P* values for 'Corr.' refer to significance values based on a correlation between total fat predicted by each technique and actual total fat extracted. Those values with an asterix (*) are significant at $P < 0.05$. Measured values are reported as ± SEM, or ± SEE (#) when regressions are used to predict TBF. PC1 and PC2 were not used to predict TBF as the regressions between TBF extracted and PC1 and PC2 were not significant.

Bird	Sex	Total Fat Ext. (g)	D_2O Regression	D_2O P/R	Body Length	PC1	PC2
1	F	359.85	-20.82	-0.18	+5.09	-8.62	+7.52
2	F	442.53	-15.38	-13.21	+13.06	-31.50	-7.67
3	F	353.78	-10.63	-1.13	+2.92	-3.44	-4.65
4	F	360.12	+15.19	-11.59	+4.78	-7.72	-6.4
5	M	370.53	-16.28	+27.2	-9.71	-17.20	+52.88
6	M	583.45	-31.07	+4.33	+6.14	-24.11	-13.76
7	M	629.89	-44.37	-13.99	-11.06	-40.87	-39.80
8	M	382.48	-17.72	+26.12	+2.79	-10.39	+59.36
9	M	525.61	-44.54	-4.84	-8.37	-19.81	-2.24
Mean			-23.6	+2.6	-0.008	-21.38	-0.65

Table IV.2: Standard algebraic % errors for predicted total fat values compared to actual fat content obtained through extraction.

Character	Mean ± SEM	Range	CV	R
Tibiotarsus	68 ± 1.4	61.1-72.1	6.1	0.95
Foot span	75 ± 1.8	65.5-81.6	7.2	0.92
Middle toe	70 ± 0.7	67.6-68.7	3.2	0.86
Head width	30 ± 0.8	26.2-33.9	8.1	0.82
Bill length	31 ± 0.4	29.6-33.3	4.0	0.81
Bill depth	17 ± 0.4	14.5-18.2	7.1	0.85
Neck circumference	36 ± 1.3	30.1-40.2	10.9	0.79
Tail length	130 ± 3.3	117.8-146.6	7.6	0.93
Wing	165 ± 4.9	145.5-181.9	8.9	0.95
Primary	398 ± 6.8	372.1-432.2	5.1	0.99
Body length	446 ± 10.7	625.7-720.1	4.8	0.62

Table IV.3: Size of various morphological characters (mm) in nine male and female captive barnacle geese, with coefficients of variation (CV) and repeatability of measurements (R).

Table IV.4: Comparisons of total body fat content for 18 species of waterfowl, taken from the literature.

Species	Body Mass (g)	Lipid Mass (g)	Fat %	Reference
Branta leucopsis	2751	455	16.5	This study
Branta canadensis	4609	875.8	19	McLandress and Ravelling (1981)
Branta bernicla	1253	78.94	6.3	Daan *et al*. (1990)
Aythya affinis	717	80.62	11	Austin and Fredrickson (1987)
Aythya collaris	670	539	7.9	Hohman (1986)
Oxyura jamaicensis	531	55.75	10.5	Euliss *et al*. (1997)
Aythya valisineria	1246	209	16.7	Hohman (1993b)
Aythya fuligula	611	91	14.9	Daan *et al*. (1990)
Aythya fulvigula	963	117.4	12.2	Moorman *et al*. (1992)
Anas clypeata	584	38.6	6.6	Euliss *et al*. (1997)
Anas acuta	891	129.6	14.5	Euliss *et al*. (1997)
Anas acuta	808	102.4	12.6	Thompson and Baldassarre (1990)
Anas rubripes	1056	100.8	9.5	Reinecke *et al*. (1982)
Anas rubripes	1204	151	12.5	Morton *et al*. (1990)
Anas discors	360	59.8	16.6	Thompson and Baldassarre (1990
Anas platyrhynchos	1099	126	11.4	Heitmeyer (1988)
Anas platyrhynchos	741	54.83	7.4	Daan *et al*. (1990)
Dendrocygna viduata	707	68.1	9.6	Petrie (2005)

List of abbreviations

TBW	Total body water
TBF	Total body fat
LBM	Lean body mass
PC1	Principal component 1
PC2	Principal component 2
D_2O	Deuterium Oxide
W	Water content of lean body mass
TBW DES	Total body water obtained through desiccation
TBW D_2O	Total body water estimated via deuterium oxide isotope dilution
D_2O P/R	Total body fat estimated using the Pace and Rathbun (1945) approach
D_2O REG	Total body fat estimated using the regression approach (the regression between total body water and total body fat extracted)

POSTSCRIPT TO CHAPTER IV

Given the unusual results that the first batch of isotope experiments produced, it was decided after some time and careful consideration to do a further batch of destructive calibrations in the geese between total body fat derived through chemical analysis, and total body fat predicted by isotope dilution. The results of this extra set of experiments were not included in the correction factor applied to total body water estimated by the isotope in Chapter V, for calculating body composition estimates. All methods used were identical as those described in Chapter IV. The results presented here include the nine birds described in Chapter IV, with the addition of four further geese.

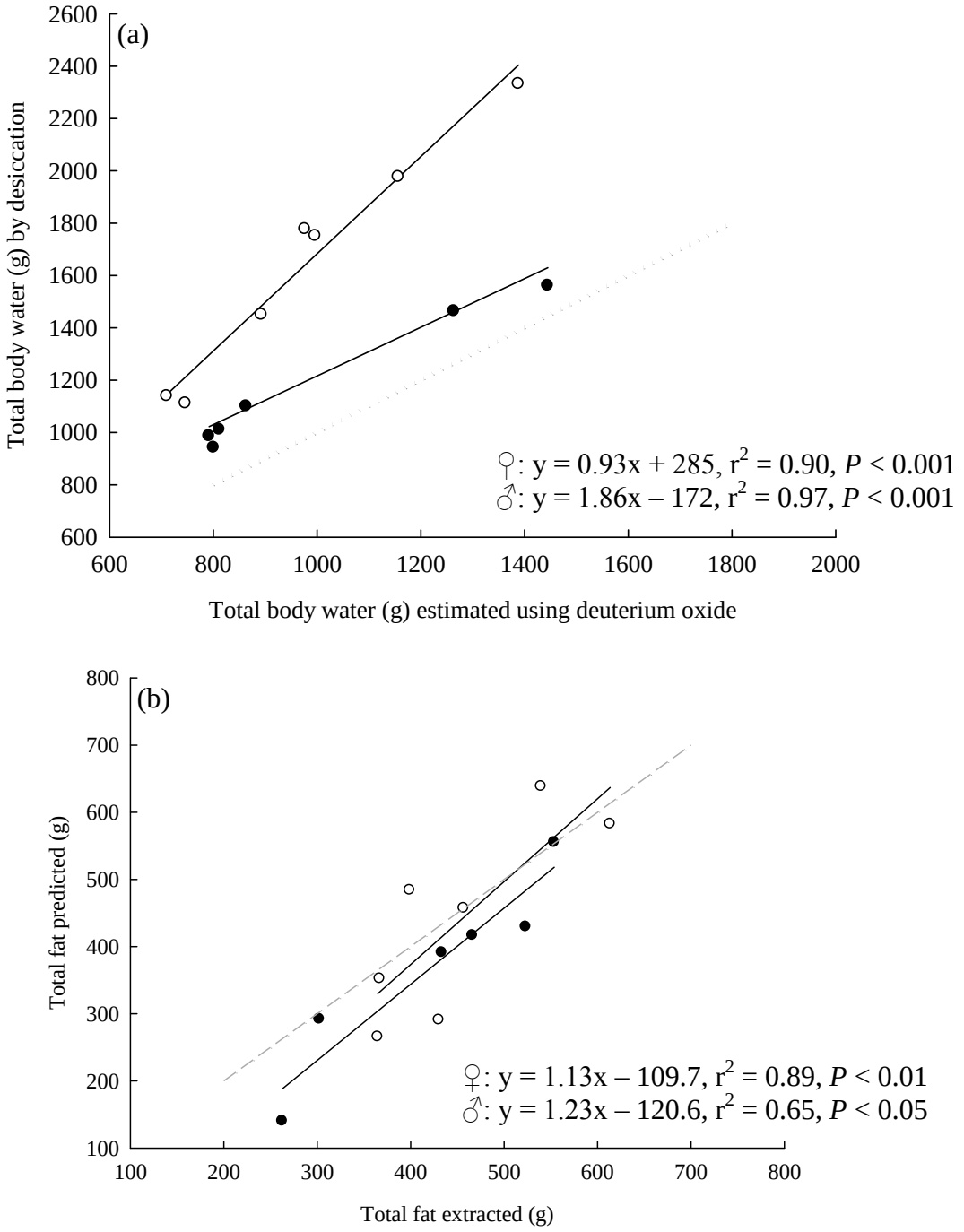
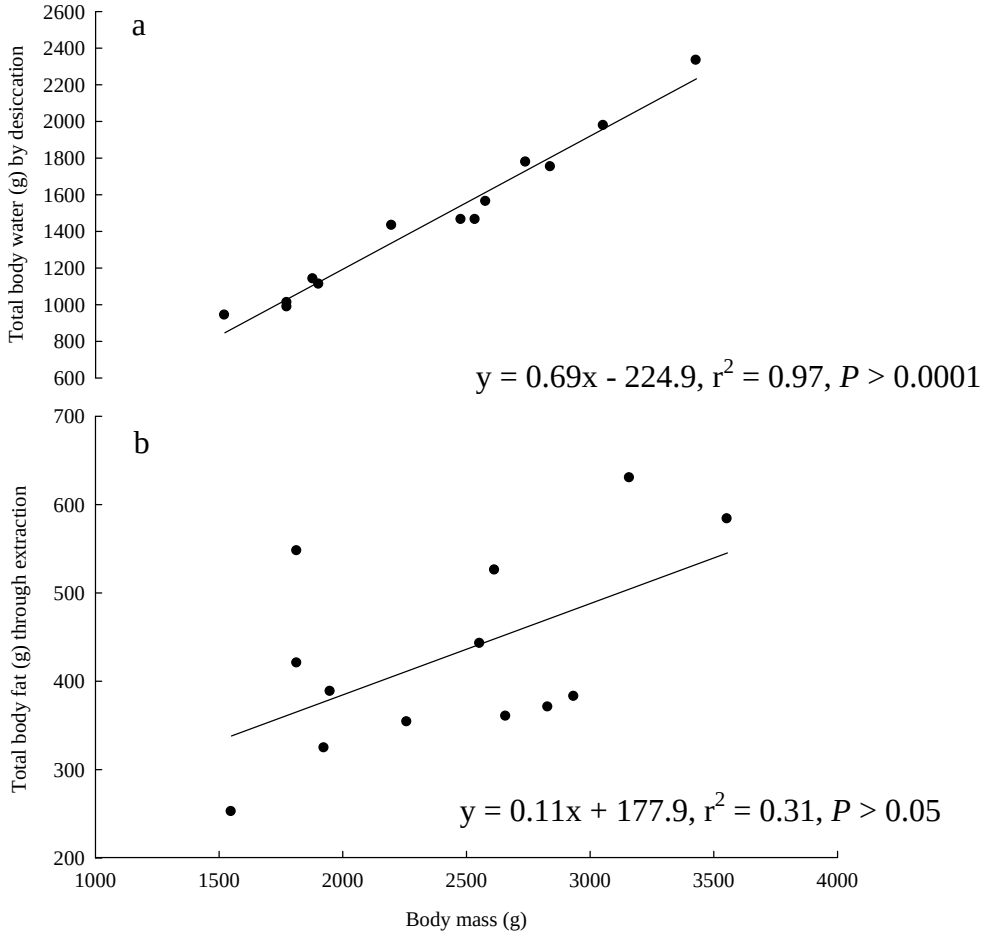


Figure IV.8: (a) total body water (TBW) estimated via D_2O isotope dilution regressed against TBW obtained by desiccation, for 13 captive barnacle geese, (b) predicted fat content regressed against actual total fat content for 13 captive barnacle geese. Predicted total fat was estimated from D_2O isotope dilution, and calculated using the Pace and

Rathbun (1945) approach. Total body water (TBW) from the D_2O dilution was calibrated for each sex against actual TBW obtained via desiccation, before total fat content was calculated. Closed circles are females (♀) and open circles are males (♂). Dashed line represents the line of equality.



Bird	Sex	Body Mass	TBW DES	TBW D_2O	Total Fat Ext.	TBF Est. D_2O P/R[#]	Error
1	F	2555	1463	1264	442.53	555 ± 19.21	+25.42
2	F	2260	1431	1003	353.78	292 ± 17.31	-17.46
3	F	2660	1562	1445	360.12	429 ± 13.32	+19.13
4	F	1550	941	801	252.13	140 ± 21.43	-44.47
5	F	1815	986	792	420.43	416 ± 19.32	-1.05
6	F	1815	1010	812	547.43	391 ± 17.32	-28.58
7	M	2830	1777	977	370.53	484 ± 24.32	+30.62

TBW D2O ♂: $y = 2.09x + 191.8$, $r^2 = 0.88$, $P > 0.01$
TBW D2O ♀: $y = 1.56x + 119.3$, $r^2 = 0.93$, $P > 0.01$
TBW DES: $y = 0.1.27x + 152.9$, $r^2 = 0.96$, $P > 0.001$

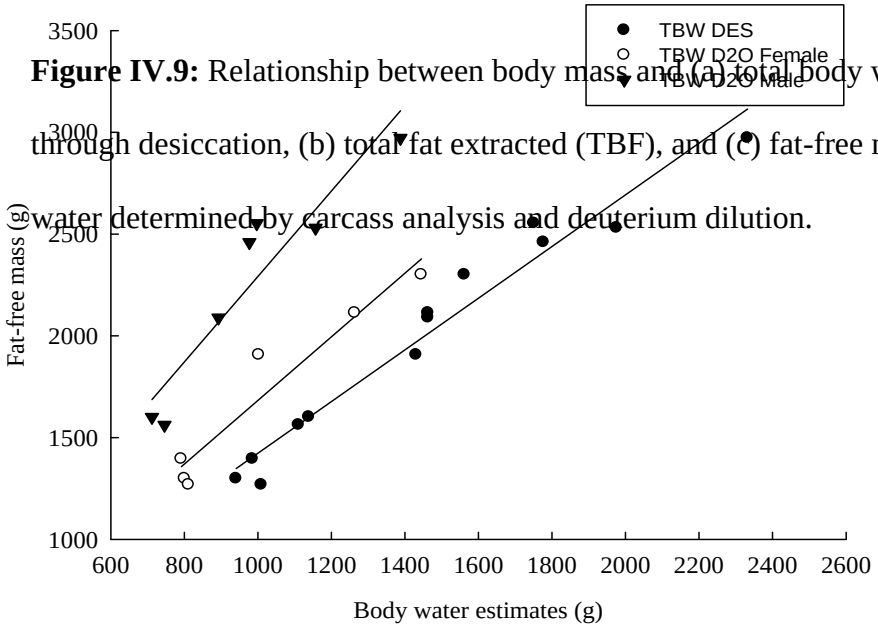


Figure IV.9: Relationship between body mass and (a) total body water (TBW) through desiccation, (b) total fat extracted (TBF), and (c) fat-free mass and body water determined by carcass analysis and deuterium dilution.

8	M	3555	2332	1388	583.45	266 ± 16.32	-54.41
9	M	3160	1976	1157	629.89	457 ± 18.43	-27.45
10	M	2935	1751	997	382.48	638 ± 14.34	+66.81
11	M	2615	1463	893	525.61	582 ± 21.43	+10.73
12	M	1950	1111	746	388.12	290 ± 16.32	-25.28
13	M	1925	1139	712	324.32	352 ± 19.43	+8.53
Mean ± SEM/SEE		2433 ± 167	1457 ± 117	999 ± 68	429 ± 31	407 ± 18	- 13.1
***t*-test**						*P = 0.51*	

Table IV.4: Summary of total fat content through extraction of 13 captive barnacle geese, compared to fat estimated using the isotope dilution technique. All values presented are in g. 'D_2O P/R' (column 7) refers to the Pace and Rathbun (1945) approach for predicting total fat content estimated from TBW derived from isotope dilution (TBW D_2O). *P* value for '*t*-test' corresponds to result from paired t-test comparisons between estimates of total fat content from isotope dilution, and actual total fat extracted. Measured values are reported as ± SEM, or ± SEE (#) when regressions are used to predict TBF. Error refers to standard algebraic % error for predicted total fat values compared to actual fat content obtained through extraction.

The results obtained from the repeat of the calibration exercise in the captive barnacle geese are consistent with the data obtained from the initial study. The deuterium oxide isotope still significantly underestimated the body water pool, and significant differences between the sexes were still apparent in the relationships between total body water estimated by the isotope, and total body water obtained through desiccation.

V. PRE-WINTER FATTENING AND FAT LOSS DURING WING MOULT: THE ANNUAL CYCLE OF FAT DEPOSITION IN CAPTIVE BARNACLE GEESE (*BRANTA LEUCOPSIS*)

Steven J. Portugal, Rona A. McGill, Paula. A. Redman, John. R. Speakman, Jonathan A. Green, and Patrick J. Butler

Many different physiological changes have been observed in wild waterfowl during the flightless stage of wing moult, including a loss of body mass. Previously we established that captive barnacle geese (*Branta leucopsis*) underwent this characteristic decrease in body mass during their wing moult, even though they had unlimited and unrestricted access to food. In the present study we aimed to determine if this body mass loss during moult comprised mainly a reduction in fat stores, and to ascertain if the captive geese undergo pre-migratory and pre-winter fattening over a similar temporal scale to their wild conspecifics. The non-destructive technique of deuterium oxide isotope dilution was employed to provide repeated measurements of estimated fat deposition from a captive flock of fourteen barnacle geese. Birds were injected with deuterium oxide at 7 distinct intervals for one annual cycle. During the flightless period of the moult, body fat decreased by approximately 40% from the pre-moult value. During late-September and early October, body fat reached its highest point in the annual cycle, both as an absolute value and as a percentage of total body mass. We propose that while the energetic cost of wing moult is not the ultimate cause of fat loss in moulting barnacle geese, the

approximate 212 g of fat catabolised during moult would provide sufficient energy to cover the cost of the replacement of the flight feathers, estimated to be 6384 kJ, over an approximate 42 day period. We conclude that the previously recorded increase in metabolism during moult in the geese, led to the use of endogenous fat reserves because the birds reduced rather than increased their food intake rates owing to the increased risk of predation when flightless. We also conclude that captive barnacle geese do undergo pre-winter and pre-migratory fattening, providing further evidence of the endogenous control of these fat deposition cycles.

SJP developed the methodology, conducted the data collection and preparation, analysed the data and wrote the manuscript. RAM assisted and supervised all isotope extractions, preparations and analysis. PAR and JRS helped develop isotope sampling protocol, procedure and analysis. PJB and JAG discussed the design of the experiment, methodology, data analysis and conclusions, and aided in the writing of the manuscript.

Introduction

Fat is the primary energy store in birds, and the benefits of fat storage have been widely addressed with respect to the quantity, composition and morphological distribution of fat stored (Blem, 1990; Witter *et al*., 1993). Benefits of fat storage include insulation, mechanical support, protection, buoyancy in aquatic birds and both sexual and social signals. Of most importance, however, is the energy it liberates when metabolised, and it is the most energy-dense substrate in the body (Blem, 1990). Birds utilise fat most during periods of energy deficit, such as during migration, reproduction and moult (Dawson *et al.,* 2000).

Geese, like the majority of avian herbivores in the Northern hemisphere, breed in the Arctic and winter in Southern, more temperate areas (Van der Graaf *et al.*, 2007; Bonier *et al.,* 2007). Being capital breeders, geese have to balance their energy expenditure and food intake throughout the winter months and early spring, to be able to migrate northwards and to breed successfully (van der Graaf *et al.,* 2007, Clausen *et al.,* 2003). In barnacle geese, *Branta leucopsis*, birds with larger fat stores have been shown to breed more successfully, suggesting fat accumulation during the non-breeding season is central to increasing fitness in this species (Cope, 2003). Once breeding has been completed, the geese must replace their feathers (i.e. moult) and accumulate sufficient fat reserves for the autumn migration, before climatic conditions on the Arctic tundra deteriorate (Bonier *et al.,* 2007). These events all require energy and thus either the utilisation of fat stores and/or an increase in food intake (hyperphagia).

Waterfowl are part of one of ten orders of birds that undergo a post-breeding simultaneous flight feather moult, rendering them flightless for a period of approximately 15-45 days (Woolfenden, 1967, Hohman *et al*., 1992). Studies on wild waterfowl have demonstrated that during this wing moult period, birds lose body mass (Geldenhuys, 1983; Sjöberg, 1986; Van der Jeugd *et al*., 2003), alter their behaviour (Kahlert *et* al., 1996; Adams *et al*., 2000) and significantly increase their rate of metabolism (e.g. Guozhen and Hongfa, 1986). Fox and Kahlert (2005) illustrated in greylag geese, *Anser anser,* for example, the majority of this mass loss during moult was a result of a depletion of fat reserves, which they proposed the geese used to meet the shortfall in normal daily energetic requirements (i.e. these stores were expended to supplement exogenous sources of energy in the diet).

Recent work on captive barnacle geese has shown that, despite having constant access to food and exposure to no predators, they respond in a similar way both physiologically and behaviourally to wing moult as their wild conspecifics (Portugal *et al*., 2007). The geese lose approximately 25% of their body mass during the flightless phase, and their rate of resting metabolism increases by 80% compared to non-moult values. Behaviourally, the birds responded to wing moult by significantly increasing their time dedicated to resting, and decreasing the time spent involved in locomotion and foraging (Portugal *et al*., 2007). Looking at the annual cycle of body mass as a whole, of particular note was the increase in body mass in the captive geese during September and early October, coinciding with the pre-migratory and pre-winter fattening period in wild barnacle geese (Portugal *et al*, 2007; Owen *et al*., 1992). Biebach (1996) noted that so far

it has been impossible to get captive animals to fatten before the onset of winter, indicating that under the conditions of captivity, factors are missing that, under natural conditions, induce the accumulation of body fat. So far, only year-round body mass has been measured in captive barnacle geese, therefore, it is not certain to what extent periods of mass loss and mass gain are the result of changes in body fat content.

The isotope dilution method is one of the most frequently used non-destructive techniques for determining body composition (Speakman *et al*, 2001). Water is not evenly distributed in body tissues, and proteinaceous tissue contains substantially more water than fat (Speakman *et al*., 2001). Isotope dilution predicts the total body water content by dilution of a stable isotope, and then estimates the total fat content based on the inverse relationship between body fat and body water (Robbins, 1993; Speakman *et* al., 2001; Mata *et al*., 2005). Previously (Chapter IV), we have demonstrated that, with the appropriate calibration and corrections applied, deuterium oxide isotope dilution can give sufficiently accurate predictions of body fat in captive barnacle geese to study the temporal changes in the 'population mean' of a group of birds.

The primary aim of this study was to document year-round total body fat in captive barnacle geese, to establish whether captive birds would follow a similar temporal pattern of fat deposition to that of their wild conspecifics. We also aimed to determine if (a) the geese would deposit fat prior to wing moult, to enable a reduction in foraging and locomotor activities during the vulnerable flightless phase by relying on endogenous fat stores (b) the body mass loss observed in moulting captive geese is primarily a result of a

depletion in fat (c) captive geese would deposit fat during September and early October to coincide with pre-migratory and pre-winter fattening in their wild conspecifics.

Materials and Methods

Birds and sampling protocol

***A captive population of 14 barnacle geese obtained as 3-week old goslings was maintained under natural light in large outdoor aviaries at the University of Birmingham. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK) which has held a self-sustaining captive population of this species since 1982. 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 libitum.**

To provide estimates of body fat content from periods of relatively stable body mass and periods of body mass change, sampling periods were selected based on year round body mass data collected from the same captive flock of barnacle geese the previous year (Portugal *et al.*, 2007). Birds were sampled in February (n = 7), March (n = 14), June (n = 11), July (n = 5), August (n = 5), October (n = 7) and December (n = 5), of 2005. Birds

were sampled at approximately the same time of day (09:00 – 12:00 GMT) to control for any natural daily rhythms and minor changes in body mass and fat content (Speakman *et al*., 2001). Sample numbers vary because of samples drying out while in capillaries and storage.

Isotope dosage and administration

For each goose, food was withheld for 7 hours prior to deuterium oxide (D_2O, Sigma-Aldrich, 99.98%) administration and the sampling of blood for D_2O analysis.

D_2O dosage was calculated using equation 12.1 from Speakman (1997);

$$\text{dosage} = \{0.65(\text{body mass (g)} \times \text{DIE}\}/\text{IE} \qquad (1)$$

where the constant, 0.65, is the approximate proportion of the body comprised of water, DIE is the desirable initial enrichment (ppm) and IE is the injectate enrichment (ppm). Birds were weighed to the nearest 5 g. Blood samples were taken from either the brachial or intertarsal vein for determination of background enrichment of D_2O, immediately prior to D_2O administration. A disposable 1 ml syringe (Terumo) fitted with a 24-gauge stainless steel hypodermic needle was used for administration of the D_2O, via an intra-peritoneal injection into the lower abdomen. D_2O was injected slowly and the needle left in for five seconds, to avoid any injectate seeping out (Speakman *et al*., 2001). The actual amount of D_2O injected was accurately determined by weighing the syringe to the nearest 0.0001 g before and after injection. Subsequent blood samples were collected 90 min

after administration of the D_2O, based on pilot data showing that 90 min was an appropriate length of time for the isotope to equilibrate (Chapter IV). Blood samples were drawn up in 50 μL non-heperinised glass capillaries (Vitrex, Cambridge, UK) and immediately shaken to the centre of the capillary, which was flame sealed with a butane gas torch (Radio Spares, Corby, UK) and then wax sealed. Capillaries were then stored in an air-tight container.

Calculation of ^2H dilution space

Isotope dilution space was calculated by the plateau method (Halliday and Miller, 1977), using equation 4 of Speakman *et al*. (2001);

$$N_{mol} = \frac{M_{mol}(E_{in} - E_p)}{(E_p - E_b)} \qquad (2)$$

where N_{mol} is the molar quantity of water present in the body, E_{in} is the enrichment of the material introduced into the animal, M_{mol} the molecular weight of the D_2O, E_b is the background enrichment of this material in the animal, and E_p the enrichment measured after the 'dispersal' process is completed.

Assuming the constancy of the water content of the lean body mass (LBM; Pace and Rathbun, 1945), estimates of body composition could be obtained as follows:

$$\%LBM = \frac{\%TBW}{w}, \quad (3)$$

$$\% \text{ TBF} = 100 - \% \text{ LBM}, \quad (4)$$

where total body fat (TBF), total body water (TBW) and LBM were expressed as a percentage of fresh body mass. Here, *w* is the mean fractional water content of LBM for nine captive geese that were sampled destructively (0.731 ± 0.013, Chapter IV).

TBW determined via D_2O dilution (hereafter referred to as TBW D_2O) was corrected (e.g. Mata *et al.*, 2005) using the relationship between TBW D_2O and TBW obtained through desiccation (hereafter referred to as TBW DES), before TBF was determined. This correction factor was achieved through calibrating the two (Chapter IV, Fig. IV.4). The relationship between TBW DES and TBW D_2O was significantly different between the two sexes, and thus TBW D_2O for each sex was corrected as follows:

F: TBW Corrected (g) = 0.25 x TBW D_2O + 1180

M: TBW Corrected (g) = 1.62 x TBW D_2O + 104

Isotope analysis

D_2O enrichment was measured using a chromium reduction furnace interfaced with a dual-inlet isotope-ratio mass spectrometer (Donnelly *et al.*, 2001), at the Scottish Universities Environmental Research Centre (SUERC), East Kilbride. Water was

extracted from the blood samples by vacuum lyophilisation. Laboratory water standards were also prepared in the same way to correct for day-to-day variations in the performance of the vacuum line. Samples and standards were collected in 50 μL non-heperinised glass capillaries, shaken to the centre of the capillary, and flame sealed with an acetylene torch. Capillaries were broken and the extracted water was then injected in to the mass spectrometer (VG Optima) (after Donnelly *et al*., 2001). Each batch of samples was analysed with triplicates of three laboratory standards, waters of known isotope composition (calibrated relative to SLAP, SMOW and GISP international water standards). These lab standards perform a dual purpose to both correct the isotope ratio of the sample relative to the international standards and to correct for daily mass spectrometer drift, and day-to-day variations in the performance of the mass spectrometer. Isotopically characterised H_2 gas was used in the reference channel. All isotope enrichments were measured in delta (per mil) relative to the reference gas and those values are in turn calibrated by the laboratory standards, and converted to ppm using established ratios for the reference materials. Measures of isotope enrichment were based on independent analysis of at least two sub-samples of the water extracted from the blood samples.

Statistical analysis

Since TBW D_2O was corrected using the calibration relationship between TBW D_2O and TBW DES, estimates of meant TBW and hence TBF are presented ± the standard error of the estimate (SEE, Zar, 1984). Estimates of fat content were compared using Woolf's test for differences with Bonferonni corrected proximate normal test (*Z*-test) post-hoc comparisons (e.g. Green *et al*., 2002).

Results

Body mass changed significantly throughout the annual cycle (Fig. V.1a, ANOVA, $P < 0.001$, see also Chapter II). Estimated total body fat content of the captive barnacle geese changed significantly between the seven sampling sessions (Fig. V.1b, Woolf's test for equality, $\chi^2 = 68.8$, $P < 0.0001$). Further *Z*-tests, with Bonferonni corrections, between the seven sampling sessions showed that total body fat content during wing moult in August (129.9 ± 22.4 g) was significantly lower than each of the other six sampling sessions. Between early July and mid-August, the geese lost approximately 212 g, or 40%, of body fat over a 5 week period, an average daily fat loss of 6 g. Fat content was at its highest in the autumn, where estimated fat content peaked at 352.1 ± 20.9 g. Consequently, over the 42 days between the August and October sampling sessions, the geese gained, on average, approximately 5.2 g per day. Body fat decreased steadily during the winter months, with total body fat values of 234.1 ± 23.4 g and 189.5 ±24.9 g being recorded for December and February respectively (Fig. V.1b). Fat content in February represented a 53% decrease on body fat at the start of the non-breeding season in October, and fat content was significantly lower in February than both July and October. Between February and March (280.9 ± 17.1 g), body fat increased by approximately 91 g, before reaching a peak just prior to wing moult in July (342.5 ± 36.6 g). Fat as a percentage of body mass followed a similar pattern to that of actual fat mass (Fig. V.1c), with body fat percentage ranging from 20.2% in October to just 8.3% in August.

Discussion

Fat loss during wing moult

Despite constant access to food, the captive barnacle geese lost approximately 212 g of body fat during the wing moult period, equivalent to 40% of their fat reserves in comparison to pre-moulting levels in July. By the end of wing moult in mid-August, body fat only constituted, on average, 8% of the bird's total body mass, compared to 16% and 22% pre-and post- wing moult. Fox and Kahlert (2005) recorded a loss of 268 g of fat on average in wild moulting greylag geese, however, no changes in fat content during moult have been identified in mallards *Anas platyrhynchos*, great-crested grebes *Podiceps crisatus*, common scoters *Melanitta nigra* or lesser scaups *Aythya affinis* (Young and Boag, 1982; Piersma, 1988; Fox *et al*., 2008; Austin and Fredrickson, 1987).

Depletion of fat stores during moult in wild birds, like body mass loss, has been interpreted to be a result of nutritional stress caused by the regrowth of the feathers (e.g. Hanson, 1962), a response to the increased risk of predation brought about by being flightless (e.g. Panek and Majewski, 1990) and an adaptation to regain flight quicker on partially regrown flight feathers (e.g. Owen and Ogilvie, 1979). Previously, we concluded that in barnacle geese at least, mass loss (and thus fat loss in this instance) was a consequence of the 80% increase in metabolism during moult, coupled with the reduction in time spent foraging, causing a decrease in body mass (Portugal *et al*., 2007). Therefore, the proximate cause of fat loss is likely to be the interaction of the decrease in food intake

and the increase in metabolic rate as a result of feather synthesis. However, the ultimate cause of fat loss in wild birds is the increased risk of predation brought about by the bird's flightless state, restricting the ability to forage, meaning the geese are not able to increase their food intake to counteract the observed increase in metabolism. As a result, they must rely on their endogenous fat reserves during the flightless phase of moult.

Further evidence that this change in behaviour and subsequent loss of body fat is a product of flightlessness and the increased risk of predation, stems from the increase in body mass and body fat in July just prior to wing moult. At this stage, the captive geese were undergoing their annual body moult, which stretched over a period of approximately 90 days between July and September. Despite regrowing heavier body feathers at this time (e.g. Panek and Majewski, 1990), the geese were gaining body fat, suggesting the cost of regrowing feather alone is not sufficient to result in a loss of body fat.

Although most of species of duck studied do not lose significant amounts of fat during wing moult, certain species of geese have been shown to also (Hohman *et al.* 1992). Therefore, it seems likely that there is a link between foraging style (i.e. how and where food is obtained), food type and fat loss during moult, and choice of moult site. Many species of duck can continue foraging throughout the duration of moult, as their food source is obtained either from the surface, or under the water, where the birds are relatively safe. Moulting barnacle geese in the Arctic rely on vegetation that is not found in water, and can often be some distance from a water body (Owen and Ogilvie, 1979). Therefore, for the geese, resting on or beside water for safety is frequently mutually

exclusive with productive foraging. The fat, and hence and body mass, increases just prior to moult are likely to serve a dual function of enabling the geese to reduce foraging when flightless and rely on endogenous reserves, while increasing the rate of feather synthesis during moult as more reserves are available for this, thus potentially shortening the flightless period – this has been suggested as the possible cause of mass increase prior to wing moult in some duck species (e.g. Van de Weetering and Cooke, 1999).

Potentially, geese may be able to build large fat stores prior to moult because of their larger size. For example, in coots and grebes, structurally larger individuals can survive longer without food, while being able to deposit larger fat stores (Biebach, 1996). It may be that ducks, being smaller, are unable to deposit sufficient fat stores, and therefore must continue foraging while flightless, albeit at a reduced level (e.g. Adams *et al*., 2001).

Fox and Kahlert (2005) described body mass and fat loss in moulting greylag geese as a representation of the nutritional or energetic "stress" of wing moult, and proposed it to be a further example of avian phenotypic plasticity. This trait enables the geese to meet a nutritional or energetic shortfall from endogenous reserves, in a way that may not necessarily be related to direct fitness costs. Therefore, the barnacle geese show adaptive fat gain prior to wing moult, and the accumulation of fat stores will enable the geese to exploit habitats that are predator free, but which perhaps do not provide the required levels of exogenous nutrient or energy to sustain them through moult.

Does fat catabolism cover the cost of moult?

The captive geese lost approximately 200 g of fat during late July and August. Although it is unlikely that the energetic cost of wing moult is the ultimate cause of body mass loss in moulting waterfowl, the increase in metabolism coupled with a decrease in foraging work together to cause a loss in body mass, and in this instance, a depletion of fat stores. Using simple calculations it is possible to calculate what the cost of wing moult in the geese would be, and to see if the fat catabolised would provide sufficient energy to 'cover the cost' of the regrowth of the flight feathers.

Plumage mass of birds can be roughly approximated by the equation, plumage mass = $0.09W^{0.95}$ (Turcek, 1966; Lindström *et al.*, 1993; Murphy, 1996), and mass of plumage is typically around 6% of the total body mass (Turcek, 1966). As demonstrated in Portugal *et al*. (2007) captive barnacle geese have a large annual variation in body mass, therefore, the average mass for all geese for the month of April 2005 (when body mass is stable, average of 1.8 kg) was used to determine approximate average plumage mass, and the value calculated was 112.37 g (6.1% of total body mass). With the associated mass of the feather sheath included (Murphy, 1996), the total plumage mass for a captive barnacle goose is 134.4 g (for comparison, Lindström *et al*., 1993, cited a plumage mass of 160.6 g for the larger greylag goose). Flight feathers typically account for about one-quarter to one-third of the total plumage mass (e.g. Newton, 1966). The midpoint of these two estimates would provide a value of 36.1g for flight feathers alone for the captive barnacle geese, or 43.2 g including sheaths.

Using equation 3 of Lindström *et al.* (1993) it is possible to calculate the rate of feather production (C_f) if mass specific metabolic rate (kJ g^{-1} d^{-1}) of the bird is known. Coupled with plumage mass, it is then possible to calculate the energetic cost of replacing all feathers, and thus the cost of moult. For the captive geese in the present study, rate of feather production was calculated to be 147.8 kJ (g dry feather) $^{-1}$. C_f typically decreases with increasing body size, for example values reported in Lindström *et al.* (1993) demonstrated 26.6 kJ (g dry feather)$^{-1}$ for ostriches, *Struthio camelus,* and 780 kJ (g dry feather)$^{-1}$ for the goldcrest, *Regulus regulus*.

With the mass of flight feathers and the rate of C_f known, the overall cost of producing the flight feathers can be estimated to be 6385 kJ, or 152 kJ d^{-1} for the approximate 42 day duration of wing moult (assuming feather growth is constant throughout a 24-hour period). If the energy density of fat is 39 kJ g^{-1}, 163 g of fat on average would be required to cover the 'cost' of re-growing the flight feathers. It is likely, therefore, that most of the 212 g of fat catabolised during the wing moult period was used to grow the new flight feathers. As foraging was greatly reduced (Portugal *et al*., 2007) during wing moult, it is likely the remaining 35 g or so of fat will contribute towards the shortfall in food intake.

Pre-winter fattening

Body fat made up the highest percentage of total body mass (21%) in late September – early October, before decreasing in December and then reaching the lowest point of the year in February (Fig. V.1c). Biebach (1996) commented that it had not been possible to

get captive animals to fatten prior to winter, however, the high percentage of body fat in autumn in the captive geese suggests that this was the case in the present study. If body fat percentage following wing moult returned to a similar level to that of late spring and early summer (e.g. 15%) it could be considered that body fat was returning to 'normal' fat levels after fat loss during wing moult. However, as post moult fat was higher both in percentage and absolute terms, it suggests that there is an element of pre-winter fattening taking place. This provides further evidence that captive geese undergo the same physiological changes as their wild conspecifics.

In the Svalbard population of wild barnacle geese, overall fat stores increased and then varied throughout the non-breeding season, with the rate of increase in fat stores being most rapid at the start and end of the non-breeding period (Cope, 2003). Owen *et al.* (1992) found that fat accumulated in wild barnacle geese during the autumn, followed by a significant decline throughout winter and an increase late spring. Phillips *et al*. (2003) noted that the geese depleted the most profitable feeding areas by around early December, after which they switched to less profitable feeding areas, and subsequently lost body fat. During the non-breeding season, peaks in abdominal profile indexes (Owen, 1981) occurred in October and November, with the lowest point coming in February.

The captive geese followed a similar pattern to the wild geese. This pattern suggests that fat levels and control of fat follows an endogenous cycle, being only slightly modified by environmental constraints. Owen *et al*. (1992) suggested that birds might lay down reserves in preparation for lean periods, and then only maintain sufficient reserves to

guarantee against predictable adversity. It is possible therefore, that once the geese have arrived in Scotland and recovered their body fat reserves from their autumn migration, a loss of unnecessary reserves may be advantageous to reduce the risk of predation, as heavier birds are probably less agile.

The one highly unpredictable event for the geese will be the conditions on arrival on their wintering grounds in Scotland following migration. During spring migration the geese take roughly 4-5 weeks to reach their breeding grounds, essentially following the new growth of vegetation northwards (Cope, 2003). Fattening for the spring migration is also a two step process, first on the Solway and then on Helgeland, a major staging area in Norway (Black *et al*., 1991). Here, fat reserves expended during the first part of the migration are at least partially replenished. In autumn however, geese migrate from Svalbard to Scotland in far shorter time, with some birds capable of achieving this is less than one week (Butler *et al*., 1998). Unlike the situation in spring, geese migrating in autumn do not know what conditions will be like on arrival at their destination. Therefore, large fat reserves are essential not only for migration but as an insurance for arrival at the wintering grounds, should conditions be adverse. Butler and Woakes (2002) demonstrated that wild barnacle geese undergo a period of seasonal hypothermia in autumn thought to aid in fat deposition, not only prior to migration but for recovery of fat reserves once the wintering grounds are reached, again stressing the importance of pre-migratory and pre-winter fattening in this species.

In the present study we have demonstrated that captive barnacle geese do deposit fat reserves prior to wing moult, and that mass loss during the flightless phase of wing feather replacement is primarily a depletion of fat. In October, fat reserves in the geese increased significantly, suggesting that the birds were depositing fat, before the onset of winter.

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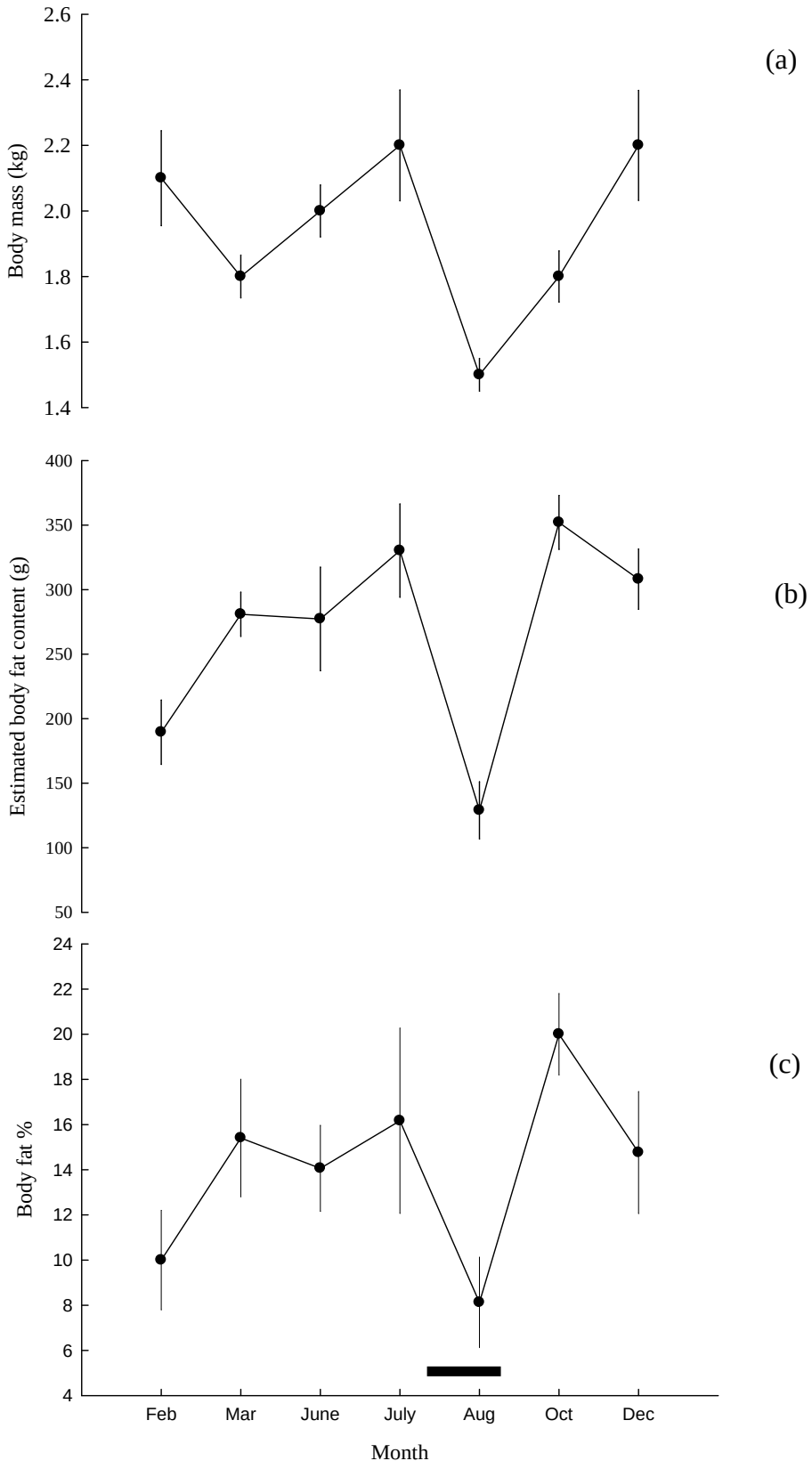
(a)
Body mass (kg)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
(b)
Estimated body fat content (g)
400
350
300
250
200
150
100
50
(c)
Body fat %
24
22
20
18
16
14
12
10
8
6
4
Feb
Mar
June
July
Aug
Oct
Dec
Month

Figure V.1: (a) Year round body mass (± SEM), (b) fat content (± SEE) and (c) fat content as a % (± confidence intervals) of total body mass, from captive barnacle geese. Fat content changed significantly throughout the annual cycle (Woolf's test for equality, $\chi^2 = 68.8$, $P < 0.0001$). Black bar represents approximate wing moult period.

VI. CHANGING BODIES, CHANGING RELATIONSHIPS? HEART RATE AS AN INDICATOR OF OXYGEN CONSUMPTION THROUGHOUT THE ANNUAL CYCLE OF BARNACLE GEESE *BRANTA LEUCOPSIS*

Steven J. Portugal, Jonathan A. Green, Peter B. Frappell and Patrick J. Butler

The heart rate (f_H) method for investigating energy expenditure in free-living organisms relies on there being a significant relationship between heart rate and rate of oxygen consumption ($\dot{V}_{O_2}$). It has been proposed that this relationship may potentially alter throughout the annual cycle of an organism, as other physiological traits change. Captive barnacle geese, *Branta leucopsis*, provide an idea model to study the $f_H/\dot{V}_{O_2}$ relationship throughout the annual cycle, as they exhibit significant changes in body mass, body composition and body temperature. Twelve captive barnacle geese were implanted with heart rate data loggers and at six points in the year, the relationship between f_H and $\dot{V}_{O_2}$ was determined using open-flow respirometry. The $f_H/\dot{V}_{O_2}$ relationship was also determined in seven wild moulting barnacle geese from the breeding population in Svalbard. General linear models of the $f_H/\dot{V}_{O_2}$ calibration lines from the captive geese showed only the session of late September – early October to be significantly different from the remaining five calibration lines. The relationship between f_H and $\dot{V}_{O_2}$ obtained from wild geese was significantly different to all of the relationships derived from the

captive geese, making it not possible to apply calibrations from captive birds to wild geese. However, the lack of significant difference in the relationship derived during moult in the captive geese to the rest of the annual cycle (bar late September – early October) means it is possible to make an assumption that the relationship between f_H / $\dot{V}_{O_2}$ during moult in the wild geese is indicative of the relationship throughout the annual cycle, and can therefore be applied to year round heart rate data to calculate estimates of $\dot{V}_{O_2}$.

SJP conducted the data collection and preparation, analysed the data and wrote the manuscript. PJB and JAG conceived the outline of the work, discussed methodology, data analysis and conclusions, and aided in the writing of the manuscript. PBF aided with data collection and analysis from the wild birds.

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Introduction

Ecophysiologists and functional ecologists seek to further our understanding of the manner in which organisms operate in their natural environment. The utilisation of energy is one of the key factors that will determine an organism's ability to survive and reproduce (Butler *et al*., 2004). Therefore, information about the energy expenditure of free-ranging animals holds the key to understanding major aspects of their behaviour, in their natural environment (Froget *et al*., 2001; Tolkamp *et al*., 2002).

The heart rate method (Butler, 1991; Butler, 1993), which uses fitted devices to transmit or record heart rate (e.g. Woakes *et al*., 1995), has proved to be an increasingly popular and powerful tool when investigating the energy expenditure of free-ranging animals (e.g. Bevan *et* al., 1995; Green *et* al., 2001; Butler *et al*., 2002; Froget *et al*., 2002). This method is based on the relationship between rate of oxygen consumption ($\dot{V}_{O_2}$) and heart rate (f_H), as described by Fick's convection equation for the cardiovascular system:

$$\dot{V}_{O_2} = f_H \times \dot{V}_S (C_a O_2 - C_{\bar{v}} O_2), \qquad (1)$$

where $\dot{V}_s$ is cardiac stroke volume, $C_a O_2$ is the oxygen content of arterial blood and $C_{\bar{v}} O_2$ is the oxygen content of mixed venous blood. If, $\dot{V}_s (C_a O_2 - C_{\bar{v}} O_2)$, the oxygen pulse ($sO_2$ pulse), remains constant or varies systematically, there will be a relationship between

$\dot{V}_{O_2}$ and f_H, hence making it possible to calculate the former from the latter (Butler, 1993).

The heart rate method for estimating field metabolic rate (FMR) has become better developed and more widely used in recent years, as it is facilitated by advances in technology for measuring and recording heart rate in the field through the use of miniaturized heart rate data loggers (e.g. Green *et al*., 2005). This technique has been used to monitor continuously the rate of energy expenditure in several species, including macaroni, *Eudyptes chrysolophus*, king, *Aptenodytes patagonicus,* and gentoo, *Pygoscelis papua*, penguins, and black-browed albatross, *Diomedea melanophyrys* (Green *et al*., 2005; Froget *et al*., 2002; Bevan *et al*., 2002; Bevan *et al*., 1995).

The heart rate method relies on accurate calibrations between $\dot{V}_{O_2}$ and f_H and a constant stroke volume of the heart. The ideal approach would be to calibrate this relationship in each animal that is to be used in the field, however, the disturbance associated with calibration, the difficultly of obtaining animals, and potential interference with selected individuals usually makes this impracticable (Green *et al*., 2001; Green and Frappell, 2007). Generally, therefore, this relationship is established in the laboratory and then applied to free-ranging animals, taking into account the associated inter and intra-individual variability (e.g. Green *et* al., 2001; Froget *et al*., 2001).

The usefulness of the heart rate method depends on this established relationship with $\dot{V}_{O_2}$ being similar under different conditions, or at least, for any differences to be quantified

and taken into account (Butler *et al*., 2002). Froget *et al.* (2001) found that the relationship between $\dot{V}_{O_2}$ and f_H in king penguins was influenced by variation in body condition and season. Moreover, Holter (1976) also noted this relationship was influenced by season in white-tailed deer, *Odocoileus virginisanus*. It appears, therefore, that determination of the $f_H/\dot{V}_{O_2}$ relationship (i.e. the changes in stroke volume) when animals are in different physiological states is essential and may explain some of the temporal differences observed in several studies.

The $f_H/\dot{V}_{O_2}$ relationship has been previously determined in captive barnacle geese *Branta leucopsis* (Nolet *et al*., 1992; Ward *et al*., 2002, see also Butler and Woakes, 1980). For this species, there was no difference in calibrations between $f_H/\dot{V}_{O_2}$ carried out on different individuals, 10 years apart (Nolet *et al*., 1992; Ward *et al*., 2002). However, the experiments in each study were conducted at similar points in the annual cycle. Recent work on captive barnacle geese has demonstrated that they undergo similar changes in body mass, body composition and body temperature to that which wild geese undergo (Portugal *et al*., 2007, Chapter VII). For example, despite *ad libitum* access to food, the captive geese lost approximately 25% of their body mass during their annual wing moult, while simultaneously experiencing an increase in resting metabolism of approximately 80% (Portugal *et al*., 2007). These findings are comparable to that found for wild moulting waterfowl (see Hohman *et al.* 1992). Therefore, captive barnacle geese present an ideal model to investigate how various changing physiological parameters such as

body mass, body composition and body temperature interact and potentially influence the relationship between f_H and $\dot{V}_{O_2}$.

The primary aims of this work, therefore, were to: (1) calibrate the relationship between f_H and $\dot{V}_{O_2}$ at set-points of interest during the annual cycle of the captive barnacle geese (2) construct general linear models that best explain any variance seen between sampling sessions in the $f_H/\dot{V}_{O_2}$ relationship, through the inclusion of body mass, body composition and body temperature (3) for the first time, calibrate the relationship between f_H and $\dot{V}_{O_2}$ in wild barnacle geese at two points in the annual cycle, to compare the calibration relationships with that of the captive geese.

Materials and Methods

Captive birds

A captive population of 44 barnacle geese obtained as 3-week old goslings was maintained under natural light in a large outdoor aviary at The University of Birmingham. The goslings were obtained from Bentley Waterfowl Park (Sussex, UK) which has held a self-sustaining population of this species since 1982. 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). Food, and water, was available *ad libitum*.

Fourteen birds out of the flock of 44 were trained for 2-3 weeks prior to the experiments to build up physical condition and to get fully accustomed to the experimental surroundings and the handler. Treadmill training consisted of walking the birds at previously determined medium speeds for 2-3 h a week. Sampling periods were selected based on year round body mass data, collected from the same captive flock of barnacle geese the previous year (Portugal *et al*., 2007). Sampling sessions were conducted in February, May, late July – early August, mid August – early September, late September – early October and November (see Table VI.1 for sample sizes).

Respirometry apparatus

Two respirometry systems were used simultaneously to record $\dot{V}_{O_2}$, in order to minimise the duration of the sampling sessions. The respirometer chambers comprised a Perspex box (74 cm high × 58 cm long × 47 cm wide) that rested on a wooden frame on a variable speed treadmill (Powerjog, Sports Engineering Ltd). The air in the chamber was mixed fully by two 12 x 12 cm fans in a side compartment. Brush style excluders ensured a good fit between the respirometer and the treadmill belt. Foam rubber seals ensured an air-tight junction between the respirometer and the frame. Data from training sessions confirmed that stable levels of gas exchange were achieved after 10 min of walking at each speed, and the flow rates used should replace 95% of the air in the chamber in 10 min. The system was calibrated with atmospheric air, nitrogen and a Wösthoff gas mixing

pump (type 2M301/a-F, Böchum, Germany). The extent to which each respirometry system leaked was determined by pumping oxygen-free nitrogen gas (BOC, UK) into the chamber at a known rate (Fedak *et al*., 1981). The calculated values of gas exchange were adjusted to compensate for the loss of chamber gas.

System 1

Respirometry system one was based on that of Wilson *et al.* (2006). The flow rate (55 L^{-1}) through the chamber was measured with an Aalborg 0-100 L min^{-1} mass flow controller, and water vapour was mathematically scrubbed to provide a flow rate corrected to standard temperature and standard pressure (STDP). Excurrent air temperature was measured by means of a negative temperature coefficient thermistor, calibrated with a Grant GR 150 precision water bath. An electronic hygrometer measured relative humidity of the incurrent air. A subsample of the air from the chamber was passed through a column of indicating Drierite® (Hammond Drierite Co., Xenia, OH, USA). Drierite was exposed to air for 5 min before being placed in the drying column **(**White *et al.*, 2006). The voltage outputs of the gas analysers and thermistor were recorded at a sampling frequency of 4Hz by a Powerlab ML750 A/D (ADInstruments) and Chart software (ADInstruments). The hygrometer reading was noted and recorded regularly. The O_2 and CO_2 signal was conditioned with a 2 Hz and 5 Hz low-pass filter respectively, and a 9-point Bartlett weighting signal average. Ambient O_2 and CO_2 were recorded for 5 min every 25 min period and the baseline check was automated with a

solenoid valve (SMC model EVT307, Radio Spares, Corby, UK), controlled by the ML750 and Chart software.

System 2

Respirometry system two was based on that of Green *et al.* (2001). Air was drawn through the respirometer using an air pump (B105, Charles Austin) and flow (45 L min^{-1}) was measured using an electronic flow meter (100 Flo-sen, McMillan Co.), calibrated at the start and end of a series of experiments using two 401 min^{-1} variable flowmeters (Fisher controls 1100). A subsample of the chamber air was drawn off and passed through a column of Drierite to a paramagnetic O_2 analyser (Servomex 570A). According to the manufacturer's specifications, the accuracy of the instrument was within +/- 0.02%. A solenoid valve (RS Components Ltd) switched between sampling outlet and inlet ambient air every 25 min. An electronic barometer transducer (Farnell Electronic Services) measured barometric pressure, and the humidity and temperature of both the inlet and outlet ambient air were continuously measured using the appropriate sensors (Farnell Electronic Services). The output signals from the O_2 and CO_2 analysers, humidity and temperature sensors, barometer and flowmeter were passed to a purpose-built interface box that amplified the signals to a standard range of -10 V to +10 V. The amplified output voltages were passed to an analogue-to-digital converter unit (DAQPad-1200, National Instruments) then to a desktop computer (Viglen Genie Professional). The computer sampled the outputs at 1000 Hz, took a mean of these values and saved them to

a file every 10 s with a program developed using a software package for automatic instrumentation (LabVIEW, National Instruments).

Respirometry protocol

Prior to the walking sessions, each goose was rested overnight between the hours of 23:00 and 07:00 (GMT) to measure resting $\dot{V}_{O_2}$ (see Portugal *et al*., 2007). Food (not water) was withheld 8 h prior to being placed in the respirometer for overnight experiments. Water was offered to the birds once the overnight sampling session had completed, and before walking began. Birds were always walked between 07:30 and 10:30 (GMT) for each session. Random number tables were used to determine the order of treadmill speeds so that the geese did not become accustomed to a set pattern of speeds. Each bird was exercised at up to seven different speeds (0.5-3.5 km/h). This speed range included the lowest speed available on the treadmill and the highest speed the birds could maintain for a sufficient time to allow for gas equilibration (minimum 10 min, maximum 20 min). Rate of oxygen consumption was calculated as a 5 min average of the steady-state values observed, once the system was in equilibrium. Birds were monitored constantly, and data were excluded if birds showed evidence of fatigue and were unable to maintain station within the respirometer for a sufficient length of time to allow for gas equilibration. In particular, on some occasions gas equilibration did not

occur at 0.5 km/h owing to the birds not walking at a constant speed. When this occurred, the data were not included in further analyses.

Respirometry analysis

The rate of oxygen consumption was calculated using the equations of Depocas and Hart (1957), as modified by Withers (1977; 2001) and Koteja (1996). As CO_2 was not measured in system 2, for these experiments equation 3a (Withers, 1977) was used to calculate $\dot{V}_{O_2}$ (Ward *et al*., 2002), where the respiratory quotant (RER) was assumed to be the mean value measured in the birds in system 1. This procedure would introduce an error of less than 1% into the calculated $\dot{V}_{O_2}$ (Koteja, 1996), given the measured variation in RER. Equation 3b from Withers (1977) was used for system 2. Data from both systems were pooled.

Heart rate

Heart rate was sampled using a miniature heart rate data logger (HRDL) which has been used successfully with this species previously (Butler *et al*., 1998; Ward *et al*., 2002). Heart rate data loggers (55 mm × 25 mm × 7 mm) were programmed to record heart rate every 5 s. The body of the logger was enclosed in wax before it was encapsulated in

biocompatible silicone rubber (see Chapter I). A long lasting antibiotic (Terramycin, Phzer Ltd, Kent) was injected (0.1 ml/kg) into the pectoral muscle at the start of the surgery, as was a long-lasting analgesic (Vetergesic, Alstoe, York). The birds were anaesthetised with isoflurane gas (3-4% isoflurane in 75% air, 25% O_2) and the incision area was washed with a hibitaine solution, which also served to deflect the feathers. The area of incision was swabbed with Pevidine solution and the sterilised data logger was implanted into the abdominal cavity via mid-line incisions in the skin and body wall. The time at which the logger was started and implanted was noted accurately, and the HRDL was fixed into place with two sutures through the body wall. The muscle layer and skin were individually closed with dissolvable suture, and wound care powder (PetMeds, UK) was used after the surgery was completed. Each logger incorporates a low power transmitter that emits a click on each QRS wave of the electrocardiogram (ECG) (e.g. Bevan *et al*., 2002). A receiver was used to confirm that the logger was correctly in position and recording the ECG. Post surgery, the birds were kept in animal carriers in warm, dark surroundings, and monitored for 3-4 hours before being returned to the outdoor aviaries. The procedure for the removal and replacement of the logger was similar to those described for implantation. On removal from a bird, the data logger was connected to an Acorn (RISC PC) computer and the data downloaded. From the timings noted when the loggers were implanted, it was apparent that their internal clock was accurate to within, on average, 45 s, over the recording period.

Other variables

Five physiological variables are included in the analysis of the captive geese data, the Methods and detailed results of which can be in the following chapters. Body mass (BM, Chapter II), lean body mass (LBM, Chapter IV), total body fat content (TBF, Chapter V), structural size (SS, Chapter IV) and abdominal body temperature (T_{ab}, Chapter VII).

Wild birds

Data were obtained from seven adult barnacle geese of the breeding population at Ny-Ålesund, which is situated in the high Arctic (79^{o} 55', 11^{o} 56'E) on the western coast of the island of Spitsbergen. The town accommodates a number of international research facilities, including the National Environmental Research Council hut. Non-flighted moulting adult birds were captured in corral nets during the post-breeding season moult, during approximately the last two weeks of July and the first week in August. Once caught, the geese were weighed and housed outdoors as a group, with food and water available *ad libitum*. All experiments were conducted with the permission of the Governor or Svalbard, and the Norwegian Animal Welfare Committee.

Respirometry

System 1 in the captive bird sampling sessions was used to measure $\dot{V}_{O_2}$ in the wild geese, with the following modifications. Air was pulled by a downstream sealed pump at a flow of about 30 L/min, controlled by a mass flow controller. At this flow the time

constant of the system was 6.3 min, which equates to a 99% washout time of about 30 min. The excurrent air was sub-sampled (100 mL/min), passed through a drying column and analysed by the O_2 and CO_2 analysers. Baseline was checked before and after each walking session. Output from the analysers, flow controller, together with ambient temperature measured with a thermocouple in the chamber, were digitally recorded at 200 Hz using the Powerlab and Chart. The recorded traces for O_2 and CO_2 were averaged every 5 s, corrected to instantaneous values as if the system had been sealed assuming a first-order linear system (see Frappell *et al*., 1989) and subsequently averaged every 2 min. Calculation of $\dot{V}_{O_2}$ was determined as previously described (see Appendix in Frappell *et al*., 1992); values for O_2 were corrected to be CO_2 free, to take in to consideration RER related errors and $\dot{V}_{O_2}$ determined from incurrent and excurrent fractional concentrations of corrected O_2 and the flow of air through the chamber. A linear drift between baseline measurements was assumed for incurrent values. As with the captive geese, birds were held in the respirometer overnight to measure resting rates of metabolism (Portugal *et al*., 2007). At 08:00 (EST) birds were offered water, before commencement of walking on the treadmill. The protocol for walking was the same as that for the captive geese.

Heart rate

Heart rate was monitored using a customised heart rate transmitter system (POLAR a3, Polar Electro Oy, Finland). The heart rate transmitter was attached dorsally to the feathers

using lightweight paper tape and custom-made brass electrodes were inserted under the skin. The transmitter unit had a functional range of approximately 1 m and the receiver unit was placed on top of the respirometer to ensure a good signal. The outputs were collected at 200 Hz (Powerlab, ADInstruments) and displayed on a computer using Chart software (ADInstruments).

Statistical analysis

The primary aim was to construct a general linear model (GLM, Zar, 1984) that examined how physiological variables could be used to predict $\dot{V}_{O_2}$ in the geese. These models examined how specific physiological variables (BM, TBF, LBM, SS and T_{ab}) affect the relationship between f_H and $\dot{V}_{O_2}$, both in their own right and between each other. Captive geese were examined first, and GLMs were constructed and run to investigate the relationship between f_H and $\dot{V}_{O_2}$ within each of the six sampling sessions. Each model was run (Minitab) with $\dot{V}_{O_2}$ as the response, firstly as absolute values, then corrected to BM (kg), LBM (kg), TBF (kg) and SS (tarsus length, mm). Each of these measurements was divided by tarsus length as an indicator of structural size (see Chapter IV), and re-ran with $\dot{V}_{O_2}$ adjusted to the resultant number from the structural correction. Each model included session as a factor, individual bird as a random factor, and several covariates (f_H, BM, LBM, TBF, SS and T_{ab}). These different measures were all incorporated to account for the fluctuating body mass throughout the annual cycle in this species (Portugal *et al*., 2007). Bird identity was a significant factor in each analysis, so it

was introduced as a random factor. A best-fit model was achieved by firstly including all possible main effects and all possible second degree interactions in the model and then removing non-significant terms, one iteration at a time, by backwards elimination (Green *et al.*, 2005). Non-significant main effects were kept if the variable in question was part of a statistically significant interaction.

Initially, the primary aim of the GLM was to find a model that eliminated session as a significant interaction with heart rate, and as a significant function in the model, therefore creating one calibration line from the six sampling sessions in the captive geese. If this proved to not be possible, then another GLM was used to compare calibration lines from each sampling period, with the appropriate Bonferonni corrections applied due to multiple tests. Those sessions where the slope and intercept were not significantly different were pooled into groups, until the calibration lines from all sampling sessions within one group were not significantly different from any other calibration line, within that group. For all statistical tests used, results were deemed significant if $P < 0.05$.

Results

General linear model showed f_H and $\dot{V}_{O_2}$ to be significantly related for each of the individual sampling sessions in the captive geese, when using either absolute or mass-specific values of $\dot{V}_{O_2}$ (Fig. V1.1, V1.2, Fig. VI.3a, VI.3b, Table VI.2). From this, the aim was initially to construct the simplest GLM based on those six calibration lines, that

eliminated sampling session as a significant factor in predicting $\dot{V}_{O_2}$ from a collection of physiological variables. In total, 205 GLMs were constructed and run.

The first set of analyses (model set 1) examined the effect of BM, LBM, TBF and SS on values of $\dot{V}_{O_2}$ between the sampling sessions. Each model in this set was run with either absolute values of $\dot{V}_{O_2}$, or $\dot{V}_{O_2}$ corrected to BM, LBM, TBF or SS. Included in these models were f_H, BM, LBM, TBF, SS and T_{ab} as covariates, and goose identity as a random factor. Of note was:

(1) the non significant main effect or interaction of TBF in any of the GLMs.

(2) in four of the models, the non-significant interaction between f_H and BM, but BM being a significant main effect in all of the models.

(3) none of the GLMs was successful in removing session as a significant effect

The second set of analyses (model set 2), also incorporated absolute values of $\dot{V}_{O_2}$ along with corrected values of $\dot{V}_{O_2}$ by BM, LBM and TBF. However, in this instance, each value of BM, LBM and TBF was corrected according to structural size by dividing by tarsal length. Each model constructed in model set 1 was thus re-run with the structurally corrected values of BM, LBM and TBF applied to correct $\dot{V}_{O_2}$. Key features from this set of analyses were:

(1) structurally correcting values of BM, LBM and TBF had no significant effect on the GLM models constructed in model set 1.

(2) after correction, TBF still had no significant main effect and no significant interactions.

(3) as a result, none of the models from this set of analyses was successful in removing session as a significant main effect, or as a significant interaction with heart rate.

The third set of analyses (model set 3) examined the exponent by which values of $\dot{V}_{O_2}$ were corrected. In the original GLM models in analyses set 1, values of $\dot{V}_{O_2}$ were corrected to an exponent of 1. However, data from experiments studying resting rate of $\dot{V}_{O_2}$ in the captive geese (Portugal *et al*., 2007) showed that this exponent potentially may change throughout the season. Therefore, this set of analyses re-constructed every model from model sets 1 and 2, and re-ran them with exponents ranging from 0.5-1.5 inclusive. Of significance was:

(1) as with model set 2, altering the exponent did not change the overall models, nor which main effects were significant, or the significant interactions.

(2) in summary, changing the exponent did not improve the accuracy of the model, nor did it remove session as a significant interaction.

As none of the GLMs constructed was successful in removing session as a significant influence of measures on $\dot{V}_{O_2}$, it was not possible to use a GLM to create one single calibration line that could be constructed from all six sampling sessions and applied to year round data sets. Therefore, a different approach was required to combine the six calibration lines from the captive goose sampling sessions.

Consequently, model set 4 compared all the regression lines from each sampling session to one another. This was done for each correction that was used in model set 1 (i.e. absolute values of $\dot{V}_{O_2}$, along with BM, LBM, TBF and SS correction values). The purpose of this analysis was to identify the corrections to $\dot{V}_{O_2}$ that produced the highest value of r^2, and yielded the most lines that were not significantly different from one another. From this analysis, $\dot{V}_{O_2}$ corrected for body mass was chosen as the model for further analysis since it had the highest r^2 and was the most parsimonious, in that it included the least additional variables and hence error terms. The key features of these analyses were:

(1) using BM corrected values of $\dot{V}_{O_2}$ meant that five out of six of the calibration lines were not significantly different from one another (more so than for any other correction factor).

(2) late September-early October was the only sampling session to be significantly different from all 5 other sampling sessions.

(3) the result of these analyses was two calibration lines (Fig. VI.4), late September-early October, and then a second calibration line incorporating February, May, late July – early August, late August – early September and November. All lines within this group were not significantly different from each other line within the group, as such, the data were combined (see Table VI.3 and Fig. VI.4 for GLM details).

(4) the resultant GLM of the two calibration lines was greatly improved by including mass as a covariate.

The final stage of the analysis, model set 5, was to compare the calibration lines gathered from the captive geese at various stages of the annual cycle to that of the wild barnacle geese, studied during their annual wing moult in mid July – early August. It was not possible to obtain a calibration relationship between f_H / $\dot{V}_{O_2}$ during the winter months, as the Wildfowl and Wetlands Trust would not permit the work to take place. General lineal model showed f_H and $\dot{V}_{O_2}$ to be significantly related (Fig. VI.4) in the wild geese, and a simple GLM model (Table VI.2) showed f_H to explain 85% of the variance. Each captive sampling session calibration line was individually compared to the wild geese session, and for this, mass-specific measures of $\dot{V}_{O_2}$ were used (Fig. VI.4). The wild goose data was then also compared to the two calibration lines constructed in model set 4. Key features from this analysis were:

(1) in four of the captive goose calibration lines, session was not a significant main effect, however, the interaction between f_H and session was.

(2) all of the calibration lines from the six sampling sessions in the captive geese were significantly different from that of the wild birds.

(3) the two calibration lines constructed in model set 4 were also significantly different from that of the wild goose data.

(4) using absolute values of $\dot{V}_{O_2}$ did not change the model.

Discussion

Using heart rate to predict rate of oxygen consumption in barnacle geese

Many authors have previously highlighted the need to establish the relationship between f_H and $\dot{V}_{O_2}$ at various stages throughout the annual cycle (e.g. Bevan *et al*., 1995; Green *et al*., 2001; Butler *et al*., 2002). Here, for the first time, we provide this relationship from six points in the annual cycle, for a species that exhibits significant seasonal changes in body mass, body composition and body temperature, even when in captivity (Portugal *et al*., 2007; Chapter V; Chapter VII).

For each sampling session in the captive barnacle geese, with both absolute and mass-specific values of $\dot{V}_{O_2}$, the relationship between f_H and $\dot{V}_{O_2}$ was significant, with the GLM for May providing the lowest r^2 value of 0.79, for absolute $\dot{V}_{O_2}$. The level of variance explained by the GLMs is consistent with that found by Ward *et al.* (2002) (0.70). Apart from the late August – early September sampling session (Table VI.2), including mass-

specific rates of $\dot{V}_{O_2}$ as opposed to absolute values greatly improved the accuracy of the models. During late August – early September, the captive geese are completing their annual wing moult, and it is likely that different individuals would be at different stages of moult completion, and thus in different physiological states. Therefore, body mass, or rather its effect on the $f_H / \dot{V}_{O_2}$ relationship, may be more complicated than at other points in the annual cycle.

The construction of the GLMs for the wild geese was not as detailed, as parameters such as TBF and LBM were not measured. Perhaps unsurprisingly therefore, the GLMs for both absolute and mass-specific rates of $\dot{V}_{O_2}$ for the wild geese explained less of the variance (0.72 and 0.85 respectively) when compared to the models constructed and run for the captive geese. Similar to the late August – early September sampling session in the captive geese, a contributing factor may have been the wide range of moult scores (Portugal *et al*., 2007) of the wild geese during the experimental period. Moult scores in the wild geese during the sampling period (late July – early August) ranged between 2-4 (see Portugal *et al*., 2007), suggesting the wild birds, as might be expected, were not as synchronous in their moulting as the captive flock. The result of this may be that birds were at different stages of mass loss/gain and feather re-growth, which may contribute to the increase in variance.

Although the difference in the $f_H / \dot{V}_{O_2}$ in the captive geese was not significant between 5 of the sampling sessions (Fig. VI.4), there was still variation. These variations may, in part, be explained by the behaviour of the captive geese. During November, for example, the birds would run at faster speeds (all six of the birds in the November session reached speeds of 3.5 km/h), compared to other sampling sessions in the year (rarely above 2.5 km/h). However, during moult (late July – early August), none of the same birds would run at this speed (i.e. they failed to maintain their station on the treadmill and as such that speed was abandoned). It was noted how different the geese responded behaviourally to handling during the moult period, with the birds not struggling or making any sound, but rather totally relaxing their body and muscles and not moving. Differences in behaviour during wing moult have been noted in both wild (e.g. Kahlert *et al*., 1996) and captive (Portugal *et al*., 2007) waterfowl. Therefore, it is possible that there is an element of a difference in stress levels and other psychological parameters that were possibly contributing to the variation in the $f_H / \dot{V}_{O_2}$ relationship.

Considering the significant differences noted in body mass, body composition and body temperature in the captive barnacle geese (Portugal *et al*., 2007; Chapter V; Chapter VII) it is of interest that the relationship between $f_H / \dot{V}_{O_2}$ generally remains constant. Green *et al.* (2001) established this relationship in macaroni penguins at three points in the annual cycle and found this to be the case. The result of these non-significant differences in the captive geese was the pooling of five out of the six calibration lines into one single calibration (Fig. VI.4). Understandably, the GLM for this was less accurate than any of

the individual calibration lines (0.79, Table. VI.3). In this GLM, body mass as a covariate increased the accuracy of the model considerably (without body mass, the r^2 of the GLM was 0.69), which, when the wide range of body mass is considered (e.g. 1.61 kg in July compared to 2.02 in February, Table VI.1, see also Portugal *et al*., 2007), is reasonable.

The effects of body fat on energy expenditure during exercise, or rather, the suggested effects of body fat, are somewhat contradictory. Fat is metabolically inactive, but can elevate overall metabolic rate by increasing the energy demands of other tissues, i.e., energy is required from lean tissues to heat and support the fat tissue (e.g. Froget *et al*., 2001; Nudds and Bryant, 2001). Therefore, increases in body mass should increase the net cost of transport (i.e. energy expenditure during locomotion), however, the changes in the cardiovascular system in response to body fat gain must be systematic, as the relationship between f_H and $\dot{V}_{O_2}$ does not change significantly between five out of the six sampling sessions, despite significant changes in body mass and total body fat (Portugal *et al*., 2007; Chapter V).

Interestingly, the $f_H/\dot{V}_{O_2}$ relationship in the captive geese that is most similar (although still significantly different) to that of the wild geese, is the relationship determined during late September – early October. This was also the only calibration line in the captive geese that was significantly different from the five other calibration lines. Late September – early October is the migratory period in the wild birds. Much evidence gathered from studying captive geese suggests they undergo similar patterns of physiological and

behavioural change to that of their wild conspecifics (Butler and Woakes, 2001; Portugal *et al*., 2007; Chapter V; Chapter VII). The difference in the relationship between $f_H/\dot{V}_{O_2}$ in the captive and wild geese suggests there is some limitation to this. However, it may be possible that in the autumn, the captive geese are also undergoing adjustments to their cardiovascular system in preparation for their 'autumn migration' as the wild geese would be doing (Butler *et al*., 1998). Data on year round body mass, body composition and body temperature suggest these factors at least are responding in the same manner as wild birds in the autumn period (Butler and Woakes, 2001; Portugal *et* al., 2007; Chapter V, Chapter VII). Just prior to their autumn migration, wild barnacle geese undergo a hypertrophy of the heart in preparation for the long migratory flight southwards to their wintering grounds (Bishop *et al*., 2002).

Rates of oxygen consumption achieved at high speeds during treadmill exercise in late September – early October are greater than those recorded at any other point in the year in the captive geese. During strenuous exercise, most birds will dissipate excess heat from the legs and feet, or through panting (Bech and Nomoto, 1982). Froget *et al*. (2002) noted that during treadmill walking, king penguins were exposed to an unnatural situation, that is, performing intense exercise in their thermoneutral zones, or possibly even above it. Running concurrently with the treadmill sessions in the current study, was a separate set of experiments studying changes in resting rates of metabolism throughout the annual cycle in the captive geese (Portugal *et al.*, 2007). These measurements filled all the criteria of measuring basal metabolic rate (see Portugal *et al*., 2007), so the exercising

geese would have been in their thermoneutral zone. Therefore, it could be a possibility that the captive geese running in the treadmill experiments in late September – early October, when they have the highest relative body percentage of fat in their annual cycle (Chapter V), were heat stressed, resulting in a change in the relationship between f_H and $\dot{V}_{O_2}$.

In wild barnacle geese, Bishop *et al.* (2002) suggested that a selective perfusion of tissues vital for support of locomotor activity could possibly occur as a strategic physiological strategy to maximise flight performance. Captive geese may undergo the same strategic physiological strategy, thus, during late September – early October are optimised for flight as opposed to leg powered locomotion. As a result, the potential increase in blood flow to the legs and feet to dissipate heat during treadmill exercise, may influence the overall cardiovascular system and in turn the relationship between f_H and $\dot{V}_{O_2}$. A cooler ambient air temperature may allow the geese to reduce their peripheral circulation, thus, optimising relative blood flow to the working muscles (flight in this case, for this time in the annual cycle), and, as a result, have a lower value of f_H and sustainable cardiac output for a given value of $\dot{V}_{O_2}$ (Bishop *et al*., 2002).

Comparing captive and wild geese

Regardless of what correction factor was applied to $\dot{V}_{O_2}$, no GLM could remove the significant difference in the f_H / $\dot{V}_{O_2}$ relationship between captive and wild barnacle

geese. The wild moulting barnacle geese have significantly lower heart rates for a given $\dot{V}_{O_2}$. This is more than likely related to the general overall fitness of the wild geese in comparison to captive birds. Generally, the more athletic, or fitter, the individual, the lower its heart rate, both during rest and exercise (Butler and Turner, 1988; McPhee *et al*., 2003). As fitness improves, the heart enlarges, ventricular stretching is enhanced, and blood volume increases, resulting in an increased stroke volume, allowing a reduction in the heart rate for a given $\dot{V}_{O_2}$ (Butler, 1991; Butler, 1993; McPhee *et al*., 2003). Butler and Turner (1988) compared various morphological aspects of the cardiovascular and locomotor system in trained and untrained captive tufted ducks *Aythya fuligula*. Trained tufted ducks had increased maximal swimming velocities, lower heart rates, higher oxygen pulses, more capillaries in their locomotor muscles, and these capillaries were present at a higher density. In the ducks, the training resulted in a greater maximal $\dot{V}_{O_2}$, but no change in maximum heart rate, leading the authors to conclude that the greater maximal $\dot{V}_{O_2}$ was a result of a greater tissue oxygen extraction, related to the increase in capillary density (Butler and Turner, 1988).

Pelsers *et al.* (1999) demonstrated that intracellular transportation of fatty acids from the capillary network to the mitochondria in the muscles is better developed in wild birds. Long-term captive barnacle geese had lower concentrations of fatty acid binding proteins and citrate synthase in the pectoral muscle than those of wild pre-migratory geese (Pelsers *et al*., 1999). Nudds and Bryant (2001) established that, after controlling for body

mass, trained zebra finches, *Taeniopygia guttata*, had significantly lower resting metabolic rates when compared to untrained finches. They concluded that the greater physical activity experienced in captivity, the more similar to wild birds the captive birds would be, i.e., it is possible to mimic wild conditions, to some extent. In the present study, it was not possible to exercise the captive barnacle geese to the extent where they would achieve the same fitness as wild migrating geese. However, year round body mass data recorded in the same captive flock were similar to those figures quoted in the literature for wild barnacle geese (see Portugal *et al*., 2007) suggesting that the birds were not overly fat. Pelsers *et al*. (1999) commented that it appeared that flight was essential for maximising the metabolic pathways and the cardiovascular system, and that the experience of flight induced more fatty acid binding proteins and citrate synthase. As the captive geese had never flown, this may be another contributing factor to the large differences observed between the cardiovascular systems of the captive and wild geese. This is despite the major locomotor muscles showing 'innate' responses to wing moult in captive birds (Chapter III).

During the autumn, data gathered from both wild and captive barnacle geese demonstrate the birds are undergoing significant physiological changes (Butler and Woakes, 2001; Portugal *et al*., 2007). In the captive geese, fat content as a percentage is at its highest point of the year (Chapter V), while in both the captive and wild geese, body temperature is decreasing significantly (Butler and Woakes, 2001; Chapter VII). However, the purpose of the GLM was to take into consideration all these factors, and these two

parameters in particular had no significant effect on the relationship between f_H and $\dot{V}_{O_2}$. Nudds and Bryant (2001) concluded that in zebra finches at least, lean body mass should be the better predictor of metabolic rate. In the captive barnacle geese, lean body mass had an inconsistent interaction with predicting $\dot{V}_{O_2}$ but at no stage did it significantly interact with f_H.

As all six of the $f_H / \dot{V}_{O_2}$ calibration lines from the captive geese are significantly different to the relationship derived for the wild geese, it would not be possible to apply calibrations obtained from captive birds to heart rate data recorded in wild geese. However, what the captive calibration lines do demonstrate, is that that the relationship between f_H and $\dot{V}_{O_2}$ determined during the flightless phase of wing moult is generally not significantly different from those derived from the rest of the year, except for the late September – early October period. Therefore, it provides evidence that it is possible to apply the wild calibration between f_H and $\dot{V}_{O_2}$ established during moult in the wild geese, to the rest of the annual cycle. Caution however, should be used when applying this calibration to heart rate data recorded in the autumn period. The autumn though is a period when wild geese are spending a larger portion of their time in flight when compared to the rest of the year, and therefore, a calibration between $f_H / \dot{V}_{O_2}$ obtained from walking data would not be appropriate at this point anyhow (e.g. Nolet *et al*., 1992; Bishop and Butler, 1995).

Nolet *et al*. (1992) commented that the $f_H/\dot{V}_{O_2}$ relationship of captive barnacle geese during terrestrial locomotion could not be the same as flight, as the extrapolation of the regression does not predict realistic values of $\dot{V}_{O_2}$ from the values of f_H recorded for flying geese, and vice versa (Butler and Woakes, 1980; Bishop and Butler, 1995; Bishop *et al*., 2002), and this was indeed later confirmed by Ward *et al*. (2002). Therefore, the $f_H/\dot{V}_{O_2}$ calibrations from the captive and wild geese would not be suitable for converting heart rate values during flight into $\dot{V}_{O_2}$. During late – September, the geese undertake a series of approximately 4-5 flights on the southward migration to their wintering grounds, over a total of approximately five days (Butler *et al*., 1998). For this period, it may not be appropriate to convert heart rate values using calibrations from walking data, however, for the majority of the annual cycle, the geese spend less than 20 minutes per day in flight (Chapter VII), which therefore, should not significantly affect the results when looking at daily means of $\dot{V}_{O_2}$, particularly as, outside migration, minutes per day in flight is also relatively consistent (Chapter VII).

Summary

We calibrated the relationship between f_H and $\dot{V}_{O_2}$ at six points in the annual cycle of barnacle geese, a species that exhibits significant changes in body mass, body composition and body temperature, even when in captivity. General linear model was unable to remove session (i.e. time of year) as a significant factor in determining the

relationship between $f_H / \dot{V}_{O_2}$, but multiple comparisons of the calibrations lines from the captive geese showed only the session of late September – early October to be significantly different from the remaining five calibration lines. Therefore, it was possible to pool these five calibrations via a GLM that explained 79% of the variance. The relationship between f_H and $\dot{V}_{O_2}$ obtained from wild geese during moult was significantly different to any of the relationships derived from the captive geese, making it not possible to apply calibrations from captive birds to wild geese. However, the lack of significant difference in the relationship derived during moult in the captive geese to the rest of the annual cycle (bar late September – early October) means it is possible to make an assumption that the relationship between $f_H / \dot{V}_{O_2}$ during moult in the wild geese is indicative of that relationship throughout the annual cycle, and can therefore be applied to year round heart rate data to calculate estimates of $\dot{V}_{O_2}$.

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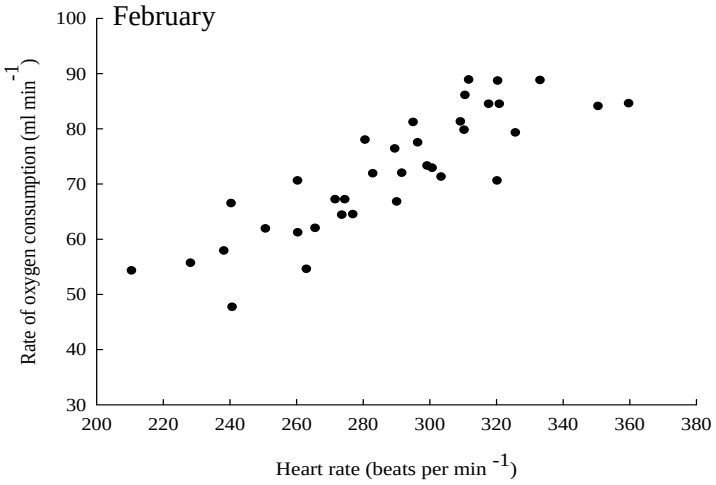
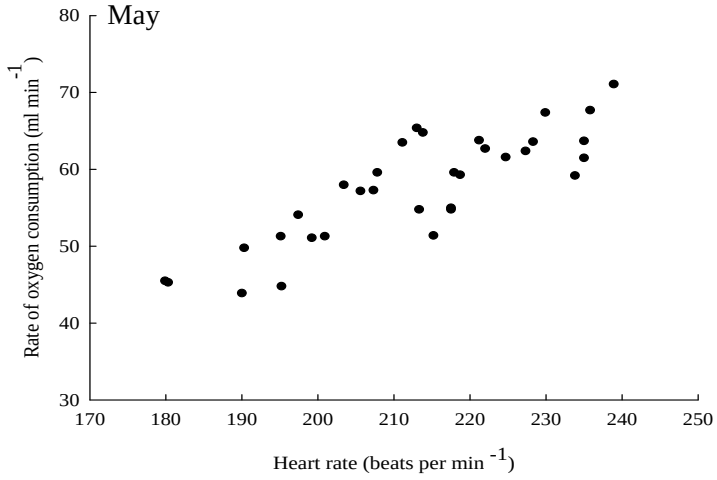
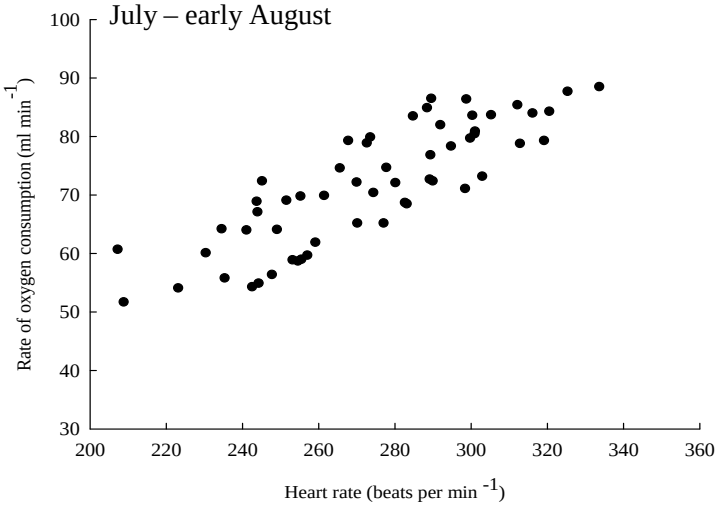
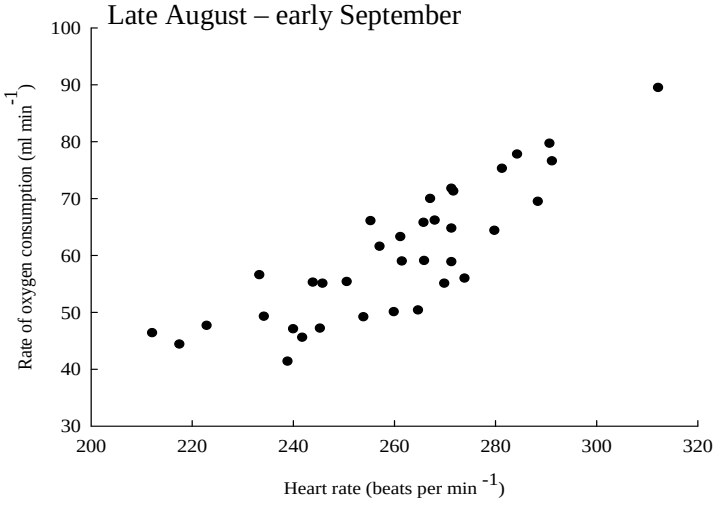

February
Rate of oxygen consumption (ml min -1)
Heart rate (beats per min -1)
100
90
80
70
60
50
40
30
200
220
240
260
280
300
320
340
360
380


May
Rate of oxygen consumption (ml min -1)
Heart rate (beats per min -1)
80
70
60
50
40
30
170
180
190
200
210
220
230
240
250


July – early August
Rate of oxygen consumption (ml min -1)
Heart rate (beats per min -1)
100
90
80
70
60
50
40
30
200
220
240
260
280
300
320
340
360


Late August – early September
Rate of oxygen consumption (ml min -1)
Heart rate (beats per min -1)
100
90
80
70
60
50
40
30
200
220
240
260
280
300
320

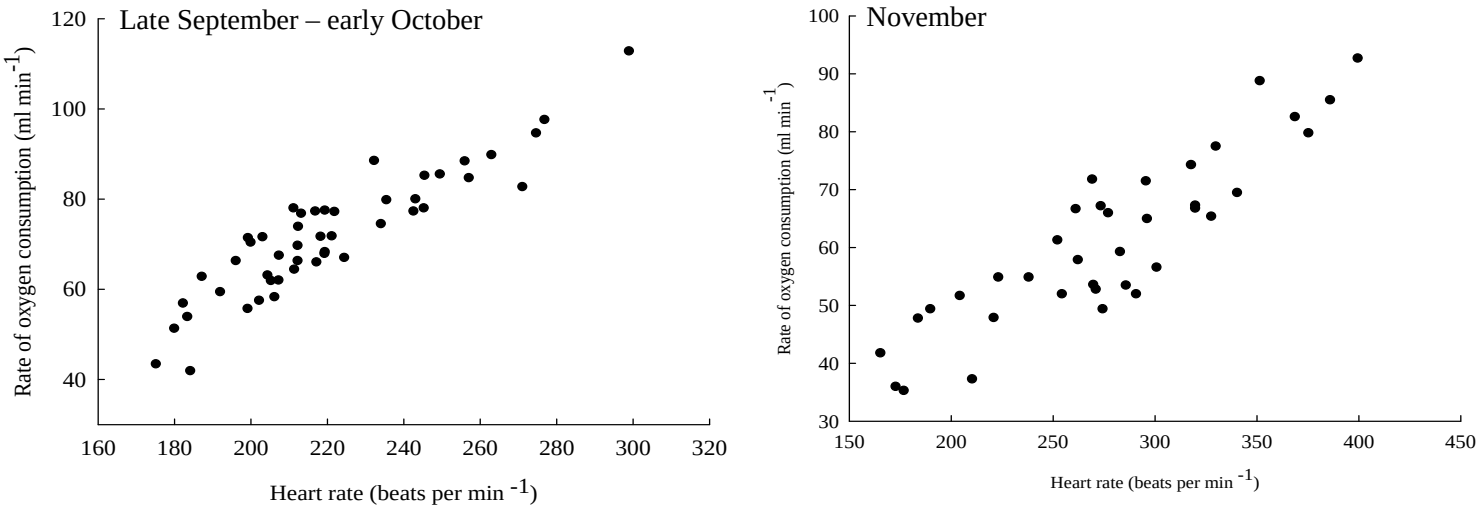
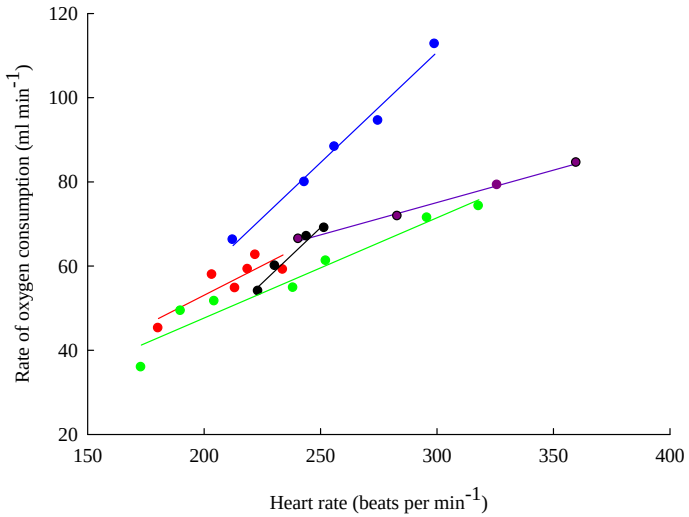
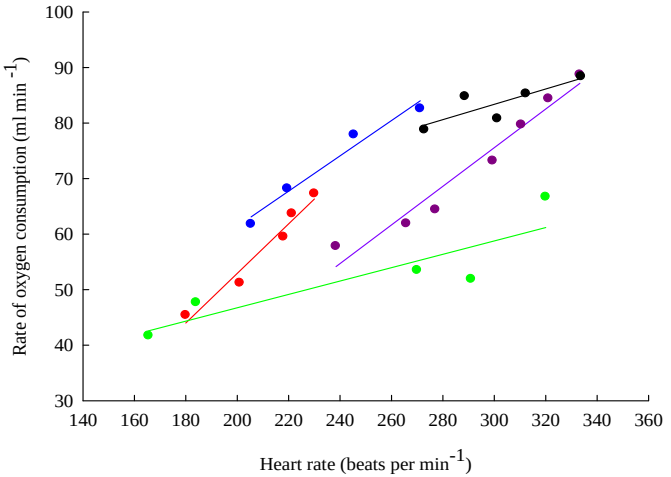
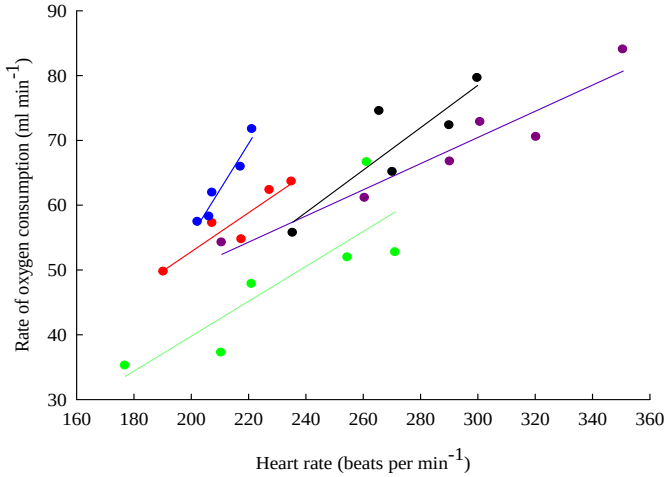


Figure VI.1: Rate of oxygen consumption as a function of heart rate, derived at six points in the annual cycle from captive barnacle geese (see legend in Fig. IV.3 for further details).


Rate of oxygen consumption (ml min-1)
Heart rate (beats per min-1)


Rate of oxygen consumption (ml min-1)
Heart rate (beats per min-1)


Rate of oxygen consumption (ml min-1)
Heart rate (beats per min-1)

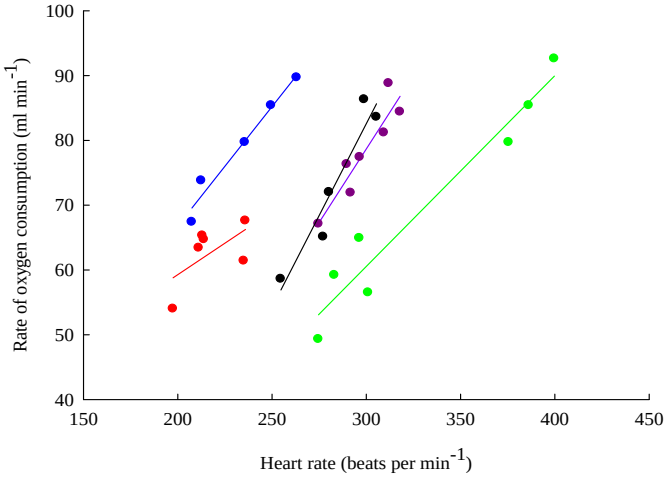

Rate of oxygen consumption (ml min-1)
Heart rate (beats per min-1)

Figure VI.2: Rate of oxygen consumption as a function of heart rate, measured in four captive barnacle geese at five points in the annual cycle. Sessions were: February (purple), May (red), late July – early August (black), late September – early October (blue) and November (green).

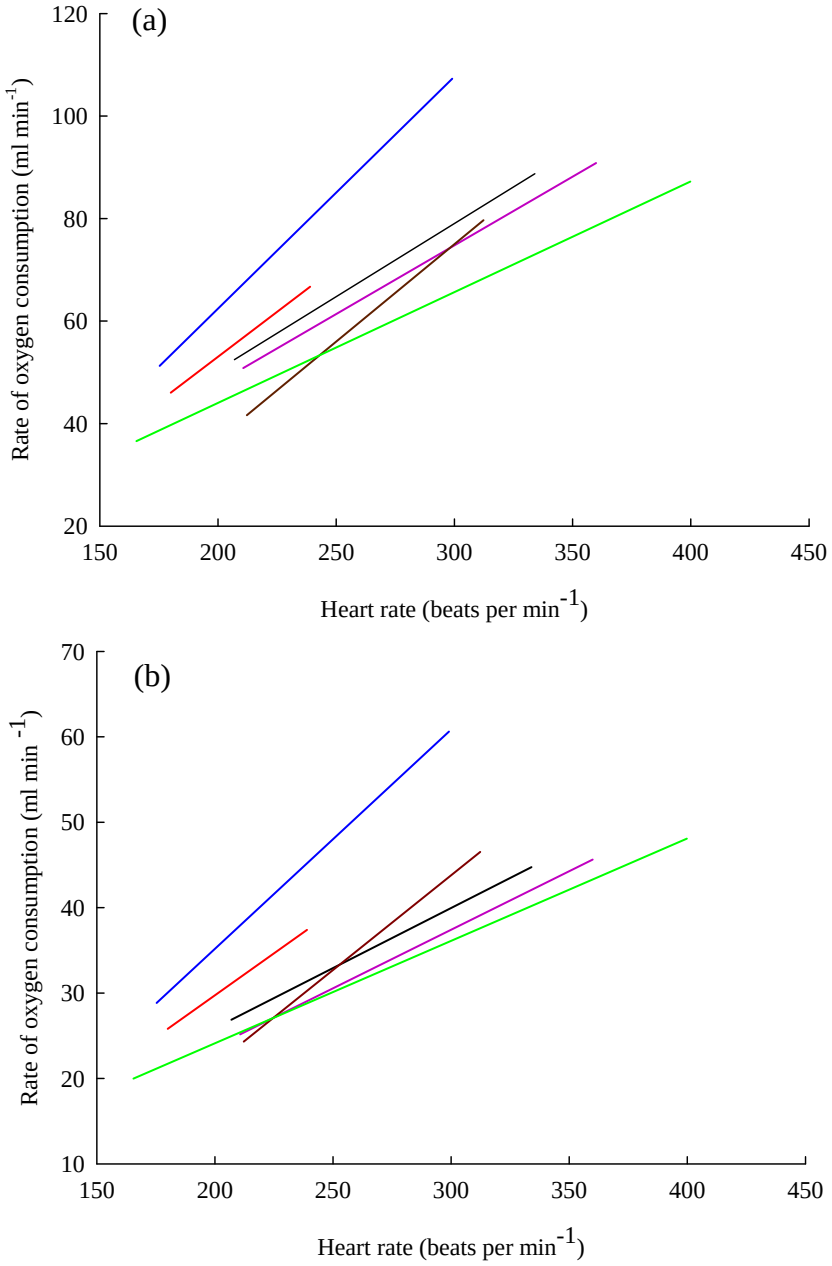


Figure VI.3: Rate of oxygen consumption as a function of heart rate, during six sampling periods throughout the year in captive barnacle geese. Sessions were: February (purple), May (red), late July – early August (black), late August – early September (brown), late September – early October (blue) and November (green). Values of rate of oxygen consumption are presented as absolute data (a), and mass-specific values (b). Equations of the lines and r^2 values are presented in Table VI.2.

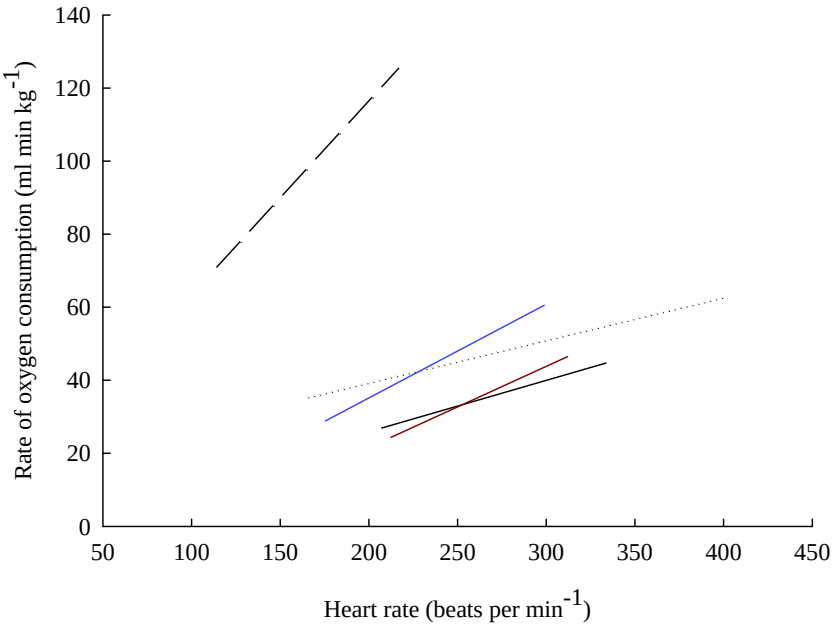


Figure VI.4: Rate of oxygen consumption as a function of heart rate, comparing the wild bird (n=7) GLM (dashed line, see Table VI.2 for details) to that of captive birds from a similar period in the annual cycle; late July – early August (black), late August – early September (brown) and late September – early October (blue). The dotted line represents the pooled GLM of February, May, late July – early August, late August – early September and November (($\dot{V}_{O_2}$ = (0.11 ± 0.007) f_H (16.05 ± 3.4), r^2 = 0.79, $F_{1,\ 182}$ = 257.61, $P < 0.001$)).

Session	N	Body mass (kg)	Lean body mass (kg)	Total body fat content (kg)	Body temperature (^{o}C)
Feb	6	2.02 ± 0.11	1.84 ± 0.10	0.18 ± 0.01	38.5 ± 0.34
May	6	1.83 ± 0.14	1.58 ± 0.12	0.25 ± 0.01	38.5 ± 0.27
Jul-Aug	12	2.02 ± 0.07	1.72 ± 0.24	0.30 ± 0.01	39.1 ± 0.18
Aug-Sep	7	1.61 ± 0.06	1.55 ± 0.05	0.16 ± 0.00	38.7 ± 0.20
Sep-Oct	10	1.78 ± 0.02	1.42 ± 0.01	0.36 ± 0.00	37.7 ± 0.39
Nov	6	1.82 ± 0.22	1.62 ± 0.08	0.19 ± 0.01	38.4 ± 0.29

Table VI.1: Samples sizes and mean (± SEM) of each physiological parameter measured for each sampling session.

CAPTIVE	Session	Equation of the line	r^2	GLM f_H	*P*
ABSOLUTE	February	$\dot{V}_{O_2}$ = (0.27 ± 0.02) f_H + (-5.623 ± 6.6)	0.87	$F_{1,29}$=139.64	*P* < 0.001
	May	$\dot{V}_{O_2}$ = (0.35 ± 0.04) f_H + (-17.026 ± 8.7)	0.79	$F_{1,26}$=73.31	*P* < 0.001
	July-Aug	$\dot{V}_{O_2}$ = (0.28 ± 0.02) f_H + (-6.625 ± 6.6)	0.88	$F_{1,44}$=140.72	*P* < 0.001
	Aug-Sep	$\dot{V}_{O_2}$ = (0.38 ± 0.04) f_H + (-39.01 ± 10.7)	0.86	$F_{1,28}$=86.23	*P* < 0.001
	Sep-Oct	$\dot{V}_{O_2}$ = (0.45 ± 0.02) f_H + (-28.07 ± 5.15)	0.83	$F_{1,28}$=82.95	*P* < 0.001
	Nov	$\dot{V}_{O_2}$ = (0.22 ± 0.02) f_H + (0.821 ± 5.3)	0.88	$F_{1,30}$=130.48	*P* < 0.01
MASS-SPECIFIC	February	$\dot{V}_{O_2}$ = (0.14 ± 0.01) f_H + (-3.708 ± 3.8)	0.90	$F_{1,29}$=109.2	*P* < 0.001
	May	$\dot{V}_{O_2}$ = (0.20 ± 0.02) f_H + (-9.522 ± 5.3)	0.91	$F_{1,26}$=61.17	*P* < 0.001
	July-Aug	$\dot{V}_{O_2}$ = (0.14 ± 0.01) f_H + (-2.239 ± 3.6)	0.94	$F_{1,44}$=111.73	*P* < 0.001
	Aug-Sep	$\dot{V}_{O_2}$ = (0.22 ± 0.02) f_H + (-22.712 ± 6.3)	0.83	$F_{1,28}$=82.95	*P* < 0.001
	Sep-Oct	$\dot{V}_{O_2}$ = (0.26 ± 0.01) f_H + (-16.128 ± 2.8)	0.96	$F_{1,37}$=398.51	*P* < 0.001
	Nov	$\dot{V}_{O_2}$ = (0.12 ± 0.01) f_H + (0.121 ± 3.0)	0.91	$F_{1,30}$=120.79	*P* < 0.001
WILD					
ABSOLUTE	-	$\dot{V}_{O_2}$ = (0.83 ± 0.23) f_H + (10.46 ± 39.6)	0.72	$F_{1,24}$=12.90	*P* < 0.001
MASS-SPECIFIC	-	$\dot{V}_{O_2}$ = (0.53 ± 0.05) f_H + (10.46 ± 8.6)	0.85	$F_{1,24}$=109.33	*P* > 0.001

Table VI.2: GLM of heart rate and oxygen consumption from walking geese (see Fig. VI.3), taken at 6 points in the year for captive birds, and during one sampling point (mid July – early August) for wild geese. Data are presented as absolute and mass-specific values of rate of oxygen consumption, and relate to Fig. VI.3a and VI.3b respectively for captive geese, and Fig. VI.4 for wild birds.

Table VI.3: Analysis of individual component factors of a general linear model (GLM) relating rate of oxygen consumption to various physiological parameters. GLM was the result of pooling 5 calibration lines that were not significantly different from one another. R^2 of the GLM was 0.79. The parameters of lean body mass, total body fat content, structural size and body temperature were removed through backwise-elimination as a result of lack of significant effect or interaction. Statistical significance at $*P < 0.001$.

Factor	df	*P*
Goose ID	14	0.000*
Session	1	0.000*
Body mass	1	0.000*
Heart rate	1	0.000*
Heart rate × Session	1	0.000*

List of abbreviations

TBF	Total body fat
LBM	Lean body mass
SS	Structural size
BM	Body mass
T_{ab}	Abdominal body temperature
$\dot{V}_{O_2}$	Rate of oxygen consumption
f_H	Heart rate

VII: GENERAL DISCUSSION

The primary objectives of this thesis were to measure various physiological parameters in barnacle geese for a full annual cycle, to investigate how these parameters change in response to different events such as moult, and in turn, the influence these changes potentially had on the energetics of the birds, and the relationship between f_H and $\dot{V}_{O_2}$. All the principle research objectives stated in Chapter I have been addressed, and as such, this study has improved our understanding of the annual cycle of barnacle geese.

Chapter II demonstrated the significance of wing moult in the annual cycle of barnacle geese, and perhaps other large species of waterfowl. This work demonstrates the amazing phenotypic plasticity (e.g. van Gils *et al*., 2006) of the avian body, and confirmed what Fox and Kahlert (2005) had proposed that the avian body is in a constant stage of flux (rheostasis). Within a very short period of time (c. 21 days), the captive geese lost a significant amount of body mass (25%), and, despite constant access to food, decreased foraging. While studies on wild moulting waterfowl have previously investigated various components of wing moult, this study has taken our understanding of this event in the annual cycle of waterfowl a step forward, by looking at a series of parameters in captive birds.

One key finding was the 80% increase in resting metabolism during moult, when compared to non-moult periods. Heart rate logger data from the captive geese (Fig. VII.1)

demonstrates that minimum daily f_H was also at its highest during wing moult, than at any point in the year. Previously, data had been gathered (but not analysed) on wild Svalbard barnacle geese (see Appendix 2). Using the same analytical approaches as described in previous chapters, it is possible to compare the annual cycle of heart rate in the captive geese, to that of wild birds (Fig. VII.1). Minimum daily f_H (see Appendix 2) during wing moult in the wild geese, like the captive birds, is also higher than at any other stage in the annual cycle.

This study challenges previous conceptions about what events in the annual cycle would be the most energetically demanding. It also stresses the importance of specific moult sites for different species of waterfowl. The behavioural response of the captive geese to wing moult suggests they are vulnerable to predation during the flightless phase. A good moult site requires safety from such predators while offering good foraging opportunities (Hohman *et al*., 2002). These sites are often limited, and the tight schedule of events for the geese in the summer months results in breeding, moulting and fat deposition prior to migration being very interlinked, and frequently, to some extent, dependant on one another. The dramatic increases in body mass and fat content observed in the captive geese in the 3-4 weeks following moult, demonstrates the limited window of opportunity the birds have to deposit sufficient fat stores to undertake the autumn migration.

The importance of saving energy during moult while avoiding detection from predators is apparent by the highly endogenous nature of this behaviour, and this indeed may only be present in certain species of waterfowl. Greylag geese, for example, have been shown to

adapt their behaviour during moult from year to year at regular moult sites in Denmark, depending on the presence or absence of predators, and disturbance by humans (Fox *et al*., 1998). If greylag geese show the ability to respond instantly to their surroundings and the circumstances under which they moult, it raises the question why captive barnacle geese do not adapt their behaviour during moult, and continue foraging in the absence of predators and disturbance. This difference in flexibility and behaviour may be linked to body size of the species, and the latitude and sites the birds moult at. Barnacle geese are considerably smaller than greylag geese, and are generally found to breed at higher latitudes, as at lower latitudes they are pushed out of suitable nest sites by larger species of geese, and cannot defend themselves against large land based predators (Owen, 1990). It may be the case, therefore, that the barnacle geese do not show such flexibility in their behavioural response to moult, since the time-window for moulting is shorter compared with species moulting at lower latitudes, and since conditions in the Arctic begin to deteriorate as autumn approaches (Bonier *et al*., 2007). Of interest would be a study looking at food intake and appetite control in captive and wild geese, to ascertain if appetite in structurally smaller species of moulting waterfowl is actually suppressed during moult, while that of larger geese (safer from predation and breeding in lower latitudes) is not. What this study contributes, is the suggestion that different approaches to wing moult will exist between waterfowl species, potentially influenced by body size, and (historic) moult locations.

Chapter II, for the first time, links body size with body mass changes during moult, and in turn, the behavioural response to being flightless. Structurally larger birds are able to,

firstly, store more fat which in turn allows them to allocate more energy per day to feather regrowth, thus quickening feather regrowth and moult. Secondly, larger birds can rely more on these fat stores to reduce percentage time spent foraging to a greater extent than smaller individuals, thus reducing the chance of being detected by predators, and saving energy by resting. This is an interesting concept, as it suggests that wing moult, and the approach to moult, both as an individual and as a species, is dictated by structural size. This, in turn, is linked to initial growth patterns as a gosling during the first month after hatching (Black *et al*., 2007). The captive birds all had equal and constant access to food throughout hatching and rearing, and in turn, during moult each year. Despite this, these individual differences in time spent resting during moult are still evident. Therefore, in one flock of captive birds, different individuals are adopting different approaches and strategies to wing moult, potentially dictated by their structural size. The approaches to moulting can be likened to capital and incoming breeding (see Chapter 1). In this instance, structurally larger birds can rely on fat stores during moult and so can reduce foraging, while smaller individuals will have to continue foraging to fuel feather growth, and incur the associated increased energy costs risk of predation. Potentially, an interesting field study would be to examine whether geese predated upon during the flightless phase of moult were structurally small, and, therefore, were having to continue foraging during wing moult.

The data from Chapter II, coupled with that of Chapter V, suggest fat has more roles to play in the annual cycle of the geese than providing fuel for migration and reproduction, and keeping warm in the winter. It dictates what moult strategy an individual goose will

adopt and in turn the ability of an individual to rest and remain undetected from predators, during one of the most vulnerable periods in the annual cycle. One question may be why simultaneous moult has evolved given the risks associated with predation and high increases per day in metabolic rate required to re-grow feathers in a short period of time. Although sequential moult (Chapter 1) has a lower increase per day in metabolic rate, the replacement of the feathers takes place over a much longer period of time. For the geese, simultaneous moult appears to be the best strategy, particularly as, at that point, the adults are rearing goslings who are also flightless.

Chapter III further confirmed the endogenous manner of these physiological changes by investigating the modifications in the key locomotor muscles in the captive geese, over the duration of wing moult. For the first time, it has been made clear that the leg and flight muscles do not just change mass in a use-disuse responsive manner to being flightless, but rather the actual architecture of the muscles adapt in a very short period of time and in a preemptive manner, to potentially provide the geese with improved physiological capabilities, to escape predation quicker on foot. This deepens our understanding of muscle atrophy and hypertrophy in general, and is consistent with what Dietz *et al*. (1999) proposed, that in birds at least, there is no training or exercise required to increase muscle mass.

Chapter IV and V provide evidence that non-destructive techniques of estimating body composition can, with the appropriate calibration, be used effectively to predict fat content throughout the annual cycle of a animal, as demonstrated here for the first time. It

also provided support that the mass loss during wing moult in captive barnacle geese at least, is a depletion of fat stores. Of particular note was the pre-winter fattening observed in the captive geese. Butler and Woakes (2001) demonstrated the potential important role seasonal hypothermia might play in assisting with fat deposition in barnacle geese (Chapter 1). Although not described in detail in the thesis, the HRDLs used to record f_H during treadmill exercise also recorded year-round T_{ab}. Therefore, it is possible to link changes in T_{ab} to periods of fat deposition, and to compare the data gathered from the captive geese with that from wild birds (Fig. VII.2). Both the captive and wild geese showed significant changes in T_{ab} throughout the annual cycle.

The reduction in T_{ab} observed in the wild geese during early autumn was not as large as that reported by Butler and Woakes (2001), who recorded a mean drop in nighttime T_{ab} of 4.4^{o}C, over an approximate 25 day period. The 1.5^{o}C drop in T_{ab} observed in the wild geese in the present study was significantly less, however, it occurred over a longer period of time, with 35 consecutive days of a decrease in T_{ab} between late August and early October. This would result in less of a decrease in MR as a result of the drop in T_{ab}, as that predicted by Butler and Woakes (2001) for the wild geese in their study. Butler and Woakes (2001) implanted three captive barnacle geese with HRDLs for comparison with wild data, and concluded that the captive birds did undergo a similar period of seasonal hypothermia, but the drop in T_{ab} was not as great as that recorded in the wild birds. In the present study, the captive and wild barnacle geese both underwent a reduction in nighttime T_{ab} of approximately 2.0^{o}C between early September and October. The lack of a significant difference between wild and captive birds is a result of the wild

geese not undergoing such a significant drop in nighttime T_{ab} compared to that reported in Butler and Woakes (2001).

The drop in T_{ab} in the captive geese in the present study is probably similar to that experienced by the captive geese in Butler and Woakes (2001), although the exact figures were not reported. What is likely is that this seasonal hypothermia is a mechanism to reduce the rate at which fat is used, and enables its more rapid replacement following and, possibly during, migration, as proposed by Butler and Woakes (2001). The fact that both the captive and wild geese in the present study underwent a reduction in T_{ab} suggests that this seasonal hypothermia is a regular annual phenomenon, in the Svalbard breeding population of barnacle geese at least. What this study also suggests is that it may be a flexible, responsive energy-saving mechanism, the extent to which the drop in T_{ab} occurs being influenced by other events over the summer period preceding it. Barnacle geese are ready to lay their eggs on arrival on the breeding grounds in late spring (Owen, 1984). Failed breeders will begin their annual wing moult earlier in the summer, to give themselves the maximum possible time to deposit fat prior to the autumn migration (Owen and Ogilvie, 1979).

It may be possible that the degree of reduction in T_{ab} will vary between years and between individuals, depending on when the birds bred and subsequently moulted, with late moulters undergoing a greater reduction in T_{ab} to help conserve fat reserves, as they will have had less opportunity to deposit fat post-moult and prior to migration. Klaasen *et al*., (2006) noted that depending on environmental circumstances, Arctic breeding geese can either be capital or income breeders, the environmental conditions in the Arctic each

year dictating which strategy the geese favour. The conditions that influence what breeding strategy the geese show a preference for each year may also govern the degree of T_{ab} reduction that subsequently occurs in the autumn.

Also of interest is the slight drop in T_{ab} observed in late March – early April (Fig. VII.2). This drop in T_{ab} would again occur with pre-migratory fattening. Spring migration, however, is a slower process than the autumn migration, and fattening occurs *en route*, as well as prior to departure from Scotland. Therefore, it may be that the degree of fattening required in spring for the first part of the migration is not as great as that of autumn, and thus the drop is T_{ab} is not as great.

Chapter VI calibrated the relationship between f_H and $\dot{V}_{O_2}$ throughout the entire annual cycle of a species for the first time. As the captive geese showed significant changes in body mass, body composition and T_{ab} throughout their annual cycle, they provided an ideal model to investigate the influences of these parameters on the $f_H/\dot{V}_{O_2}$ relationship. The lack of a significant difference in this relationship between five out of the six sampling sessions, suggests that the $f_H/\dot{V}_{O_2}$ relationship remains relatively constant for a large portion of the annual cycle. What was apparent was the difference in the $f_H/\dot{V}_{O_2}$ relationship between the wild and captive geese. Although the annual cycle of many of the physiological parameters measured in this study showed remarkable similarities between wild and captive geese, one factor it seems may be impossible to recreate in

captive conditions is the overall physical fitness that the wild birds posses. However, although the $f_H/\dot{V}_{O_2}$ relationship was significantly different between the wild and captive birds, the line derived in captive geese during moult, was not significantly different to the majority of the $f_H/\dot{V}_{O_2}$ calibrations derived from the rest of the year. This provides some confidence when using the $f_H/\dot{V}_{O_2}$ calibration established in the wild geese during moult, to provide year round estimates of $\dot{V}_{O_2}$ from f_H data. However, because one of the calibrations was significantly different from the rest, it does suggest that changes in body mass and fat content in particular can affect the $f_H/\dot{V}_{O_2}$ relationship. This may be a result of a change in stroke volume, brought about the atrophy and hypertrophy of the heart, observed in the wild geese just prior to autumn migration (Bishop and Butler, 1995), and which may potentially be occurring in the captive geese.

What this study demonstrates is the endogenous nature of the annual cycle in barnacle geese. Typically in birds, the structure of the 24 hour period, and in turn, the annual cycle, is governed by the avian circadian pacemaking system, a complex system consisting of three components that contribute to the regulation of overt behavioural and physiological rhythmicity: (1) the pineal gland, by rhythmically releasing melatonin; (2) the hypothalamic oscillator, possibly acting through neural output pathways; and (3) the retinae of the eyes, acting either via periodic melatonin secretion or neural output signals (Gwinner and Brandstätter, 2001; Dawson *et al.*, 2001; Brandstätter, 2002). Several lines of evidence suggest that these components interact with each other to produce a stable

organismic circadian rhythmicity (Gwinner and Hau, 2000; Gwinner and Brandstätter, 2001; Brandstätter, 2002). Most birds have pineal glands that are well-developed, and rhythmically release melatonin (Gwinner and Hau, 2000). Even when birds are under constant conditions (e.g. continuous darkness), rhythmic melatonin can be found in the blood, demonstrating that melatonin production is regulated by endogenous circadian oscillators (Kumar *et al.*, 2000; Brandstätter, 2002). This is particularly important for birds, like the barnacle geese, that inhabit polar regions.

Polar regions are challenging environments for circadian systems because they lack diel light-dark changes twice each year (Hau *et al*, 2002). In the high-Arctic, light intensity remains below civil twilight for more than two months in winter, and in late spring and summer there is continuous light for five months (Reierth and Stokkan, 1998), although daily changes in light intensity do occur (Vleck and Hook, 2002). Studies conducted in the laboratory have demonstrated that exposure to continuous periods of light or dark can suppress or disrupt circadian function in many animals (Yamada *et al.*, 1988; Vleck and Hook, 2002; Hau *et al.*, 2002). In addition, the melatonin rhythm is dampened or absent under the continuous light of the polar summer in seals (Barrel and Montgomery, 1989), penguins (Cockrem, 1991; Miché *et al.*, 1991), Lapland longspurs, *Calcarius lapponicus*, (Hau *et al.,* 2002) and Svalbard ptarmigan, *Lagopus mutus hyperboreus*, (Reierth *et al.*, 1999).

Svalbard ptarmigans, the only resident bird on Svalbard, exhibit reduced day-light amplitudes and mean daily melatonin secretions in midwinter, as well as nearly

undetectable melatonin secretion in summer, presumably as an adaptation to life in the Arctic (Reierth *et al.*, 1999). It has been suggested that there is a causal relationship between melatonin and locomotor activity, in the sense that high activity may depend on low levels of plasma melatonin (Oshima *et al.,* 1989; Yamada *et al.,* 1988). Therefore reduced amplitude and production of melatonin observed in the Svalbard ptarmigan in midwinter could be a mechanism for coping with the environment when it becomes arrhythmic and unpredictable (Gwinner *et al.,* 1997). This may enhance adaptation by allowing the birds to forage whenever physical conditions are favourable (Gwinner *et al.*, 1997; Reierth *et al.,* 1999). The ptarmigan activity was intermittently continuous around the clock during the more than two months of continuous darkness in midwinter, suggesting that the reduced amplitude of melatonin production enabled the birds to be active in the dark (Reierth *et al.,* 1999). It could be suggested therefore that high levels of melatonin production and activity are mutually exclusive.

Reierth and Stokkan (1998) proposed that feeding itself could potentially act as a *zeitgeber* in polar birds. With animals that have continuous access to food in their natural environment (e.g. the herbivorous Svalbard ptarmigan and barnacle geese), it would be advantageous to have a *zeitgeber* that were not light only, particularly during the autumn when fat deposition is imperative. By using food availability as a *zeitgeber*, it may allow the birds to enjoy a longer feeding period each day than if the rapidly declining daylength was the only *zeitgeber* (Reierth and Stokkan, 1998). Svalbard ptarmigans, for example, feed continuously throughout the summer months. In late winter, spring and autumn, when there is a daily light-dark cycle, Svalbard ptarmigans are mainly active during the

light part of the day, with peaks of activity in the morning and evening (Stokkan *et al*., 1986). The photoperiod and the activity period do not change in parallel, however, causing the phase relationships between the morning and evening peaks of activity and the light-dark cycle to change progressively (Reierth and Stokkan, 1998). Captive ptarmigans tended to begin and end their daily visits to their food box at about the same time as they did the previous day, suggesting feeding itself acts as a *zeitgeber* in Svalbard ptarmigans (Reierth and Stokkan, 1998). During *ad libitum* feeding and light-dark cycles, Svalbard ptarmigan displayed a bimodal, diurnal activity pattern that is typical for diurnal birds. Through controlling their access to food, Reierth and Stokkan (1998) were able to shift the morning and evening peaks of activity in the ptarmigan, indicating that the timed access to food acted as a *zeitgeber*. When the birds had established a stable phase relationship to the food access interval, a marked anticipatory activity preceded the feeding interval by approximately 1 h (Reierth and Stokkan, 1998).

The present study lends support to the theory that at certain points in the annual cycle, birds can use different cues to structure their day, and, in turn, season. It is likely that the barnacle geese will use different cues and stimuli at different stages in the year for the control and organisation of their annual cycle, depending on whether they are in Scotland or Svalbard. During the summer months when in constant daylight in the Arctic, other factors may control their behaviour, such as food availability and development of goslings. It could be argued that because of the shortness of the Arctic summer, a circadian rhythm is not required *per se*. The geese are in pairs, the conditions are fairly constant throughout a 24 hour period (i.e. no rhythmic or regular temperature changes

associated with day and night), and to take full advantage of the constant daylight, they are active the majority of the time. This high level of activity would result in a decrease in melatonin levels, thus enabling the geese to use other factors, such as food availability, as a cue. Therefore, there is no requirement to have a structure to the 24 hour period. However, even if a circadian rhythm is temporarily not required, knowledge of the passing of the season is. The circadian rhythm that is so evident during the winter months in both T_{ab} and f_H in the wild geese vanishes after the first long duration flight (i.e. the flight that results in the geese departing Scotland and flying to Helgeland, Norway). It is possible that this move triggers a chain of hormonal events that 'take over' control of the annual cycle during the summer months (i.e. breeding and wing moult), before the return of twilight to the Arctic breeding grounds signals for the geese to deposit fat and migrate south.

How this 'chain reaction' of events is then controlled in the constant conditions of the Arctic summer is then largely dictated by reproductive state and the onset of twilight in late summer. Almost all seasonally breeding birds use changes in photoperiod as the major initial predictive cue to time reproduction (Gwinner, 1996; Gwinner, 2001; Brandstätter *et al.*, 2001; Gwinner and Brandstätter, 2001). Other cues, such as food supply, temperature, nest site availability or social interactions, tend to modify seasonal changes in reproductive physiology and behaviour but these are principally driven by changes in photoperiod (Gwinner, 1996; Gwinner, 2001; Brandstätter *et al.*, 2001; Gwinner and Brandstätter, 2001). Seasonally breeding birds experience short days in late winter, and during this period they are in a state of responsiveness to long days, and are

said to be photosensitive (Gwinner, 1996; Dawson and Sharp, 1998; Shackleton *et al.*, 2003). In spring, when days become long enough to illuminate the photo-inducible phase of the circadian cycle, birds show a surge in gonadotrophic hormones, grow their gonads, increase circulating sex steroid levels and engage in reproductive behaviour (Nicholls *et al.,* 1988; Dawson and Sharp, 1998; Shackleton *et al.,* 2003). As the season progresses through the summer and the days are still long, birds cease responding to long days, regress their gonads and initiate feather moult (Shackleton *et al.,* 2003). During this period, the bird is said to be photorefractory, and in some species, birds must experience short days before they will cease being photorefractory and once again become photosensitive (Nicholls *et al.,* 1988; Gwinner, 1996; Dawson and Sharp, 1998; Shackleton *et al.,* 2003).

Migrant birds, like the barnacle geese, typically undergo their annual moult prior to their autumn migration, once reproduction has been completed. At temperate latitudes, seasonal breeding in many birds is terminated by the development of absolute photorefractoriness; absolute photorefractoriness is the condition in which the gonads regress in summer and early autumn when days are longer than those that stimulated gonadal growth in spring (Dawson and Sharp, 1998). The moult typically follows absolute photorefractoriness.

Two mechanisms are involved in the timing of the moult: photoperiod, and the relationship between breeding and moult (Dawson and Sharp, 1998; Dawson *et* al., 2000; Dawson *et al.,* 2001). Long photoperiods are required to initiate moult. If breeding

activity continues beyond the photoperiodic timing of moult, the start of moult is delayed (Dawson *et al*, 2001). Moult in birds is dependent upon, and inducible by, thyroid hormones, and is inhibited by oestrogen and testosterone (Dawson and Sharp, 1998). By delaying the moult in blue tits, *Cyanistes caeruleus*, Nilsson and Svensson (1996) were able to demonstrate the importance of the timing of the moult on the year round condition of the birds. Delayed birds had higher thermoregulatory costs in the following winter, reduced both –over-winter survival, and breeding success the following year (Nilsson and Svensson, 1996). Nilsson and Svensson went on to propose that there was a trade-off between the energetic costs of reproduction and of moult such that the parental effort of late breeding was, in some way, detrimental to the insulative quality of the plumage. In general, later-moulting birds moult more rapidly because the rate of moult is influenced by the seasonal changes in daylength (Dawson *et al.*, 2000). The start of moult is triggered by long days, but once it has started, decreasing daylength increases the rate of moult (Dawson and Sharp, 1998; Dawson *et al*., 2000). By decreasing the daylength after the start of the moult in European starlings, *Sturnus vulgaris*, Dawson *et al.* (2000) were able to decrease the duration of the moult. The decrease in duration of the moult resulted in a poorer quality of the primary feathers, the new primaries being shorter, with a narrower rachis, lighter, less rigid structure, and consisting of softer keratin (Dawson *et al*., 2000). A reduction in plumage quality may influence survival by decreasing flight performance and increasing thermoregulatory costs.

For the geese, the slight changes in light intensity and the onset of ‘twilight’ in late August on Svalbard will be vital cues to finish moulting and start depositing fat for the

autumn migration. Once at the wintering grounds, circadian rhythms may play an important social role for the geese, and be a significant energy saving mechanism. It has long been proposed that flocks in winter can act as information centres for birds about good foraging sites etc, while being in flocks also offers protection against predators. During the winter, the wild barnacle geese leave the roost sites on the marshes and split into smaller groups to forage on neighbouring farmland (Phillips *et al*., 2003). To maintain being in a flock, it is important to have cues to return to the roost sites at the end of the day, and, as the geese cannot feed in the dark, the onset of twilight in the mid-afternoon will act as a signal to the birds to return to their roost site.

Of interest are the regular and rhythmic peaks in T_{ab} and metabolic rate (MR) observed in both the captive and wild birds during the winter months. Data on MR and T_{ab} from the summer show that if circumstances allow, geese will forage and be active throughout the 24 hour period. In the winter, the geese stop foraging when night falls, because, as diurnal foragers, they cannot see in the dark (Owen and Black, 1992). However, it may be that on clear nights when the moon is bright, conditions make it possible for the geese to forage, thus giving them the opportunity to increase their daily food intake. As these peaks in T_{ab} and MR are at regular intervals, it suggests that the geese are responding to a rhythmic lunar cycle, with the moon producing different light levels, at different stages in the month. The lunar cycle (moon phases) repeats with a period of 29.5 days (the synodic period). The geese, therefore, may be responding to the increased light levels of a full moon and, become active. However, it is not necessarily the full moon that is the brightest moon each month, but rather perigee, when the moon is closest to the Earth

(Elkins, 1983). Therefore these regular peaks in T_{ab} and MR may be the geese taking advantage of brighter conditions and foraging.

What is apparent from this study is the suggestion that certain aspects of the annual cycle are under different endogenous processes and control. Decreases in T_{ab} appear to be flexible and respond to the immediate conditions of that year. Other aspects however, such as behavioural responses during moult, appear to less flexible. Findings from this study also provide strong evidence that there is much potential for using captive birds to research complex events in the annual cycle that would not be possible to study fully in wild birds.

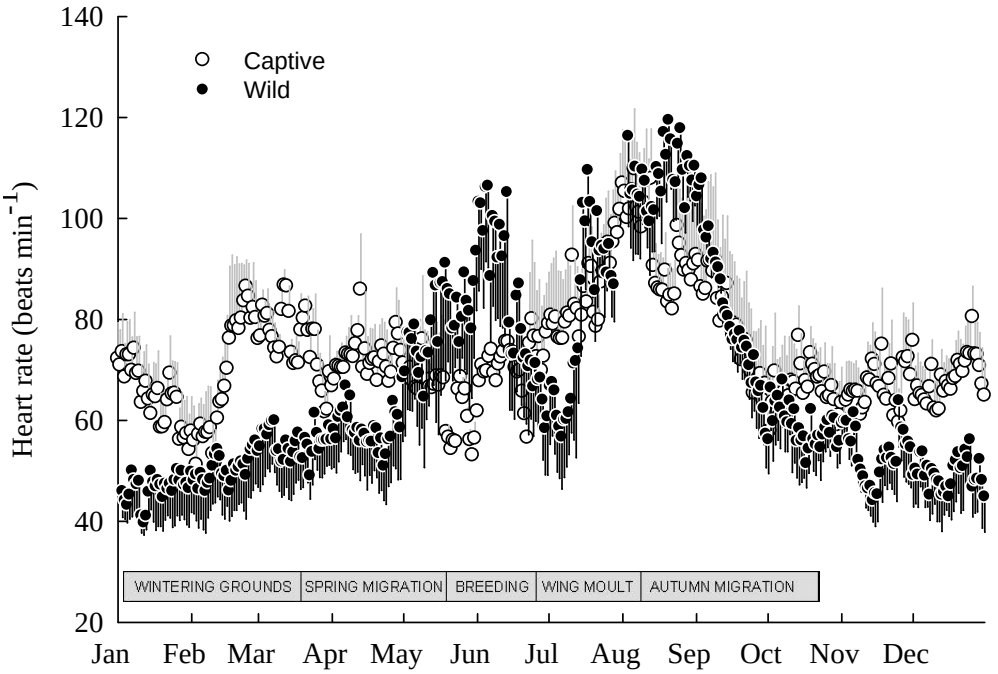


Figure VII.1: Year-round minimum daily heart rate (± SEM) from six wild and 20 captive barnacle geese. Closed circles refer to wild birds, and open circles, captive birds. Minimum daily heart rate changed significantly throughout the annual cycle (repeated measures ANOVA, $P < 0.001$ for both) and was at its highest during the annual wing moult for both the captive and wild birds.

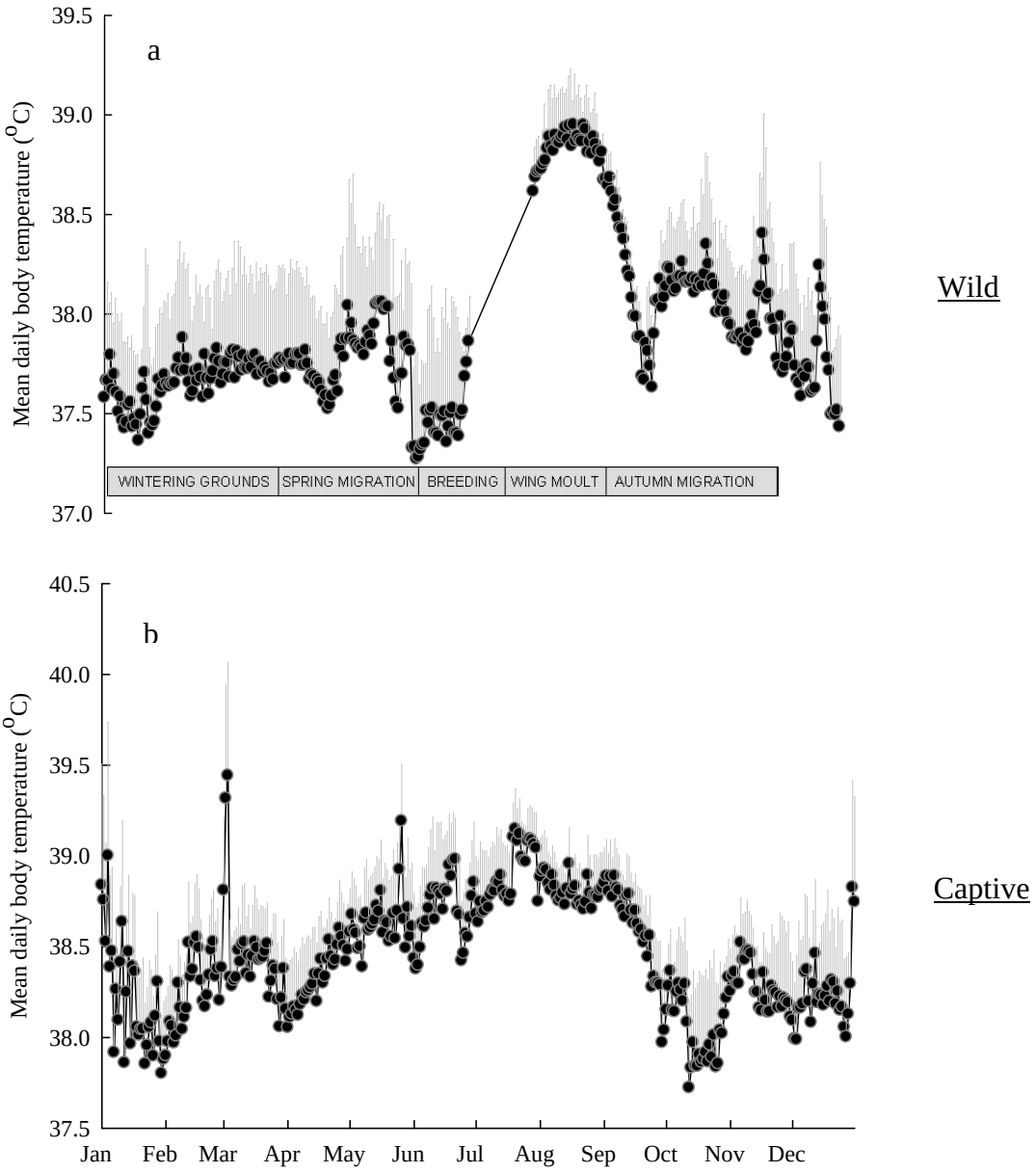


Figure VII.2: Year-round mean (± SEM) daily abdominal body temperature in (a) six wild and (b) 20 captive barnacle geese. There was insufficient data from the wild birds for the month of July due to logger failure. Mean daily abdominal body temperature changed significantly throughout the annual cycle in both wild and captive geese (repeated measures ANOVA, $P < 0.001$ for both).

This thesis so far has produced the following papers:

White, C. R., Cassey, P., Schimpf, N., Green, J. A., Halsey, L. G. & Portugal, S. J. (2013) Implantation reduces the negative effects of bio-logging devices on birds. *Journal of Experimental Biology*. **216**: 537-542.

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Portugal, S. J., Green, J. A., White, C. R., Guillemette, M. & Butler, P. J. (2013) Wild geese do not increase flight behaviour prior to migration. *Biology Letters*. **8**: 469-472. (http://rsbl.royalsocietypublishing.org/content/early/2011/11/07/rsbl.2011.0975.full).

Guillemette, M. G., Richman, S. E., Portugal, S. J., & Butler, P. J. (2012) Behavioural compensation reduces energy expenditure during migration hyperphagia in a large bird. *Functional Ecology*. **26**: 876-883.
(http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2435.2012.01993.x/abstract)

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Portugal, S. J., Butler, P. J., Green, J. A. & Cassey, P. (2011) Indications of phenotypic plasticity in moulting birds: captive geese reveal adaptive changes in mineralisation of their long bones during wing moult. *Journal of Ornithology*. **152**: 1055-1061. (http://www.springerlink.com/content/6437466461022617/)

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(http://www.springerlink.com/content/b5154v68m9322324/)

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(http://jeb.biologists.org/content/212/15/2403.short)

Halsey, L. G., Portugal, S. J., Smith, J. A., Murn, C. A. & Wilson, R. P. (2009) Recording raptor behaviour on the wing via accelerometry. *Journal of Field Ornithology*. **80**: 171-177.

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