The causes of individual and seasonal variation in the metabolic rate of knot calidris canutus

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by

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Abstract

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By Colin Selman **Ph.D. 1998**

Basal metabolic rate (BMR), an individual bird's minimum rate of energy expenditure, was followed in adult and juvenile captive Knot throughout their annual cycle, in conjunction with measurements of total body mass (BM) and body composition (lean mass and fat mass, as predicted using Total Body Electrical Conductivity). Adult captive Knot increased significantly in BM during spring, primarily due to fat deposition. Most juvenile Knot did not display fat deposition in their first spring in captivity.

A seasonal peak in BMR, often double the seasonal minimum, occurred during spring but typically took place, on average, 5,11 and 4 days (respectively) after the seasonal peaks in BM, lean mass and fat mass. Little of the variation in BMR seen within or amongst captive Knot, irrespective of physiological state, was explained by variation in a single parameter (BM, lean mass or fat mass). As variation in BMR was not simply a consequence of variation in total lean mass, the average metabolic output per gram of the lean tissues must also have altered seasonally.

During fat deposition in spring, Knot exhibited a significant increase in liver mass and a significant elevation (approximately 50% higher) in the activity of succinate dehydrogenase (SDH, an indicator of metabolic activity) in the small intestine. Such adaptations may have assisted an increase in fat deposition rate. SDH activity decreased by approximately 60% in the pectoral muscle of Knot during this period. Such a reduction in SDH may also aid fat deposition as it lowered an individual's overall BMR. As Knot BM decreased after the spring peak, their BMR decreased in parallel with a decrease in SDH activity in their pectoral muscles.

The spring peak in overall BMR may indicate an increase in the maximal sustainable metabolic rate (MMR) of an individual during migratory flight. If a relationship exists between BMR and MMR, then variation in metabolic activity rather than variation in the mass of various lean tissues (e.g. pectoral muscle) will increase metabolic scope without increasing the energetic costs of flight.

No part of this thesis has previously been submitted for a degree in this or any other university. The work described is my own except where duly acknowledged.

CONTENTS

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To my family and my brilliant friend Kev O'Reilly (1972-1998)

General introduction

1.1 Overview

This thesis is concerned with variation in basal metabolic rate (BMR), both amongst and (seasonally) within individuals of the same species and the factors that lead to this variation. This study examined the role that seasonal variations in body mass and body composition (total lean mass and total fat mass, predicted using Total Body Electrical Conductivity, TOBEC) have in altering an individual's BMR. The lean tissues are known to generate almost all of the metabolic heat produced by an individual and differences in the metabolic output per gram of various lean tissues are known to exist. Therefore this thesis reports investigations to test whether intraspecific and seasonal variation in BMR can be explained by differences in the relative masses of various metabolically active organs and tissues that make up total lean mass. Some of the variation in BMR seen amongst and within individuals may also be due to differences occurring in the metabolic activity per gram of these various lean tissues and organs. Therefore differences in BMR occurring amongst and within individuals were investigated through the measurement of the mitochondrial volume composition of various lean tissues and through the measurement of aerobic enzyme activities per gram of these lean tissues. Both procedures give indirect measures of metabolic activity.

1.2 Interspecific variation in basal metabolic rate (BMR)

Basal metabolic rate (BMR) is the minimum rate of energy expenditure by a non-growing, non-reproductive homeotherm and is measured under postabsorptive and thermoneutral conditions, in the inactive phase of the circadian cycle (Aschoff & Pohl, 1970; Speakman *et al*, 1994; McNab, 1997). BMR has been described as the minimum energetic cost of maintaining cells and organs in readiness for higher levels of activity (Ricklefs *et al*, 1996).

The first study of metabolic rate and its relationship with total body mass was carried out over 60 years ago by Kleiber (1932). He investigated the relationship in different species of homeotherms, by surveying a range of mammals varying in size from rats to cattle. Between them they exhibited a 4000-fold difference in BM. Kleiber (1932) expressed his findings allometrically and found that the line of best fit for his data was described by the equation:

$$\mathbf{MR = 73.3\ BM^{0.74}},$$

where MR was metabolic rate (kcal/day) and BM was body mass (kg).
The mass exponent of 0.74 so derived was later increased to 0.75 (to aid ease of calculation), and this value of 0.75 has since been cited as a classic example of a physiological variable scaling to body mass (Hayssen & Lacy, 1985). The relationship is generally expressed logarithmically as:

$$\mathbf{Log\ MR = Log\ a + b\ Log\ BM},$$

where a = mass coefficient and b= mass exponent.

Kleiber's (1932) findings were later extended by far more extensive studies (Brody *et al*, 1934; Benedict, 1938). These workers found that amongst species of both birds and mammals, the metabolic rates tended to lie very close to a regression line with a slope of 0.75, and that the relationship between BMR and BM was not simply therefore a consequence of body surface to volume ratio, where the mass exponent would be closer to 0.67 (Rubner, 1883). This interspecific allometric equation with a slope of 0.75 is often termed the Brody-Kleiber Law.

The relationship has been shown to explain some 80% of the observed variation in BMR between different species of homeotherms (McNab, 1988). Much attention has been focused on the theoretical basis of this 0.75 mass exponent, but with little agreement on the physiological reasons behind it (Scott, 1991; West *et al*, 1997). It has been postulated (McMahon, 1973; Speakman *et al*, 1994) that as animals become larger they do not retain geometric similarity, which would predict a slope of 0.67, but are designed with elastic similarity which predicts a mass exponent close to the 0.75 calculated by Kleiber (1932; 1961). There

appears however to be little direct empirical support for the elastic similarity hypothesis (see Norberg, 1981). Porter & Brand (1993) postulate that some of the differences seen in metabolic rate between mammals of different mass, may be attributable to differences between them in the rate of "proton leak". They suggest that this proton leak, the futile cycle of proton pumping and proton leak across the mitochondrial inner membrane, may account for a significant proportion of the oxygen consumed by a mammal at rest and that proton leak decreases with increasing body mass.

1.3 Deviations from the interspecific mass exponent of 0.75

Recently it has been shown, both statistically (Heusner, 1982, 1991a) and empirically (Bartels, 1982; Hayssen & Lacy, 1985; Daan *et al* 1989, 1990; Kirkwood, 1991; Piersma *et al* 1995; 1996; Weber & Piersma 1996; Scott *et al*, 1996) that considerable deviations from the interspecific slope of 0.75 exist, and that the 0.75 mass exponent is not a constant characteristic of entire classes, orders or even species (Hayssen & Lacy, 1985). In mammals it has been shown that the mean mass exponent increases from 0.60 for species within a genera to 0.83 for orders within a class (Elgar & Harvey, 1987). On the contrary, the mean mass exponent in birds decreases from 0.82 for species within a genus, to 0.62 for orders within a class (Bennet & Harvey, 1988). The mass exponent for all birds has been calculated as being between 0.66 and 0.68 (Kendeigh *et al*, 1977; Daan *et al*, 1989).

It has been widely accepted that passerine bird species have higher BMRs than do nonpasserines of a comparable BM (Lasiewski & Dawson, 1967; Aschoff & Pohl, 1970). Shorebirds, of the family *Charadriidae*, are often cited as a group of birds which tend to have higher BMRs than expected allometrically (Castro, 1987; Kersten & Piersma, 1987; Scott, 1991). Recently however, doubts have arisen regarding the differences thought to exist in BMR between passerines and nonpasserines of a given mass. Reynolds & Lee III (1996) suggest that once both phylogeny and body mass effects are accounted for, no differences in the

metabolic rates of passerines and non passerines actually exist and that no differences exist in metabolic rate between 'extreme' groups of birds, e.g. shorebirds and 'conventional' groups. Other reasons why orders, families and species deviate from the 0.75 interspecific mass exponent have been cited, e.g. the effect of diet and habitat preference (McNab, 1988) and latitude (Weathers, 1979; Piersma *et al*, 1996). Potential theoretical problems may also arise in the study of the relationship between BMR and BM, due to small sample sizes (Scott, 1991) and also the repeatability of the indirect calorimetry measurements used in the analyses (Hayes *et al*, 1992, Speakman *et al*, 1993).

Only very recently have studies begun to investigate the intraspecific and intraindividual relationships that exist between BMR and body mass. Most of these studies have investigated this relationship within species of birds (Daan *et al*, 1989; Scott, 1991; Piersma, 1994; Piersma *et al*, 1995, 1996; Scott *et al*, 1996). Considerable deviations from the mean interspecific mass exponent calculated for all birds of around 0.67 (Aschoff & Pohl, 1970) have been shown to exist when the relationship between Log BMR and Log BM is analysed both intraspecifically (Scott *et al*, 1996) and particularly when this relationship is examined intraindividually (Daan *et al*, 1989; Piersma *et al*, 1995; Scott *et al*, 1996). Daan *et al* (1989) reported that the intraspecific mass exponent (± standard error) calculated for 20 captive Kestrels *Falco tinnunculus* was 0.790(±0.226), a value not significantly different from that calculated for all birds (Aschoff & Pohl, 1970). Recently Weber & Piersma (1996) found that the intraspecific mass exponent for 14 captive Knot (subspecies *islandica*), losing mass after spring BM peak was 0.690(±0.223), which although lower than that calculated by Daan *et al* (1989) and Aschoff & Pohl (1970), was not significantly different from the interspecific mass exponent (0.729±0.214) calculated by Kersten & Piersma (1987) for 6 shorebird species. Scott *et al* (1996) recently showed that the mean mass exponent produced for 21 captive Redshank *Tringa totanus*, of 1.02(±0.21), did differ significantly from the interspecific value calculated for all birds or indeed for shorebirds alone (Kersten & Piersma, 1987). This exponent of 1.02 is significantly greater than that for homorphic change

(0.667; Heusner 1984).

The mass exponents produced when Log BMR is regressed against Log BM intraindividually has consistently produced values in excess of 1.0 (mass proportionality). Daan *et al* (1989) calculated that the mean mass exponent produced for 4 captive Kestrels was 1.66(±0.190), with similarly high mean mass exponents being produced for 3 captive Knot (1.38±0.398, Piersma *et al*, 1995) and 21 captive Redshank (1.23±0.110, Scott *et al*, 1996). Piersma *et al* (1995) suggested that the high mean value calculated in captive Knot arose because the mass of the metabolically active lean tissues altered more in the course of a individual Knot's annual cycle than its body mass (although body composition was not followed in their study). Some of this intraindividual variation in BMR has been shown to occur on a seasonal basis (Daan *et al*, 1989; Piersma *et al*, 1995) and these findings and the possible causes for this will now be discussed.

1.4 Seasonality in BMR within individuals and its consequences

Seasonal variation in BMR has been shown to occur in certain species of birds (Daan *et al*, 1989; Cadee, 1992; Piersma, 1994; Piersma *et al*, 1995) and mammals ('resting metabolic rate', McDevitt & Andrews, 1995). Daan *et al* (1989) followed BMR and total body mass in 4 captive Kestrels at fortnightly intervals throughout their annual cycle. They found that BMR tended to be elevated, for a given body mass, during the moult (June-October). Variation in BMR has also been reported to occur within individual captive Knot during their annual cycle (Cadee, 1992; Piersma, 1994; Piersma *et al*, 1995). Indeed Piersma *et al* (1995) reported 'pronounced seasonal variability' in BMR existing in 3 captive Knot, with peak values occurring 'during the early summer peaks in BM'. The BMR in each of their 3 Knot was only measured once every 6 weeks, although BM was measured weekly. Recently Weber & Piersma (1996) showed that a single individual captive Knot decreased from a peak body mass in spring of 214 grams to a BM of 98g in only 24 days. Because of this rapid rate of BM change, it is likely that the extent of seasonal variation in BMR during the spring

period of BM increase and then decrease was underestimated due to the infrequency of BMR measurements.

Although seasonal variation in BMR has been shown to take place within individual birds (Daan *et al*, 1989, Cadee, 1992; Piersma *et al*, 1995), these findings have all been based on extremely small sample sizes, and no attempts were made to monitor any seasonal changes within individuals in body composition, i.e. lean mass and fat mass. Both the relative and absolute masses of the metabolically active lean tissues (Marsh, 1983; Evans, 1992; Piersma & Lindstrom, 1997; Piersma & Gill Jr, 1998) and the mass of the fat stores are known to vary seasonally, even in captivity (see Scott *et al* 1994). Although avian adipose tissue is metabolically relatively inactive (Scott & Evans, 1992), the indirect costs of carrying and maintaining this fat may be considerable (Witter & Cuthill, 1993; Scott *et al*, 1996). There is also strong evidence that the metabolic activity per gram of various lean tissues, as indicated by both aerobic enzyme activity (Lundgren & Keissling, 1985; 1986; Lungren, 1988) and mitochondrial volume composition (Evans *et al*, 1992) may alter seasonally, leading to variation in the metabolic output per gram of the lean tissues.

Piersma *et al* (1995) calculated that the BMR of individual Knot increased on average by over 200% from its seasonal minima to its seasonal maxima, despite the seasonal increase in BM only being around 50%. Therefore, there is good evidence that the relationship between BMR and BM may change seasonally within an individual, and that the factors that lead to differences in BMR, both between and within individuals, may also differ at different times of the year, when individuals are in different physiological states, e.g. waders during mid winter and during pre-migratory fattening. These findings make it imperative that any study that attempts to investigate the causes of variation in BMR both between and within individuals or produce valid intraspecific or intraspecific regression equations between BMR and BM must compare individuals that are in similar physiological states to one another.

Although Daan *et al* (1989) were probably the first to show that seasonal variation did occur in BMR within individuals, it appears that they did not control for the physiological state of an individual when calculating the intraspecific mass exponent. They also pooled the data collected for each sex together, even though Kestrels are known to be sexually dimorphic with males, for a given mass, tending to have BMR on average some 12% higher than females (Daan *et al*, 1989). Many of the interspecific studies produced in the recent past do not seem to have paid attention to the potential problems that may be introduced by seasonality in BMR (e.g. Bennet & Harvey, 1988; Daan *et al*, 1989; 1990; but see also Bryant & Tatner, 1991). Kersten & Piersma (1987) for instance formulate the energetic margin hypothesis to help explain the higher than average BMRs seen, for a given mass, in 6 species of shorebirds. They themselves measured BMR in Turnstone *Arenaria interpres*, Grey Plover *Pluvialis squatarola* and Oystercatcher *Haematopus ostralegus*, all of which varied in sex, age and date of capture, i.e. some individuals were likely to be summer passage birds and some likely to be overwintering residents. Kersten & Piersma (1987) also do not give any indication of the time of year when BMR was measured, although it is known that the study lasted from October 1980-July 1983. Therefore, it is not known whether the waders used to produce their interspecific allometric equation were in a similar physiological state, both intraspecifically and interspecifically.

Recently, attempts have been made to control for the inherent seasonality in BMR within individuals, by using individuals in similar physiological states to one another. Scott *et al* (1996) investigated how variations in body composition affected the BMR of 21 captive Redshank *Tringa totanus*. They controlled for seasonal variation in BMR, by using mean values of BMR and body mass components for each individual bird measured repeatedly outside the non-breeding season, i.e. a period of relatively stable BM and body composition. Scott *et al* (1996) did not however indicate whether the 21 Redshank used in their study were of the *robusta* or *britannica* race. Mitchell (1996) showed that Redshank of the subspecies *robusta* had significantly higher mass-specific metabolic rates than the *britannica* subspecies, although no significant difference in BM existed

between the two subspecies. Therefore using a mixture of both races may have affected the validity of their findings (see Scott, 1991). Weber & Piersma (1996) have also recently addressed the potential problems posed by seasonality in BMR by using in their study only captive Knot that were decreasing in mass after the spring migratory peak, thereby being in a similar physiological state to one another.

1.5 The ecological implications of a seasonally varying level of BMR

Much recent research into the energetics of mammals and birds has sought to investigate the presence of a relationship between BMR and daily energy expenditure (DEE). The amplitude through which metabolic rate can vary is termed the metabolic scope (Fry, 1947). The first attempt to quantify the relationship between BMR and DEE was carried out by Drent & Daan (1980). They suggested that four times BMR was the optimal working capacity, beyond which energy expenditure in the long term would inflict some subsequent fitness cost, i.e. reduced survival. More recently it has been shown that 4 times BMR is not an ubiquitous upper limit to sustained work rate in small birds, and that in the short term the peak metabolic rate can be considerably higher, although this peak rate cannot be maintained for periods greater than several minutes or hours (Peterson *et al*, 1990). Indeed, Bryant & Tatner (1991) reported that DEE during brood provisioning exceeded 4 times BMR in 48% of birds species investigated in their study.

As mentioned previously, Kersten & Piersma (1987) postulated the energetic margin hypothesis to help explain the higher than expected BMR, for a given body mass, seen in some species of shorebirds that experienced energetically costly climatic conditions in the non breeding season and/or long-distance migratory flight. They suggested that during such periods of high energetic demand, a high DEE (primarily generated by the skeletal muscles) would be required to increase the maximal sustainable working level and thereby, enable

these birds to cope energetically. A high DEE was acted on by natural selection, and the elevated BMR measured in these shorebirds was simply a consequence of the high level of support needed during periods of peak energy demand. This support was provided by the highly metabolically active organs of the abdominal cavity, e.g. liver, heart and kidney. The energetic margin hypothesis is similar to the 'power strategy' theory proposed by Gnaiger (1987). He suggested that species adopting a power strategy (high total power output), due to a high daily energy expenditure, would require adaptations for this, that included large muscle masses, high mitochondrial volume composition in the lean tissues and a high degree of alimentary tract digestive efficiency. These adaptations will in turn support the high rates of energy acquisition and processing required, leading to a high BMR, assuming there is a constant ratio between BMR and DEE.

A major assumption of the energetic margin hypothesis (Kersten & Piersma, 1987) is that there is constant proportionality between BMR and DEE. The evidence of such a relationship does not generally have sound experimental support in the literature (Koteja, 1991) and recently it has been shown statistically that there appears only to be an interspecific relationship between maximal sustainable metabolic rate (MMR) and BMR in mammals, but not in birds (Ricklefs *et al*, 1996). Meerlo *et al* (1997) recently failed to find either an intraspecific or an intraindividual association between BMR and overall energy expenditure in the Field vole *Microtus agrestis*. Contrary to the findings of Ricklefs *et al* (1996), Dutenhoffer & Swanson (1996) reported that a significant correlation (r=0.861) existed interspecifically between BMR and maximal cold induced 'summit' metabolism in 10 species of passerine. Dutenhoffer & Swanson (1996) controlled for any complications that may have arisen from using different experimental techniques (see Daan *et al*, 1990) by using open-flow respirometry to measure BMR and MMR (maximum cold induced 'summit' metabolic) throughout their study. The data used by Ricklefs *et al* (1996) was originally collected from a wide range of studies, using a wide range of experimental techniques, by Daan *et al* (1991).

The energetic margin hypothesis of Kersten & Piersma (1987) suggests that individuals increase their MMR by increasing their total lean mass or by altering the percentage composition of the tissues that make up this metabolically active lean mass. Recent studies (Daan *et al*, 1990, 1991; Piersma, 1994; Lindstrom & Kvist, 1995; Piersma *et al*, 1995,1996; Weber & Piersma 1996, Lindstrom, 1997) all suggest that the mass of the metabolically active tissues and organs may increase during periods of peak energy demand, i.e. migratory flight, when an increased MMR is required, with BMR increasing as a consequence. There is however is little direct evidence that the mass of the various organs are increased during migratory flight itself. None of these studies, with the exception of Weber & Piersma (1996), investigated whether variation occurred in the metabolic activity per gram of the various lean tissues and few took into consideration the physiological state of the study species used. Weber & Piersma (1996) measured metabolic activity in various lean tissues of captive Knot by assaying for the aerobic enzyme cytochrome c-oxidase.

1.6 The possible causes of intraspecific and seasonal variation in BMR

As mentioned previously, only fairly recently have comparative studies attempted to unravel the factors that lie behind intraspecific and seasonal variation in BMR. Most studies have attempted to identify the factors that led to differences in BMR amongst individuals, by using individuals of the same species but differing in physiological condition (Daan *et al*, 1989; Konarzewski & Diamond, 1994; Speakman & McQueenie, 1996), or by comparing individuals of distinct subspecies (Konarzewski & Diamond, 1995; Piersma *et al*, 1995). Few studies have attempted to identify the causes of variation in BMR amongst or within individuals that are in physiologically similar states (see Scott *et al*, 1996; Weber & Piersma, 1996), i.e. little attention has been paid to the potential effects of seasonality. No study has actually attempted to identify whether the factors that lead to differences amongst individuals in one physiological state, are the same as those that lead to differences in BMR in another physiological state.

Most lean tissues and organs such as liver and skeletal muscle, in both birds (Scott & Evans, 1992) and mammals (Field *et al*, 1939; Krebs, 1950; Wheeler, 1984), have considerably higher metabolic activities per gram than tissues such as white adipose tissue, skin and bone. Therefore, the vast majority of the metabolic heat produced by an individual is derived from its lean tissues. Seasonal variation in the mass of these metabolically active organs is known to take place, particularly in premigratory and moulting birds (Evans, 1969; Fry *et al*, 1970; Marsh, 1984; Gaunt, 1990; Evans, 1992; Piersma & Lindstrom, 1997; Piersma & Gill Jr, 1998). Therefore, seasonal variation in the absolute and/or relative mass of these organs may lead to intraspecific and seasonal variation in the BMR.

There is evidence from the literature that some of the variation seen in BMR, usually between physiologically distinct groups of individuals, can be explained by differences amongst them in the mass of the metabolically active lean tissues. Daan *et al* (1989) surmised that the variation in BMR seen between two distinct physiological groups of captive Kestrel (n = 4 in each, high maintenance or low maintenance diets), primarily reflected differences between the groups in the mass of the heart and kidney. The mean differences recorded in BMR between two subspecies of Knot (*islandica* and *canutus*, both wild and captive) by Piersma *et al* (1996), were highly correlated with mean differences between groups in both total lean mass and mass of the 'nutritional organs', i.e. stomach, intestine, kidneys and liver. Similar work has recently been carried out in mammals, e.g. Konarzewski & Diamond (1994) reported that mice *Mus musculus* with higher than average 'resting' metabolic rates tended to have 'unusually' large hearts, kidneys and intestines. Follow up work by Konarzewski & Diamond (1995) discovered that subspecies of mice with exceptionally high (or low) BMRs tended to have disproportionately large (or small) intestine, liver, heart and kidney masses and Speakman & McQueenie (1996) suggested that the hypertrophy of the alimentary tract and associated organs led to a consequential increase in the BMR of gestating and lactating mice. Meerlo *et al* (1997) also put forward the idea that intraspecific variation in BMR amongst Field vole *Microtus agrestis* reflected differences amongst them in the mass of the metabolically active organs, although

they found a positive correlation only between lean dry heart mass and BMR. None of the above workers attempted to investigate whether intraspecific variation in the metabolic activity per gram of various lean tissues existed and whether this could have explained some of the variation in BMR seen.

Recently Scott *et al* (1996) showed that the variations in BMR amongst captive Redshank, measured outside the breeding season and therefore similar in physiological state, were explained more by variation in total lean mass (predicted using Total Body Electrical Conductivity), than by differences in total body mass or predicted fat mass. However, no attempt was made in their study to determine whether differences in organ masses or metabolic activity per gram of the lean tissues could explain any of the intraspecific variation in BMR. Weber & Piersma (1996) also attempted to determine the causes of intraspecific variation in BMR in a group of 14 captive Knot (subspecies *islandica*), which were all in a similar physiological state, i.e. losing body mass after spring peak. They examined whether the variability seen in BMR amongst these birds was; a function simply of body mass or body composition (total lean and fat mass), due to alterations in the mass of various metabolically active organs and tissues, or was due to changes in the metabolic activity per gram of these lean tissues (measured by cytochrome c oxidase activity, an indicator of metabolic activity). They concluded that intraspecific variation in BMR was better explained by variation in the mass of the heart (r^2 =0.441) and flight muscle (r^2 = 0.551), rather than by variation in the metabolic activity per gram of these tissues (r^2 = 0.001 and 0.072 respectively). The levels of cytochrome c oxidase activity measured by Weber & Piersma (1996) were highest in heart and pectoral muscle and lowest in liver and kidney. These findings are slightly strange given that in most vertebrate tissues the liver and kidney tend to have the highest metabolic activities per gram measured of any tissues (Field *et al*, 1939; Else & Hulbert, 1981).

Variation amongst individuals in the metabolic activity per gram of the lean tissues could be indicated by variation in the volume composition of mitochondria contained within the lean tissues, and/or by variation in activities per gram of

various aerobic enzymes contained within the mitochondria, e.g. citrate synthase (CS) and succinate dehydrogenase (SDH). The volume density of mitochondria is known to be an adequate estimator of oxygen consumption in mammalian muscle (Mathie *et al*, 1981). Evans *et al* (1992) examined mitochondrial volume composition in flight muscle of Dunlin *Calidris alpina* and Sanderling *Calidris alba*, during winter and just before spring migration. The proportion of myofibrils in the flight muscles decreased in spring, with a compensatory increase in the proportion of mitochondria. This would suggest that the metabolic activity per gram of the flight muscle increased prior to migration, possibly to increase power output of the flight muscles during migratory flight (Pennycuick & Rezende, 1984).

There is some direct evidence from the literature that the activities per gram of various aerobic enzymes alter on a seasonal basis. Saunders & Klemm (1994) showed that CS activity in the flight muscle of wild Blue-winged teal *Anas discors* was significantly lower during moult, a period which coincides with flightlessness and atrophy of the flight muscle. During this period the activity of CS in the leg muscle of these birds actually increased. A seasonal increase in CS activity has been reported in the pectoral muscle of migratory Reed warblers *Acrocephalus scirpaceus,* when compared to premigratory conspecifics (Lundgren & Keissling, 1986). The activity of CS was also higher in several migrating species of passerines when compared to breeding conspecifics (Lundgren & Keissling, 1985), and higher in migratory individuals, when compared to non-migratory birds of the same species (Lundgren, 1988). These authors suggest that the increases in CS seen during migration are due to the high energy consumption rate of the flight muscle during this time. In other studies however, there is scant evidence that the activities of various enzymes alter amongst individuals on a seasonal basis (Marsh, 1981; Yacoe & Dawson, 1983; O'Connor, 1995; Weber & Piersma, (1996). Indeed, Marsh (1981) suggested that any increase in the total aerobic capacity of flight muscle in Grey catbirds *Dumetella carolinensis* was due solely to flight muscle hypertrophy and not to changes occurring in the metabolic activity per gram of the muscles.

Few studies have attempted to address the causes of seasonal variation in BMR within individuals. Piersma *et al* (1995), in following BMR throughout the annual cycle of 3 captive Knot, implied that the seasonal variation seen in BMR was due to variation in the mass of the lean tissue, although this was not measured in their study and BMR was measured only at 6 week intervals. Scott *et al* (1996) reported that the variation in BMR within captive Redshank, measured during the non-breeding season, was predicted better by differences in total fat mass carried than by differences occurring in lean mass. Lean mass actually remained fairly stable within individual Redshank during the period of study. The direct contribution of fat to an individual's BMR is likely to be small because white adipose tissue has a relatively low metabolic activity per gram, when compared to various lean tissues and organs (Krebs, 1950 for mammals; Scott & Evans, 1992 for birds). Therefore, any effects that fat has on an individual's BMR are likely to be indirect and result from carrying and maintenance of this fat mass (see Witter & Cuthill, 1993), rather than due to the direct respiration of the adipocytes themselves. The class Aves also do not appear to possess the thermogenic brown adipose tissue, so typical of hibernating and neonatal mammals (Saarela *et al* 1989; Brightman & Trayhurn, 1994).

The findings of Scott *et al* (1996) demonstrate that the average metabolic activity of the lean tissues must be changing seasonally within individuals. This variation is due either to the metabolic activity per gram of the lean tissues and organs changing seasonally, or the relative composition of the individual tissues and organs that make up total lean mass varying seasonally, or a combination of both. Therefore, it can clearly be seen that there is a dearth of published literature into what causes variation in BMR both amongst and within individuals of the same species, whether the causes are the same in animals in different physiological states and what leads to the clear seasonal variation that has been seen within individuals.

1.7 Aims of study

This study aimed to identify the causes of individual and seasonal variation in basal metabolic rate (BMR) in captive adult and juvenile Knot *Calidris canutus*. BMR, as determined through open-flow respirometry, was followed intensively throughout an individual's annual cycle, in conjunction with long-term measurements of total body mass and body composition (Predicted total lean mass and predicted total fat mass, as determined using Total Body Electrical Conductivity, 'TOBEC'). TOBEC enables the role that seasonal variation in body composition has on an individual's BMR to be investigated non-invasively. The frequency of BMR and TOBEC measurements taken on individual birds was increased significantly during the period in spring of considerable body mass increase then decrease. This period coincides with that period in the wild of preparation for, and then migration to the breeding grounds. A comparison of birds in three distinct physiological states was made: **i)** outside the spring migratory period, **ii)** the period of BM increase to peak in spring **iii)** the period of BM decrease in spring, to determine whether seasonality in BMR could be explained by:

1. Seasonality in BM, lean mass or fat mass
2. Seasonal variation in the masses of metabolically active organs and tissues that make up total lean mass.
3. Seasonal variation in the metabolic activity per gram of these tissues as indicated by volume composition of mitochondria and/or seasonal variation in the activity of aerobic enzymes within the mitochondria.

The causes that lead to variation in BMR amongst individuals in the same physiological state were also examined, and I investigated whether the causes that lead to differences seen in BMR amongst individuals in one particular physiological state were the same as those that lead to variation amongst individuals in another physiological state. Differences amongst individuals in BMR were examined using the same criteria (1-3), employed to identify the causes of

variation within individuals.

1.8 Study species

Knot are long-distance migrant birds that breed in the high Arctic tundra and spend the nonbreeding season at a wide variety of lower latitudinal estuarine sites. The Knot used exclusively in this study were of the sub-species *islandica*, which breed in the high Greenland and Canadian Arctic and winter in Western Europe. Knot exhibit clear seasonal fluctuations in total body mass, due to variation in both the lean and fat body mass components (Evans 1992; Piersma, 1994; Piersma *et al*, 1995; 1996), and are a species of wader that are known to adapt well to the conditions of captivity (Cadee, 1992; Piersma, 1994) with their total body masses being seen to follow closely the seasonal pattern of change observed in wild conspecifics (Piersma & Davidson, 1992). Captive Knot have also be shown to exhibit seasonality in BMR, with levels of BMR tending to be elevated within individual birds during the spring, coinciding roughly with the seasonal peak in total body mass (Cadee, 1992; Piersma, 1994, Piersma *et al*, 1995). Therefore, because of the above factors, Knot are an excellent model species to use to try and identify the causes of both intraspecific and intraindividual variation in BMR.

Chapter 2.0: Materials and methods

2.1 Captive Knot

A total of 53 Knot *Calidris canutus* were caught under licence (English Nature) by cannon-netting on Teesside, north-east England and held in captivity for periods ranging from 6-30 months between November 1994 and July 1997. These birds were held in groups containing between 6-10 individuals in indoor aviaries of 2.4m (l) x 1.2m (h) x 1.3m (w) , under artificial light of a 'normal' day-length period (varying seasonally) and temperature close to ambient. Food was provided *ad libitum* and consisted of blow-fly larvae *Calliphora sp.* and commercial trout pellets (Trout Excel 23, Trouw Aquaculture, Nutreco, UK). A mineral supplement SA-37 (Intervet, UK Ltd, Cambridge) was applied to the food monthly. Fresh water flowed through the cages continuously for drinking purposes and additional baths were provided to enable bathing to take place.

2.2 Measurement of Total Body Electrical Conductivity (TOBEC)

Total Body Electrical Conductivity (TOBEC) was measured using a SA-1 Small Animal Body Composition Analyser (EM-SCAN, 3420 Constitution Drive, Springfield, Illinois 62707, USA). The SA-1 is a portable machine which can be used either in the laboratory or in the field. When used in the field the SA-1 was powered by a 12V battery via a converter (Oertling PC-01), to produce 240V AC. TOBEC measurements require the animal (Scott *et al*, 1991; Skagen *et al*, 1993; Mitchell, 1996) to be restrained and placed in a measurement chamber, which is surrounded by a solenoid. The Knot used in this study were restrained in a plastic cuff that was fastened around their body and legs with Velcro. The presence of the animal in the chamber acts as a conductor which alters the electromagnetic inductance of the

solenoid coil. This alteration is proportional to the total electrical conductivity of the animal's body. It is known that the electrical conductivity of lipids is around 4-5% that of lean tissues, body fluids and bone (Pethig, 1979). Therefore, the primary determinant of the TOBEC signal output is total lean mass.

When measuring an individual bird's TOBEC index, attention has to be paid to several factors. The subject has to be dry (Scott *et al*, 1991) and of a 'normal' hydration state (Walsberg, 1988). The presence of British metal identification rings may also increase TOBEC indices by up to 45% (Scott *et al*, 1991), although Castro *et al* (1990) found that metal identification rings had no effect on the TOBEC output. This is probably because the metal rings used in Castro *et al's* (1990) study were manufactured in the U.S.A., hence consisted of metals of low magnetic susceptibility, e.g. Aluminium (see Scott, 1991). Finally, care has to be taken to ensure that the horizontal position of subjects along the length of the TOBEC chamber is standardised. Scott *et al* (1991), employed the protocol (adopted in this study) whereby specimens were inserted head-first and keel down into the chamber, until a maximum difference in the TOBEC index was obtained between the empty and full chamber. Each pair of readings of empty and full chamber was repeated four times to avoid drift in the base-line.

An index of TOBEC (I) was calculated using the formula in equation 2.1;

$$\mathbf{I = (E - B) / R * a} \qquad \mathbf{2.1}$$

where **R** is the mean of two reference numbers taken before and after each set of four readings. **E** is the reading of the empty chamber and **B** is the reading with the subject in the chamber. A normalisation constant **a** was provided by EM-SCAN. Predicted total lean mass (PTLM), in grams was calculated from the linear regression equation 2.2, as seen in Appendix II (Equation 1). (This equation is specific to Knot).

$$\mathbf{PTLM = (0.199 * I) + 64.929} \qquad \mathbf{2.2}$$

The power of the intraspecific equation (2.2) to predict TLM was tested using an independent sample of 9 captive Knot (see Appendix II). The absolute mean error produced from using this intraspecific linear regression model of TLM and TOBEC (I) was 1.4±0.7g (95%CI), over a TLM range of 91.6-104.8g.

Predicted mass of fat (PFM), in grams was calculated by subtracting PTLM from total body mass (BM) (equation 2.3).

$$\mathbf{PFM = BM - PTLM} \qquad \mathbf{2.3}$$

2.3 Measurement of Basal Metabolic Rate: Open-Flow Respirometry

Basal Metabolic Rate (BMR), as mentioned in Chapter 1, is the minimum rate of energy expenditure in the thermoneutral zone (TNZ) of a non-growing, postabsorptive homeotherm at rest. In studies that measure BMR, it is therefore imperative that the experimental conditions applied are clearly defined and strictly adhered to.

BMR measurements were carried out on one or, more commonly, two birds/day, in an open-flow respirometry system. To keep the description of the methodology simple, the protocol is given for a single bird. Protocol for a pair of birds followed exactly the same pattern. Individual birds were placed in one of two identical sealed cylindrical metabolic chambers measuring 24.5 cm (height) and 21cm (diameter). All measurements were taken in complete darkness and at a constant temperature of 25°C in a controlled temperature cabinet (LMS, Sevenoaks, Kent). The temperature of 25°C is well within the thermoneutral zone (TNZ) of Knot (see Piersma, 1994) and therefore sufficient to counter any possible seasonal variations in the upper and lower limits of the TNZ within individual birds. BMR was measured throughout the annual cycle of captive Knot, with an increase in frequency during the spring period of fat

deposition and fat loss. During the 30 months of this study over 520 individual measures of BMR were carried out.

The amount of metabolic heat produced by a bird was estimated by measuring its oxygen consumption, using a paramagnetic oxygen analyser (Servomex plc, Crowborough, East Sussex, Model 1111D/OOO) and the amount of carbon dioxide it produced was measured using an infrared analyser (Mine safety Appliances Company, Pennsylvania, USA, Lira 3000). Dry air was drawn through the metabolic chamber at a rate of 60 $l.h^{-1}$. Gas analyses were performed on samples of both the inlet and outlet gases, regulated for flow using mass flow controllers (Brooks Instruments, Netherlands, 5878 & 5850 TR series) at rates of 4.8 $l.h^{-1}$ for O_2 and 36 $l.h^{-1}$ for CO_2. Both inlet and outlet gases were dried prior to measurement by passing them through columns of dried silica gel. Calibration of the two analysers was carried out daily, prior to measurement using both 100% N_2 (BOC), and a certified mixture of 21.2% O_2, 0.0311% CO_2 in N_2 (SIP Analytical Ltd).

Prior to measurement of BMR, a bird was removed from the aviary at 0900 GMT and kept in isolation for a minimum of 2 h^{-1} without food. Around 1100 GMT the bird was weighed to the nearest gram using a Pesola spring balance, a TOBEC measurement was then taken to determine body composition and then the subject was placed in one of the two identical metabolic chambers. Metabolic measurements commenced around 1400 GMT, after a period of around 4-5 h^{-1} fasting with around 3 h^{-1} acclimation to the metabolic chamber. The throughput of food in the gut of Knot has been estimated to be, at most, around 2 h^{-1} (Weber & Piersma, 1996). Therefore, the fasting time of 4-5 h^{-1} used in this study was sufficient to render all individuals postabsorptive and thereby remove any effect on O_2 consumption caused by the heat increment of feeding.

A measurement of BMR on a single individual was taken over a period of time ranging between 1-3 h^{-1}. This ensured that a uniform and stable measurement of BMR was obtained for all birds. When measurements were carried out on two birds on the same day, a period of 20 min^{-1} was required between the first and second individual's BMR measurement to enable the levels of O_2 and CO_2 to return to the pre-measurement baseline levels. To ensure that the individual not undergoing a BMR measurement did not suffer from hypercapnia due to a build up of CO_2, a constant flow of dry air was maintained using a simple diaphragm pump.

A key assumption when measuring BMR is that the subject is at rest, or that at least no 'abnormal' activity is taking place (Kleiber, 1961). Cursory checks of activity were not possible in this study because during the BMR measurements individuals were kept in opaque metabolic chambers, within a constant temperature cabinet. BMR was however measured in the dark, which is likely to minimise locomotory activity (see Bryant & Furness, 1995), although no indirect estimate of locomotory activity during a BMR measurement was employed during this study, e.g. Doppler radar or infra-red activity recorders. Occasionally however, transient increases in O_2 consumption were noted during a BMR run and were assumed to be brief bouts of activity. Due to the relatively small metabolic chamber used during this study, allied to the fairly high flow rates and the rapid response time of the Servomex oxygen transducer (Response time of 4 sec^{-1} from N_2 to 100% O_2 at 80ml min^{-1} flow, Servomex User Manual), such periods of elevated O_2 consumption were easily identified and were eliminated from BMR measurements. In figure 2.3.1, a typical trace for an individual Knot (Knot WGG) of both O_2 consumption and CO_2 production can be seen, with the arrow pointing to a brief period of elevated metabolism. Typically these periods of elevated metabolism (assumed to be due to activity) lasted no longer than 5 min^{-1}, with the metabolic rate increasing by around 10-15% of the basal level recorded. There was no evidence of any increase in the frequency of the periods of bouts of 'activity' during the spring, which may have been associated with Zugunruhe (migratory restlessness). In this study, captive Knot generally did not exhibit Zugunruhe (*pers. obs.*). This finding is consistent with studies of passerines, which tend to exhibit low levels of

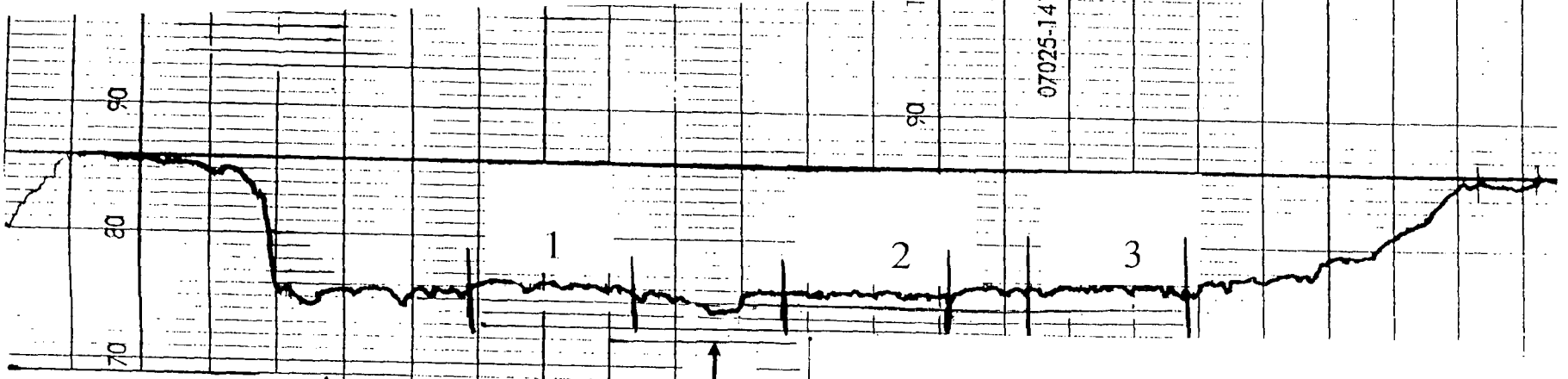
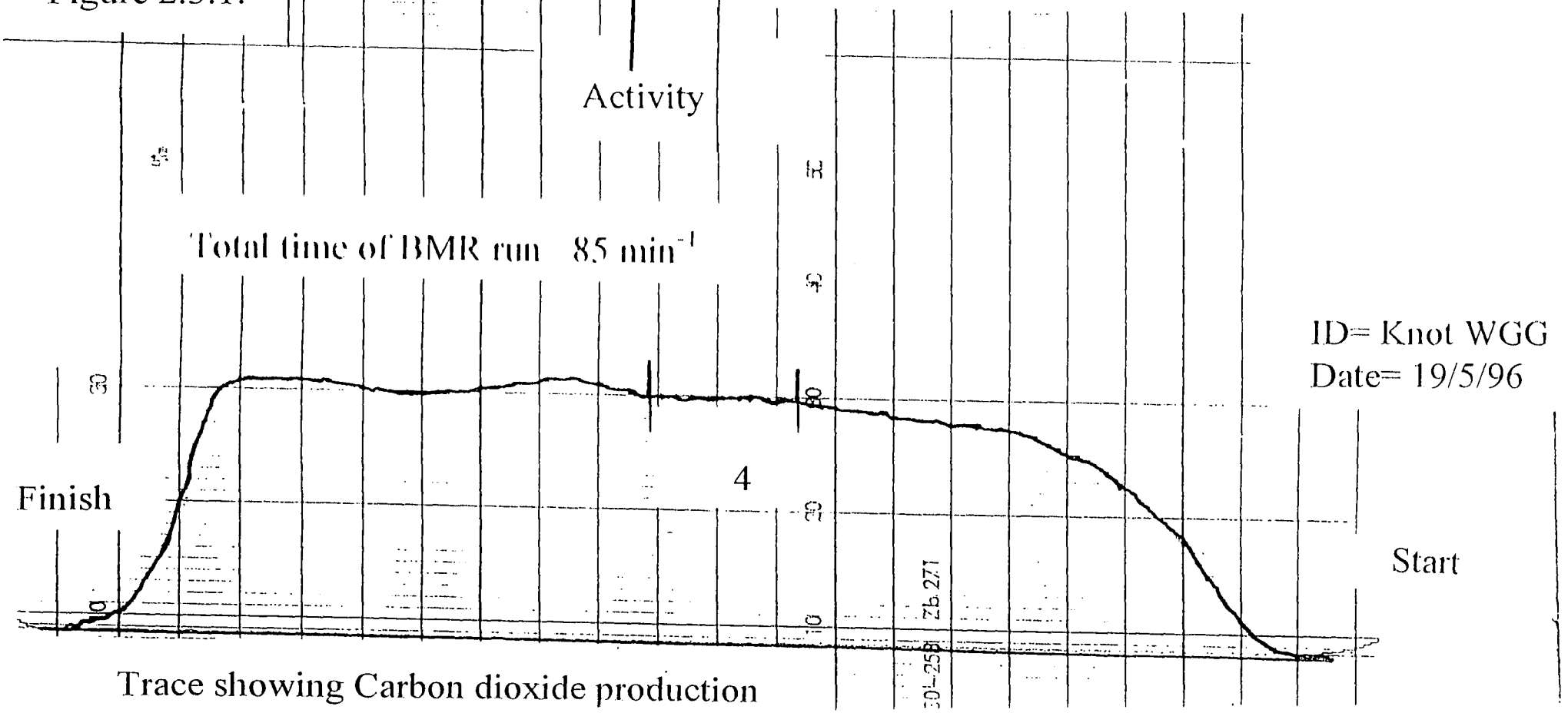


Figure 2.3.1: Trace showing Oxygen consumption

Activity



migratory restlessness if they have access to *ad lib* food (see Lindstrom & Kvist, 1995).

The levels of O_2 and CO_2 measured during a BMR run were recorded directly onto a flatbed recorder (Kipp and Zonen, Delft, Netherlands, Model BD 112). The mean O_2 level was then calculated from thirty readings (3 x 10 minute periods, see Figure 2.3.1., Points 1, 2, & 3) and CO_2 from 10 readings (Figure 2.3.1., Point 4), where the trace recorded was both stable and basal. Mean O_2 consumption was then used to estimate BMR (expressed in Watts) and CO_2 production/O_2 consumption was used to calculate RQ; using an energy value of:

Table 2.1

19.6kJ per litre O_2 consumed appropriate for a RQ of 0.70
19.8kJ per litre O_2 consumed appropriate for a RQ of 0.75
20.1kJ per litre O_2 consumed appropriate for a RQ of 0.80
20.3kJ per litre O_2 consumed appropriate for a RQ of 0.85
20.6kJ per litre O_2 consumed appropriate for a RQ of 0.90
20.8kJ per litre O_2 consumed appropriate for a RQ of 0.95
21.1kJ per litre O_2 consumed appropriate for a RQ of 1.00

Mass-specific BMR was calculated as BMR/BM^x expressed in mW/g, where x was the mass coefficient for BMR, i.e. the slope of the relationship between Log_{10} BMR and Log_{10} body mass in Knot. The relationship between BMR and mass is known to be allometric not isometric, i.e. the ratio between BMR/mass does vary with mass. It is therefore, not correct simply to divide BMR by mass as this does not remove all the variation due to mass (Packard & Boardman, 1987; Scott, 1991). Lean mass-specific BMR was calculated as $BMR/PTLM^{x\,1}$ expressed in mW/g of predicted lean tissue,

where x1 was the mass coefficient for BMR i.e. the slope of the relationship between Log_{10} BMR and Log_{10} PTLM in Knot.

2.4 Carcass and organ analysis and relationship between organ masses and BMR

All individuals sacrificed for scientific purposes (Sections 2.4, 2.5, 2.6 and Appendix II), were killed by cervical dislocation under licence from English Nature. Individuals that were used for carcass analysis, measurement of aerobic enzyme levels (Section 2.5) and mitochondrial counts (Section 2.6), were sacrificed 18 hours after measurement of BMR. This was to counteract any dehydration experienced immediately after a measurement of BMR. Knot on average lost around 6g in body weight during a BMR measurement and a large amount of this loss was probably due to dehydration, which could have interfered with estimation of TLM using TOBEC measurements.

On the day of sacrifice, individuals were removed from the aviaries, weighed to the nearest gram using a Pesola balance and a TOBEC measurement taken. Birds were then killed by cervical dislocation and the various organs removed immediately after death. The carcass and the various organs (liver, brain, gut, stomach, left pectoralis major, left supra-coracoideus, left kidney and heart) were then weighed to the nearest mg on a torsion balance. It is important to note that as much superficial lipid as possible was removed from the stomach and gut before these were weighed. The bird was then sexed by gonadal inspection, the gut length measured and four skeletal measurements were taken to the nearest 0.01mm using vernier callipers, following the methods of Piersma *et al.* (1984), in order to calculate a standard muscle volume SMV (Evans & Smith, 1975). The mass of one lean dry pectoral muscle block was then expressed as a proportion of the SMV. This produced an estimation of pectoral

muscle size independent of total body (skeletal) size, known as the standard muscle index (SMI).

The carcass and the tissues were then either sealed individually in plastic bags and frozen at -20°C until carcass analysis could be carried out at a later date, or they were immediately dried to constant dry mass at 40°C in a vacuum oven prior to solvent extraction. Storage lipids (tryglycerides), were extracted from the carcass and the dissected organs using a Sohxlet extractor with petroleum ether as a solvent (see Appendix II). All the dissected organs were ground in a pestle and mortar prior to fat extraction. The carcass and organs were subsequently dried once again to constant mass at 40°C in a vacuum oven and the fat-free masses were then obtained. Some individuals provided liver or heart tissue for enzyme assays, as well as for analysis of the relationship between BMR and organ masses. In these birds the wet mass of the intact organ was measured as well as the mass after removal of some tissue for enzyme analysis. Assuming that the water and fat content of the liver and heart tissue was uniformly distributed the actual dry and fat-free mass of the whole organ could be extrapolated.

2.5 Measurement of aerobic enzyme levels: Succinate dehydrogenase

Birds were killed by cervical dislocation and then the left deep aspect of the pectoralis muscle (see Deaton *et al*, 1996), gut, liver and heart were dissected out and weighed. Tissue samples, between 1.0-1.5g, were then frozen in liquid nitrogen (-196°C) within 5 minutes of death. Tissues were stored for 1-3 months at -80°C until enzyme analysis was carried out. The rate of decrease in light absorbancy at 550ηm, due to ferricyanide reduction by succinate, was used as a measure of enzyme activity.

Turn on spectrophotometer and allow it to warm up for at least two hours.

At the time of enzyme analysis the tissues were thawed, minced with a razor-blade, and then homogenised in 10 volumes of 20mM sodium phosphate buffer using a hand-held glass-glass homogenizer, maintained on ice. The homogenate was then sonicated in glass tubes for 3 x 15 sec, with a 45 sec pause on ice between each sonication. Homogenate was then transferred to 15 ml Eppendorf tubes and stored on ice (maximum of 1 h^{-1}), until the enzyme assays were performed.

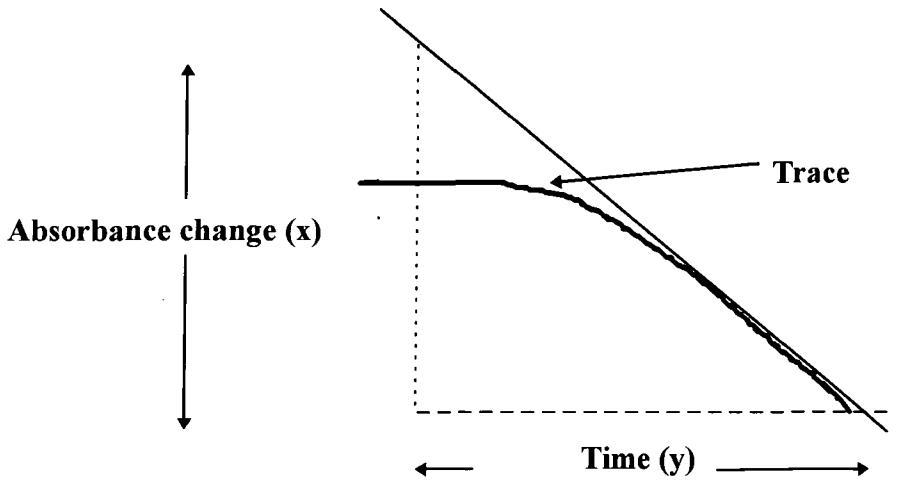
Spectrophotometric assays were then performed in duplicate/triplicate on the crude homogenates. Two cuvettes were prepared, each containing 0.6 mM sodium phosphate/ 1% (w/v) bovine serum albumin (pH 7.0) and 0.05 ml of 1% (w/v) cytochrome c. After mixing, the cuvettes were equilibrated at 37°C in a water bath. Membrane suspension (25µl) was then added to each cuvette and mixed well. The cuvettes were then read against each other in a dual beam Pye-Unicam SP8100 ultraviolet spectrophotometer. Two and a half minutes later, 0.2 ml of 10mM Potassium cyanide was added to the reference cuvette and 0.2 ml of 10mM KCN containing 50mM succinate was added to the test cuvette and both mixed well. The increase in absorbency at 550ηm was then measured at 37°C over the next 3-4 min.

Steps 1-6 follow calculations of R. Manning (*pers. com*).

1. Calculate the gradient $\frac{x}{y}$ at the steepest part of chart recorder trace (see Figure 2.5).

This will provide absorbance (Abs) change over time.
(Time = 1 minute to ease calculations).

Figure 2.5. Example chart recorder trace



N.B.: Prior to <u>each</u> enzyme assay manually alter level of absorbancy, e.g. 0.0 and 0.4, and record the distance (cm) that the two traces are apart from each other on the chart-recorder paper. The distance apart will then give a measure of absorbance change per cm on chart recorder paper.

2. If $\frac{\text{Abs change}}{\text{time}} = x_1 (\text{Abs min}^{-1})$, then from the molar extinction coefficient of Cytochrome C (29705) at 550 ηm and using a light path of 1cm, the absorbance of a 1 Molar solution is calculated:

$$= \frac{x_1}{29705}$$

$$= x_2 \ \mu\text{mol/litre.}$$

3. Calculate amount of reduced Cytochrome C in cuvette:

$$= \frac{x_2}{1000} * 0.875$$

$$= x_3 \ \mu\text{mol.}$$

Cuvette contains:	
0.6ml buffer	0.025ml tissue homogenate
0.05ml cytochrome c	0.2ml KCN and succinate

4. $\text{Rate} = \frac{x_3\ \mu\text{mol (Amount)}}{1\ \text{min}^{-1}\ \text{(Time)}}$,

$= x_4\ \mu\text{mol min}^{-1}$

This rate (x_4) is calculated for 25 µl of tissue homogenate (Aliquot volume).

5. Total enzyme activity in original sample is :

$$= x_4 * \frac{\text{(Total volume of original sample + (10 vols) sodium phosphate buffer)}}{\text{Aliquote volume}}$$

$= x_5\ \mu\text{mol min}^{-1}$

6. Therefore, total activity of Succinate dehydrogenase per gram of sample tissue

$$= x_5 * \left(\frac{1\text{g}}{\text{tissue sample g}}\right)$$

$= x_6\ \mu\text{mol g}^{-1}{}_{\text{wet}}\ \text{min}^{-1}$ tissue.

2.6 Measurement of aerobic enzyme levels: Citrate synthase

Tissue collection followed exactly the procedures described in Section 2.5, with tissues samples of 1.5-2g being immediately frozen in liquid nitrogen (-196°C) within 5 minutes of death. Samples were then stored at -80°C until enzyme analysis was carried out.

Enzyme activity was determined using between 100-400mg of thawed tissue, which was then homogenised on ice using a glass-glass homogenizer. 10 volumes of buffer (100 mM PO_4, 2mM EDTA) was then added to the homogenate and sonicated for 3 x 15 sec with three 45 sec pauses on ice. The homogenate was then diluted 200 fold , using a serial dilution of the original homogenate-

1. 25µl homogenate + 225µl buffer =1:10,
2. 25µl homogenate (1:10) + 475µl buffer, giving total dilution factor of 1:200.

N.B. In highly aerobic tissues the activity of citrate synthase may be considerable. Therefore in the initial evaluation of this procedure the activity of a homogenate should be checked at several different dilutions, in order to find the lowest dilution which gives the maximal citrate synthase activity. If the homogenate concentration is too high, the apparent activity of the enzyme will be decreased due to the non-linearity of the reaction and subsaturating concentrations of substrate. i.e. the reaction will be over before it can be monitored.

As Tris buffers have a substantial temperature coefficients, if assays are carried out at temperatures other than 25°C the pH of the buffer solution should be adjusted accordingly.

Cuvettes were then prepared containing 0.6ml reaction buffer, 0.1ml 1mM DTNB and 0.1 ml of diluted homogenate. After mixing the cuvettes were equilibrated at 25°C in a water bath and then 0.1 ml of a 2mM Acetyl-CoA solution was added and a control absorbency was read at 412ηm for 2-3 min. Finally, 0.1 ml 5mM oxaloacetic acid was added to the cuvette and the absorbancy increase was read for a further 4-5 minutes.

Calculations follow those of O'Connor (1995, 1996 and *pers com*):

1. As with step 1 of section 2.5, calculate gradient $\frac{x}{y}$. This will give the absorbance change over time.

2. Citrate synthase activity (μmol gram^{-1} $_{wet}$ min^{-1}) of the tissue sample is then calculated according to Equation 2.6 below:

Equation 2.6:

$$\frac{\left(\frac{\text{Change in Abs}}{\text{Min}}\right) * 0.07353 * \left(\frac{\text{volume of buffer + mass of tissue}}{\text{mass of tissue}}\right) * \text{dilution factor}}{\text{0.1 ml diluted homogenate}}$$

2.7 Measurement of mitochondrial volume composition: Electron microscopy

Birds were killed by cervical dislocation and the left pectoralis major muscle and the liver were dissected out and weighed as quickly as possible (Tissues were also taken for enzyme analysis at this time). Serial transverse sections of pectoralis muscles used for electron microscopy were made from muscle blocks (See figure 2.7) taken from both a superficial, dorsal aspect (A) and from the deep ventral region (B) of the muscle. Sections were cut along muscle fibres, stretched on white card to prevent their contraction and then fixed in Karnovsky fixative. This whole procedure was carried out as quickly as possible, usually in under 3 minutes, to prevent the mitochondrial membranes from collapsing.

The following protocols were then followed:
[All times below are minimum times required]

1. Fix in Karnovsky fixative for 1.5 hours on a rotator at room temperature

2. Post-fix in 1% buffered osmium tetroxide for 1 hour on rotator at 4°C (Tissues could then be stored at 4°C for several weeks in 0.1M Sodium cacodylate)

3. Serial dehydrate at room temperature

70% alcohol	**3 x 5 min**
95% alcohol	**3 x 5 min**
100% alcohol	**3 x 10 min**

4. Infiltrate with intermediate solution

100% alcohol/propylene oxide	**3 x 10 min**
propylene oxide	**3 x 10 min**

5. Infiltrate with Araldite in oven at 45°C

propylene oxide/Araldite in glass bottles with lids off	**30 min**
pure Araldite in bottles with lids off	**30 min**

6. Orientate samples and embed in a suitable mould and then cover with fresh Araldite

polymerise at 45-60°C **48 hours**

7. Ultra-thin transverse sections were then cut on a Reichert ultracut S and stained with:

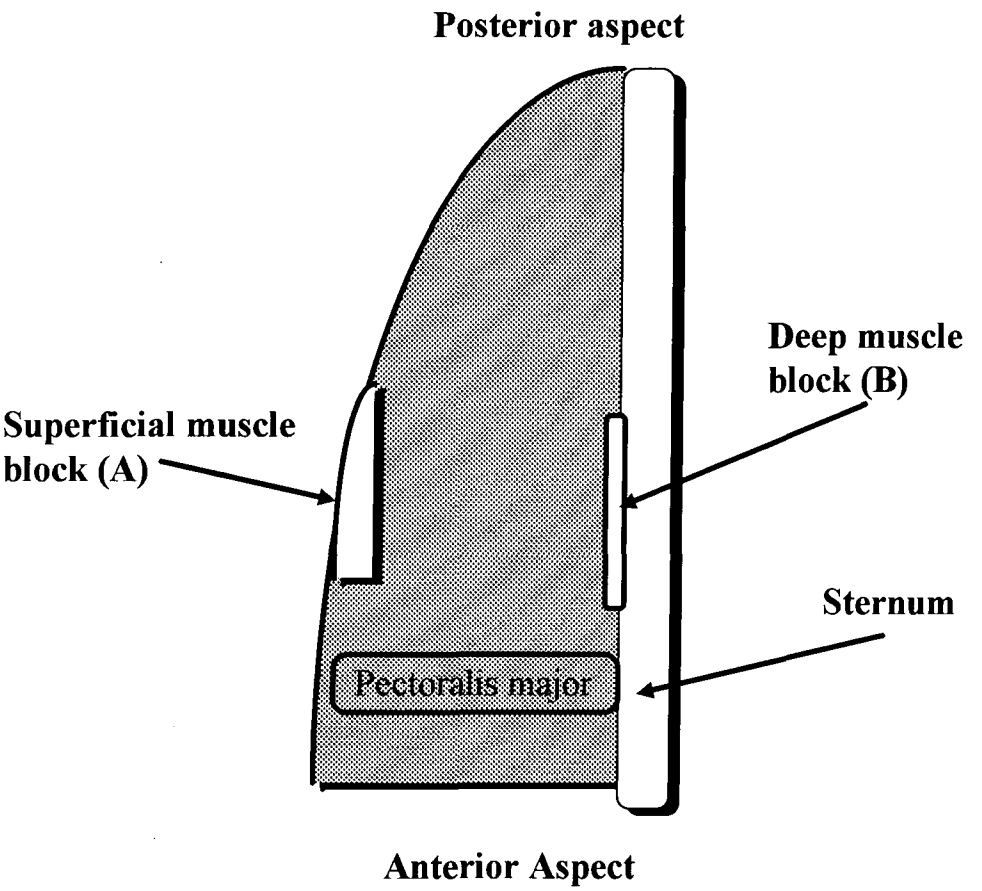
uranyl acetate	**10 min**
lead citrate	**10 min**

Sections were then viewed on a Phillips 400 transmission electron micrograph and photographs were taken at x6000 magnification. 10 randomly selected areas from each of the tissues sectioned from each bird were then enlarged to x12000 for stereoscopic examination (Weibel *et al*, 1966). An acetate sheet with a 240 point square grid was then placed over each micrograph and the number of points which fell upon mitochondria was measured. Mitochondrial area per micrograph was then calculated:

$$\text{Area of mitochondria} = \frac{\text{No. of points covering the mitochondria}}{\text{Total no. of points covering micrograph}}$$

A mean volume of mitochondria was then calculated from the 10 micrographs taken for each individual, assuming mitochondria were distributed homogeneously throughout the tissues sample. The mean value was multiplied by 100 and expressed as a percentage of the total micrograph.

Figure 2.7: Identifies the areas sampled from the pectoralis major muscle of Knot to enable mitochondrial volume to be calculated.
Adapted from Deaton *et al* (1996)



2.8 Glossary of abbreviations used throughout this thesis.

BM	Total body mass
TLM	Total lean mass
TFAT	Total fat mass
TLDM	Total lean dry mass
TOBEC	Total body electrical conductivity
PTLM	Predicted total lean mass
PFAT	Predicted total fat mass
BMR	Basal metabolic rate
DEE	Daily energy expenditure
MMR	Maximal sustainable metabolic rate
LMSBMR	Lean mass-specific BMR (mWatts g^{-1}) $\frac{BMR}{PTLM^{1.011}}$
RQ	Respiratory quotient
BMR*	(BMR measured) - (Estimated direct oxygen consumption of adipose tissue)
SMV	Standard muscle volume
SMI	Standard muscle index
SDH	Succinate dehydrogenase
CS	Citrate synthase

Chapter 3.0: Seasonal variation in Basal Metabolic Rate (BMR) within individual Knot

3.1.1 Introduction

The aim of the work presented in this chapter was to determine the factors that account for seasonal variation in basal metabolic rate (BMR) within individual birds of a single species, the Knot *Calidris canutus*, of the sub-species *islandica*, a long distance migrant which breeds in Greenland and the high Canadian Arctic and winters in Western Europe (see Section 1.8).

The primary objectives of studies reported in this chapter (see Section 1.7) were to address the following questions:

Does BMR of an individual bird change seasonally?
Does a relationship exist between BMR and the size of particular body components and (if so) does this relationship vary at different times of the year?

TOBEC was used to follow seasonal changes in the lean and fat components of body mass of individual birds and investigate whether BMR was simply a function of lean mass. I also examined the possibility that seasonal changes were occurring in the metabolic activity of these lean tissues, particularly during the period of fat deposition and utilisation, by measuring both mitochondrial volume and the levels of two aerobic enzymes in various lean tissues of Knot.

3.1.2 Seasonal changes in body mass and body composition

It has been well documented (Dugan *et al*, 1981; Blem & Shelor, 1990; Scott *et al*, 1994), that many species of birds, particularly migrant songbirds and shorebirds, undergo significant variation in total body mass during their annual cycle, to levels far exceeding those seen in other vertebrate classes (Blem, 1976). These seasonal variations in body mass are thought to be under endogenous control in many species (Gwinner, 1990; Alerstam, 1990), with birds regulating their overall mass in a 'pre-programmed' manner at specific times in the circannual cycle coinciding with periods of harsh environmental conditions, such as winter at high latitudes, during moult (Murphy, 1996) and during migration. On a proximate level, photoperiod, diet and environmental temperature (Rogers, 1995) are thought to be the most important factors influencing body mass changes. Body mass may double in the 2-3 week period prior to migration in many long distance migrants, but after 2-3 days of continuous migratory flight levels return to starting mass (Davidson & Evans, 1988; Evans, 1992; Alerstam & Lindstrom, 1990).

Shorebirds such as Dunlin *Calidris alpina* and Knot, wintering at Teessmouth, Northeast England, increase in body mass between arrival at the wintering grounds and late December or early January. Body mass then declines until the end of February-early March before increasing rapidly in the period during late March-May (Pienkowski *et al*, 1979; Scott *et al,* 1994). Such seasonal changes in body mass have also been found in captive populations of waders and generally follow those of their wild conspecifics (Scott, 1991; Scott *et al*, 1994; Mitchell, 1996), both in intensity and timing. Such seasonal fluctuations in body mass have been reported in captive Knot (see Appendix III for review). Piersma (1994) reported that all adult captive Knot of the subspecies *islandica* exhibited a single peak in body mass occurring between late May and early June, which was 'consistent and synchronised between years' and a smaller increase in body mass

during early winter. He reported that free-living adult Knot exhibit 3-4 fattening episodes during the breeding season, two between April and July, before and during the migratory flight northwards to the breeding grounds and one or two, later in the year which coincided with migration to the wintering grounds. Peaks in body mass were also found in juvenile Knot but only during their second spring in captivity (Piersma, 1994). Juvenile Knot do not tend to migrate to the breeding grounds until after their second winter. Captive Knot also exhibited both flight feather and pre-nuptial body moult at times similar to those seen in wild conspecifics (Piersma, 1994).

3.1.3 Seasonal changes in body mass due to deposition of fat

The seasonal fluxes in body mass of wild migrant shorebirds such as Knot generally, but not exclusively (Piersma, 1990; Davidson & Evans, 1988), involve the deposition and utilisation of fat (primarily triglycerides) as a fuel for sustained flapping flight (Dugan *et al*, 1981; Grimiger, 1990). Flapping flight is thought to be the most expensive mode of locomotion per unit time (Saunders & Klemm, 1994). Dry fat is a rich source of energy which, when completely oxidised, releases up to 40kJ/g of energy compared to 5 kJ/g from wet protein (Piersma, 1990). Fat reserves are stored prior to migration and also at stopover sites along the migration route. They are deposited mainly in the subcutaneous and abdominal regions of the body. Such reserves of fat (and protein) can also be used during periods when the intake of food is insufficient to balance nutrient and/or energy requirements (Dugan *et al*, 1981). The deposition of fat is achieved mainly by an increase in adipocyte volume without an increase in adipocyte cell number (Odum 1960; Blem 1976). On the Banc d'Arguin in West Africa, shorebirds increased their daily food intake by increasing their total feeding time per day before spring migration, by feeding both during the night and during neap tides (Ens *et al*, 1990). In Turnstone *Arenaria interpres*, however, birds preparing for migration

increased feeding rates by reducing time spent in vigilance (Metcalfe & Furness, 1984).

Other strategies that may be adopted to aid fat deposition (see Biebach, 1996 for review) include a reduction in locomotor activity with a concomitant reduction in DEE (Stokkan *et al*, 1986, Cherel *et al*, 1987; Lindgard *et al*, 1992). During the breeding season Oystercatchers *Haematopus ostralegus* spend considerably more of their time being inactive because they suffer from a 'digestive bottleneck', where they collect food faster than they are able to process it (Kersten & Visser, 1996) and it is thought that fuel deposition is limited by a ceiling in the level of food intake and when this ceiling is reached, the rate of fuel deposition is negatively affected by daily energy expenditure rate (Klassen *et al*, 1997). However, such a decrease in locomotor activity may not occur in other wader species, as the feeding rate of wild Redshank *Tringa totanus* and Ringed plover (*Charadrius hiaticula*) increased during the period of spring fat deposition (I. Scott, unpublished results), although this is only true if birds had to move further in order to feed faster. A switch in diet, such as that seen in small passerines from an insectivorous to frugivorous diet in autumn (Bairlein, 1990), might aid fat deposition possibly because sugars are more rapidly digested or by allowing a reduction in energy expenditure when feeding, because more energy may be required to obtain mobile insect prey (Biebach, 1996). There may also be an increase in the efficiency of food uptake by the gut during fat deposition (see Appendix III, Bairlein 1985, 1990).

3.1.4 Seasonal changes in body mass resulting from changes in mass of lean tissues

Although for many years it was assumed that only fat was stored prior to migratory flight in passerines (Odum *et al*, 1964), it is now known that protein

(lean mass) also increases during periods of fat deposition in some passerines (Evans, 1969; Fry *et al*, 1970; Marsh, 1983), geese (Newton, 1977; McLandress & Ravelling, 1981, Dubowy, 1985) and shorebirds (Davidson *et al* 1986, Klassen *et al*, 1997). Increases in lean mass of up to 50% of total mass increase prior to migration in certain shorebirds were claimed (Piersma & Jukema, 1990; Piersma & van Brederode, 1990; Zwarts *et al* 1990) but these are now thought to be overestimates as the studies 'gave little consideration to interindividual variation or timing of mass gain' (Lindstrom & Piersma, 1993).

Amongst the increase in total lean mass, the mass of the pectoral muscles is known to increase in some species of wader prior to migration (Davidson, 1981a; Davidson & Evans, 1988; Evans, 1992). A single reason for such an increase has proved difficult to isolate and its function may vary between species (for review see Evans *et al*, 1992). Such hypertrophy in the pectoral muscles will increase the power output of the muscles which, in turn, will enable a migrating bird to carry a larger load of fat (fuel) at the start of a long flight (Evans, 1969; Marsh 1981, 1983; Davidson & Evans, 1988; Driedzic *et al*, 1993). However, the increase in lean mass prior to migration is not accounted for solely by increases in the flight muscles (McLandress & Raveling, 1981; Davidson & Evans, 1988). Evans (1992) calculated, in Knot at a stop-over site that increased their body mass by an average of 64 grams (g), 49g consisted of fat and 15g consisted of lean tissue. The pectoral muscles increased in mass by only 3g's (see Lindstrom & Piersma 1993). Pectoral muscle mass is also lost during flight as fat mass is decreasing but by far less than expected from theoretical flight mechanics (Pennycuick, 1978; Davidson & Evans, 1988; Evans *et al*, 1992). Other possible reasons for increases in lean mass before migration may be that muscle acts as a protein store for use during flight to maintain protein turnover and muscle repair (Piersma, 1990), or as a store of amino acids to aid egg formation at the breeding grounds (McLandress & Raveling, 1981), although Evans *et al* (1992) found no differences in the mass of the protein stores between sexes of the waders they studied. Protein stores may also provide glucogenic precursors to maintain glucose homeostasis during flight,

as it is thought that birds are incapable of catabolising fat exclusively (Klassen *et al*, 1997). It would appear that none of the reasons cited above are mutually exclusive to one another.

3.1.5 Seasonal and intra-individual variation in Basal Metabolic Rate (BMR)

A general review of the extensive literature concerning basal metabolic rate (BMR), its allometric relationship with body mass, and the application of this relationship to physiological, theoretical and ecophysiological studies has been addressed in Chapter I (General Introduction) of this thesis. To summarise, BMR is the most commonly employed parameter in the study of homeothermic energetics (Meerlo *et al*, 1997), and is defined as the minimum level of energy expenditure that proceeds in an post-absorptive animal under thermoneutrality, while at rest during the inactive phase of the circadian cycle (Aschoff & Pohl, 1970).

3.1.5.1 Effects of captivity on basal metabolic rate

There have been conflicting reports on the effects on BMR of captivity, measured when comparing captive individuals with wild conspecifics. Weathers *et al* (1983) in their study of 4 captive and 4 wild Apapanes *Himatione sanguinea* reported that the BMR of individuals held in captivity for a period of one year did not differ significantly from those of 'freshly caught individuals' (see also Dawson & Carey, 1976; Wasser, 1986). Merlins *Falco columbarius* held in captivity for periods ranging from 7 months to 3 years however had significantly higher BMRs and body temperatures than freshly caught birds (Warkentin & West, 1990). In their study, only 4 captive birds of mixed sex and differing in age were used; they were

compared with 9 wild conspecifics. None of the captive birds was able to fly, due to wing fractures. Long-term captive Knot had lower BMRs than those of wild conspecifics (Piersma, 1994; Piersma *et al*, 1996). This was claimed to be due to a decrease in mass of the digestive organs. Metabolic intensity of lean tissues is known to decrease with age (Rolfe & Brown, 1997), which may be another possible or additional mechanism involved in this decrease seen in captivity In my study, I was concerned with the seasonal variation in metabolic rate, so that even if the general level of BMR decreases in captive birds, the processes that cause seasonal variation should be the same in captive and wild birds.

3.1.5.2 Effects of seasonality on basal metabolic rate

Seasonality in BMR has been reported in certain species of birds e.g. Kendeigh *et al*, (1977); Cadee, 1992; Piersma, 1994; Liknes & Swanson,(1996) and Swanson & Weinacht, (1997), although the factors underlying this seasonality have proved difficult to elucidate. Seasonal variation in resting metabolic rate (RMR) seen in Long-eared owls *Asio otus* was correlated with variations in overall energy expenditure (Wijnandts, 1984) and in Kestrels *Falco tinnunculus* RMR was correlated with the period of moult (Dietz *et al*, 1992). The effect of moult on BMR has been reviewed by Murphy (1996). It is thought to have no effect on certain species (Brown & Bryant, 1996) but in others increases oxygen consumption by between 9-111% (Lindstrom *et al*, 1993). Only fairly recently have studies been made of the relationship between BMR and body mass within individuals (Daan *et al*, 1989; Scott 1991; Cadee, 1992; Piersma, 1994, Scott *et al*, 1996) and of these studies few have involved the continuous monitoring of metabolic rate and body composition variations within an individual throughout its annual cycle. Both Cadee (1992) and Piersma *et al* (1996) claimed that in captive Knot, seasonal peaks in BMR coincided with seasonal peaks in total body mass, although in both studies BMR measurements were only taken infrequently- at 6

week intervals. BMR also decreased with a decline in fat free mass (Weber & Piersma, 1996).

3.1.5.3 Intra-individual studies on the relationship between BMR and body mass

The first detailed study on the relationship in birds between BMR and body mass within individuals of the same species (see Section 1.3) was of Kestrels *Falco tinnunculus* by Daan *et al* (1989). They reported that Mean(±SE) BMR varied in individual Kestrels in proportion to body mass to the power of 1.67(±0.190), an exponent considerably higher than that applying to the relationship between Kestrels (0.786±0.226)) and higher than mass proportionality (1.00). Both Scott *et al* (1996) studying Redshanks *Tringa totanus* and Piersma (1994) studying Knot found that the mass exponent of BMR within individuals was also higher than mass proportionality at 1.26 (±0.110) and 1.38(±0.398) respectively. No studies have actually followed the relationship between BMR and body composition (using TOBEC, see Appendix III) within individual birds over the annual cycle. As seasonal fluctuations in BMR could occur as a result of either or both plasticity in the mass of metabolically active lean tissues and variation in the intensity of metabolism within unit mass of these lean tissues, information relating to these two mechanisms for altering overall BMR will now be discussed.

3.1.5.4 Evidence that seasonal variation in BMR is due solely to variations in the mass of the metabolically active lean tissues

Long-distance migratory flight is known to be a very strenuous form of exercise, with oxygen consumption increasing by 5-14 fold above that at rest (Saunders & Klemm, 1994). This very high level of energy expenditure is fuelled primarily by

the large fat depots deposited prior to migration (see section 3.1.3) with the organs of the abdominal cavity providing support during this time both in the form of fuel supply and waste degradation (Kersten & Piersma, 1987; Jenni-Eiermann & Jenni, 1991; Konarzewski & Diamond, 1995).

It is well known that different organs have very different rates of oxygen consumption (Terrione & Roche, 1925; Krebs, 1950; Wheeler, 1984; Schmidt-Nielsen, 1984; Scott & Evans, 1992), with organs such as the brain, liver , gastrointestinal tract and kidney (Aschoff *et al*, 1971) having mass-specific oxygen consumption rates some 100 times greater than skin, fur and bone (Schmidt-Nielsen, 1984). It is claimed that a high DEE generated mainly by the skeletal muscles (Kersten & Piersma, 1987), is acted on by natural selection and that a high BMR simply reflects the mass of the metabolic machinery needed to provide a high level of support by the organs of the abdominal cavity (Daan *et al*, 1990). Thus, although organs such as the kidneys and liver may account for only a small proportion of the overall body mass, their contribution to BMR may be disproportionally large (Daan *et al*, 1990; Konarzewski & Diamond, 1995; Meerlo *et al*, 1997). In an interspecific study of 22 species of birds, Daan *et al* (1990), showed that lean dry heart and kidney mass were better predictors of BMR than total body mass. The overall contribution of skeletal muscle to the total oxygen consumption during a measurement of BMR may be fairly low because of its low rate of metabolism per gram (Rolfe & Brown, 1997), although during activities such as during flight, flight muscle will be the main contributor to the maximum level of oxygen consumption (Else & Hulbert, 1985). However, Ricklefs (1996) argues that although variation in BMR among species may be correlated with the relative sizes of certain organs, it 'evidently depends more on variation in the metabolic intensity of larger organs , such as the muscles and viscera'.

The 'Energetic margin hypothesis' proposed by Kersten and Piersma (1987) to explain the higher than predicted levels of BMR seen in shorebirds stated that it was an adaptation found in shorebirds that experienced energetically costly climatic conditions or long-distance flight (see General Introduction). In species facing predictable periods of high energy demand, the maintenance of a high mass of metabolically active lean tissues would be necessary, together with supporting tissues and organs, which produce a high BMR (Drent & Daan, 1980; Kersten & Piersma, 1987; Daan *et al*, 1989, 1990, Lindstrom & Kvist, 1995; Weber & Piersma, 1996; Lindstrom, 1997). Piersma *et al* (1995) found that Knot wintering in West Africa had lower BMR and lean masses than conspecifics wintering in western Europe. They suggested that a low lean mass and hence a low BMR in Knot wintering in West Africa would reduce the problems of overheating and reduce the need for evaporative water loss at high ambient temperatures. Temperate wintering Knot, they argued, would be at a selective advantage if they could maintain slightly higher lean mass and BMR to cope metabolically with periods of inclement weather. They claimed that this was why the two subspecies of Knot that have significantly different levels of lean mass in the wild converged to similar levels under common conditions in captivity (Piersma *et al,* 1995).

A major assumption in the energetic margin hypothesis is that there is a constant ratio between BMR and daily energy expenditure (DEE), i.e. if BMR doubles then DEE doubles (see General Introduction). Various values for the ratio between DEE during periods of high energy demand (maximal sustainable metabolic rate or MMR) and BMR have been calculated to be between about four (Drent & Daan, 1980) and slightly higher at up to 7 times BMR (see Bryant & Tatner, 1991) with short-term peak levels in peak metabolic rate being even higher (Peterson *et al*, 1990). It is thought that values greater than 4 times BMR cannot be maintained indefinitely without the individual incurring some long term detrimental effect (Drent & Daan, 1980), with some evidence having been produced to support this (Daan *et al*, 1996). It has been suggested that while a relationship may occur between MMR and BMR in mammals (Ricklefs *et al*,

1996, but see also Meerlo *et al*, 1997), no significant relationship occurs in birds (Ricklefs *et al* 1996). Ricklefs *et al* (1996) extrapolated their results from the data of Daan *et al* (1991) in which the experimental protocols used to measure MMR and BMR varied between individuals, and often measurements of BMR and MMR were compared from data collected on different individuals and in different studies. Therefore, there was no attempt to control for intraspecific variation in BMR or MMR. However a recent study on 10 species of passerines, measuring both BMR and MMR in the same individuals, has reported that a highly significant relationship (r=0.861) does actually exist between MMR and BMR in birds (Dutenhoffer & Swanson, 1996).

It has also been shown recently in mice that peak sustainable metabolic rate can alter depending on the what energy stress is acting on the individual (Hammond *et al*, 1996). Peak sustainable metabolic rate was higher in lactating mice at 5°C than that measured in either virgin mice at 5°C or lactating mice at 23°C. Energy assimilation was higher in the former due to increases in the masses of the small intestine, liver and kidneys.(Hammond *et al*, 1994). So it can be seen that there is some evidence that BMR of an individual bird can change as a result of altering the mass of metabolically active lean tissues. This may enable them to cope with various ecological conditions encountered during their annual cycle.

3.1.5.5 Evidence that seasonal variation in BMR is due to variations in the metabolic output per gram of the lean tissues

Evidence that an increase in metabolic rate results from increasing metabolic intensity of the lean tissues has also been shown in studies such as that of Scott *et al* (1996). They found that the BMR of individual Redshank was significantly correlated with the mass of fat being carried by Redshank that exhibited large seasonal variations in their fat mass. Lean mass tended to remain constant within

individual Redshank during these seasons. This suggests that the average metabolic output per gram of the lean tissues must have altered, as the fat mass itself is unlikely to contribute much to overall metabolic rate (Scott & Evans, 1992). Scott & Evans showed that the oxygen uptake rate of avian adipose tissue was less than 2% of the rate of uptake by liver and 10% of that found in skeletal muscle slices. In view of this, any increase in BMR associated with fat deposition is unlikely to be due to respiration of the adipocytes alone but is more likely to be an indirect cost. There is scant evidence (Saarela *et al*, 1989; Brightman & Trayhurn, 1994) that birds possess brown adipose tissue similar to that which has such an important role in non-shivering thermogenesis in neonates and hibernating mammals (Nicholls & Lockie, 1984) and is implicated in energy balance regulation (Rothwell & Stock, 1979). The cost of being fat may increase an individual's BMR simply through the costs of carrying the fat mass and maintaining posture (Scott *et al*, 1996). Another reason why fat mass may affect BMR indirectly (for reviews see Scott *et al*, 1996, Witter & Cuthill, 1993) may be an increase in thermal conductivity resulting from an increase in body size without a comparable increase in the feather mass. Fat could reduce heat loss to the environment if it acts as an insulator; however there is little evidence of this in birds (Blem & Shelor, 1990), except for one paper in the literature (Mortensen & Blix, 1986). More heat may also be required to heat a larger body mass (Witter & Cuthill, 1993). Extra heat will be provided only by an increase in the metabolic output of the lean tissues. Scott *et al* (1996), however, argued that an increase in BMR to warm fat tissue would not account for an increase in the BMR/body mass exponent above unity. A review of the factors that may be involved in mass-independent variation in BMR have been reviewed by Lindstrom & Kvist (1995).

The organelles responsible for the consumption of oxygen, the production of ATP and which contain the enzymes of the tricarboxylic acid cycle are the mitochondria (Else & Hulbert, 1985). If the metabolic intensity of the lean tissues does change seasonally it may be that variations in the volume of mitochondria and/or the levels of key aerobic enzymes alter seasonally. An implicit assumption

of this work is the theory of symmorphosis (Taylor & Weibel, 1981), where an animal incurs a selective penalty for maintaining structures in 'excess' of their immediate demand. Both the resistance of skeletal muscle to fatigue (Parkhouse, 1987) and the specific power output of aerobic muscle is related to the density of mitochondria (Pennycuick & Rezende, 1984; Hoppeler & Billiter, 1991). Indeed the volume density of mitochondria is known to be an adequate estimator of oxygen metabolism in muscle (Mathie *et al*, 1981). Mitochondrial volume was examined using electron microscopy in the pectoral muscle of both Sanderling *Calidris alba* and Dunlin *Calidris alpina* caught during the winter and just before spring migration (Evans *et al*, 1992). The proportions of myofibrils in the muscle decreased in spring with a compensatory increase in the proportion of mitochondria. In contrast, mitochondrial volume did not alter significantly in Knot caught on arrival and immediately before departure from a staging site in spring. The results from the Sanderling and Dunlin (Evans *et al,* 1992) appear to indicate that the mass-specific metabolic output of the flight muscle must have increased before migration, as indicated by the increase in the proportion of mitochondria. If hypertrophy of the flight muscles occurred only during the migratory period and this increase in mass of lean tissue caused BMR to increase (Kersten & Piersma, 1987), one would expect that the volume density of mitochondria would remain constant. This was indeed what Gaunt *et al* (1990) found in Eared grebes *Podiceps nigricollis*. During the flightless period of wing moult, when flight muscles atrophied by up to 50%, the relative volume of mitochondria in the flight muscle remained stable (27% vs. 33% in migratory condition), although the absolute volume was reduced because of muscle atrophy. Possibly a minimal volume of mitochondria must be present in avian skeletal muscle to maintain muscle function even in atrophied muscle.

Another technique employed to investigate whether seasonal changes occur in metabolic output of the lean tissues is that of enzymatic assay of key aerobic enzymes of the tricarboxylic acid (TCA) cycle. It is thought that the activities of catabolic enzymes such as citrate synthase (Yacoe & Dawson, 1983; Marsh,

1981; Lundgren & Kiessling, 1985; Lundgren, 1988; Saunders & Klemm, 1993, O'Connor, 1995, 1995a, 1996), cytochrome c oxidase (Weber & Piersma, 1996) and succinate dehydrogenase (Bass *et al*, 1969; Mathie *et al*, 1981) constitute useful indicators of rates of energy consumption in the lean tissues. Seasonal variation in citrate synthase activity has been shown to occur in the Blue-winged teal *Anas discors* (Saunders & Klemm, 1994), where citrate synthase activity was significantly lower in flight muscle during the period of moult in the wild and significantly higher in the leg muscle *Iliotibialis cranialis*. Moult coincides with a period of flightlessness and atrophy of the flight muscle, during which Blue-winged teal rely on running as their main mode of locomotion and escape from predators (Saunders & Klemm, 1994). An increase in citrate synthase activity in 5 species of bird prior to migration was 'attributable to the high energy consumption of the muscle during flight' (Lundgren & Keissling , 1985). A difference in catabolic capacity, with increased activity of CS in migratory Reed warblers *Acrocephalus scirpaceus* when compared to premigratory birds has been documented by Lundgren & Keissling (1986). In other studies, however, the activity of aerobic enzymes do not appear to change significantly on a seasonal basis (Marsh, 1981; Yacoe & Dawson, 1983; O'Connor, 1995). Indeed Marsh (1981), said that the fact that the levels of activity of CS and cytochrome c oxidase did not change during premigratory fattening or in relation to muscle hypertrophy indicated that total aerobic capacity of flight muscle of Grey catbirds *Dumetella carolinensis* increased in direct proportion to muscle mass.

3.2 Methods

The protocols that were employed throughout this chapter were described in Chapter 2- sections 2.1-2.7.

The seasonal periods used in this study were divided into:

1. Period of body mass (BM) increase to peak in spring- BM increasing

2. Period of BM decrease, after spring peak in mass- BM decreasing

3. Any period outside time period 1 and 2- Outside-

During the periods of pre-migratory fat deposition and subsequent loss in mass, the weight of individual birds was monitored daily and their TOBEC indices measured every few days. During periods of rapid change in mass, TOBEC measurements were also taken daily. Measurements of BMR in individual Knot, outside the pre-migratory period, were taken monthly from January 1995 until September 1996 and then less frequently. During the periods of fat deposition and loss in spring/early summer measurements of BMR were taken every few days. At least 36 hours was allowed between measurements on the same individual to avoid complications arising from possible dehydration and stress. Individuals that were sacrificed for enzyme assays and mitochondrial volume counts were deemed to be increasing in mass during the spring if body mass increased by over 10g in a week. Losses in mass, immediately after peak mass, were so rapid that these individuals were easily identifiable.

Individual Knot were consigned to an age class, i.e. Adult or juvenile by plumage characteristics at the time of capture in the wild by P.R.Evans and R.M.Ward. The extent of breeding plumage was categorised between classes 0-5, where 0 was no breeding plumage present and 5 being full breeding plumage achievable in captivity.

3.2.1 Application of lean-mass-specific BMR

Lean mass-specific BMR was calculated as $BMR/PTLM^{x}$ expressed in mWatts per gram of predicted lean tissue, where x was the mass coefficient for BMR i.e. the slope of the relationship between Log_{10} BMR and Log_{10} PTLM in Knot. The relationship between BMR and mass and BMR and lean mass is known to be allometric not isometric, i.e. the ratio between BMR/lean mass does vary with lean mass. It is therefore, not correct simply to divide BMR by mass or lean mass as this does not remove all the variation due to this mass (Packard & Boardman, 1987; Scott, 1991). The lean mass exponent calculated intraspecifically, however, was very close to the value of 1 (isometry) anyway, being 1.011. Packard & Boardman (1988) also admit that 'ratios are adequate for scaling data when the coefficient of variation for the numerator is substantially greater than the coefficient of variation for the denominator variable'. As can be seen in the following chapter this certainly occurs in this data set. The use of ANCOVA would also be difficult with this data set as, as can be seen later in this chapter, only one bird actually exhibits a significant relationship between BMR and PTLM, out of a total of 19. Lean-mass-specific BMR was used despite this because it gives some indication of whether the average metabolic rate per gram of the lean tissues does in fact alter.

3.3 Results

3.3.1 Seasonal variation in body mass and body mass composition in captive Knot

Adult Knot: typical examples

From the Figures 3.3.1.A-B (Knot GG and Knot OO) it can clearly be seen that captive adult Knot in this study maintained seasonal cycles in body mass (BM) similar to those measured in wild birds in the non-breeding areas, both in terms of timing and mass (Piersma, 1994). Clear peaks in BM between May-June occurred in both in year 1 (mean day number = 154±0.56, where day number 0= 1 January 1995) and somewhat later in year 2 (176±0, where day number 0= 1 Jan. 1996) of captivity in adult Knot GG and OO. Note that although a peak in BM was seen in both Knot GG and OO, the rate of BM gain was not steady (see also other examples in Appendix I) but showed periods of rapid increase interrupted by periods of BM stability or even loss before a maximum BM was reached. Increases in BM during spring (which tended to be slightly lower in year 2) were due almost entirely to the deposition of fat, although peaks in estimated total lean mass (PTLM) also occurred during this time in the first year of captivity (see graphs 3.3.1A-B) but generally not in year 2. It can be seen that PTLM was a far more stable component of body mass than was fat mass. Fat mass (PFAT) accounted for 43% of spring peak BM in Knot GG during year 1 and 36% during its second year in captivity. Data for other individuals are shown in Table 3.3.1.1. Autumnal peaks in mass that are presumed to occur in wild Knot before southward migration from the breeding grounds were not evident in captive adult Knot and the elevated BM typical of wild Knot wintering at high latitudes were not evident in these captives. Few adult Knot moulted completely into breeding plumage in either their first or second year of captivity, with most individuals

Figure 3.3.1.A-F: Seasonal changes in body mass and body mass components in typical adult and in typical and atypical juvenile Knot.

Upper line denotes overall body mass.

M= Prenuptial body moult PM= Flight feather moult

Figure 3.3.1.A: Knot GG -typical adult

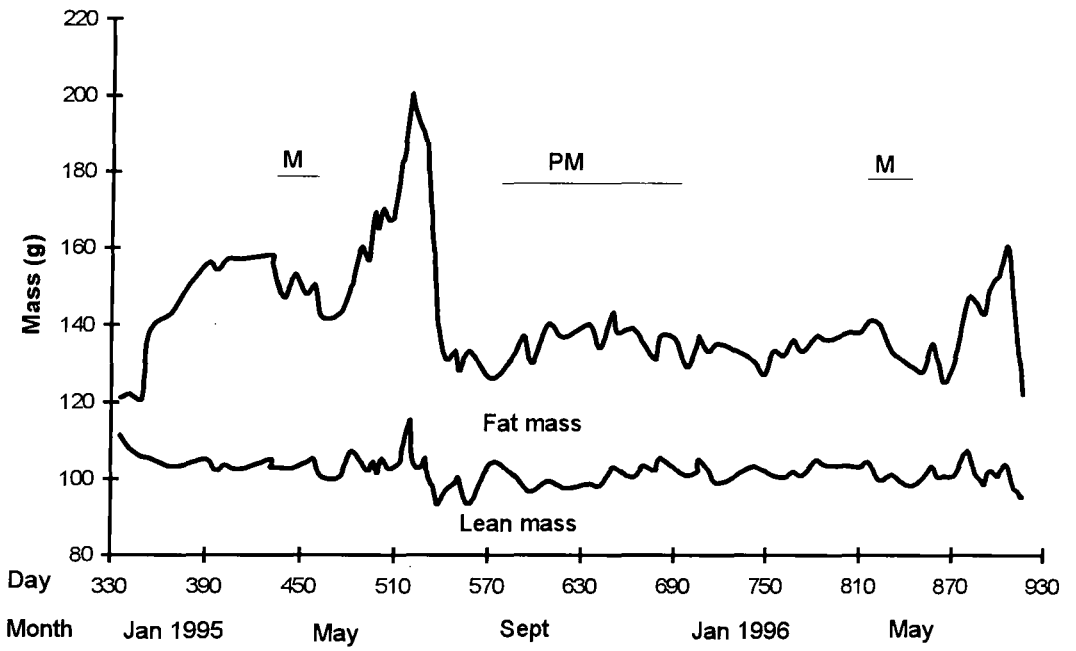
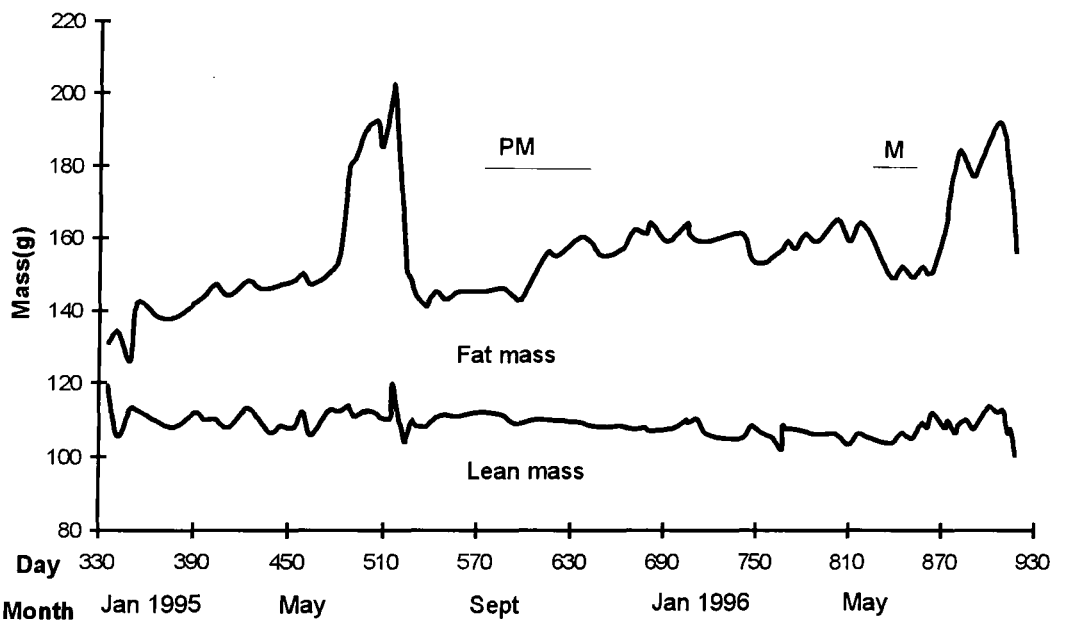


Figure 3.3.1.B; Knot OO -typical adult



retaining some 'old' feathers from the non-breeding season. All adult Knot however underwent flight feather moult, generally during autumn, over a period of 2 to 3 months. The mean(SE) time from the start of body mass gain in spring to peak mass and then through mass loss to stabilisation of BM at a level similar to that measured before spring mass increase was 32(5.3) days in 5 adult Knot during 1995.

Additional Graphs of individual Knot showing seasonal variations in body mass, body components and BMR can be seen in Appendix I.

Juvenile Knot: typical examples

Graphs 3.3.1.C-D show the typical seasonal pattern of body mass changes seen in Knot that were brought into captivity as juveniles (Knot GL and Knot GO, first-year birds). Juveniles typically did not show migratory fattening during their first spring in captivity but clear spring peaks in mass occurred during their second year. Peaks in PTLM could not be detected during the period of fat deposition in the second spring. Fat mass formed a percentage of peak body mass similar to that seen in adults e.g. 37% for Knot GL and 43% for Knot GO (Table 3.3.1.1). Juvenile Knot underwent flight feather moult during their first autumn in captivity but, as with adults, did not achieve complete breeding plumage in the following spring.

Juvenile Knot: atypical examples

Graphs 3.3.1.E-F (Knot GF and Knot WGL) show that some juvenile Knot underwent seasonal variations in BM atypical of most juveniles but typical of adult Knot. Three juveniles (see Knot WYY also, in Appendix I) showed distinct and considerable peaks in fat mass during their first spring in captivity, with

Figure 3.3.1.C: Knot GL -typical juvenile

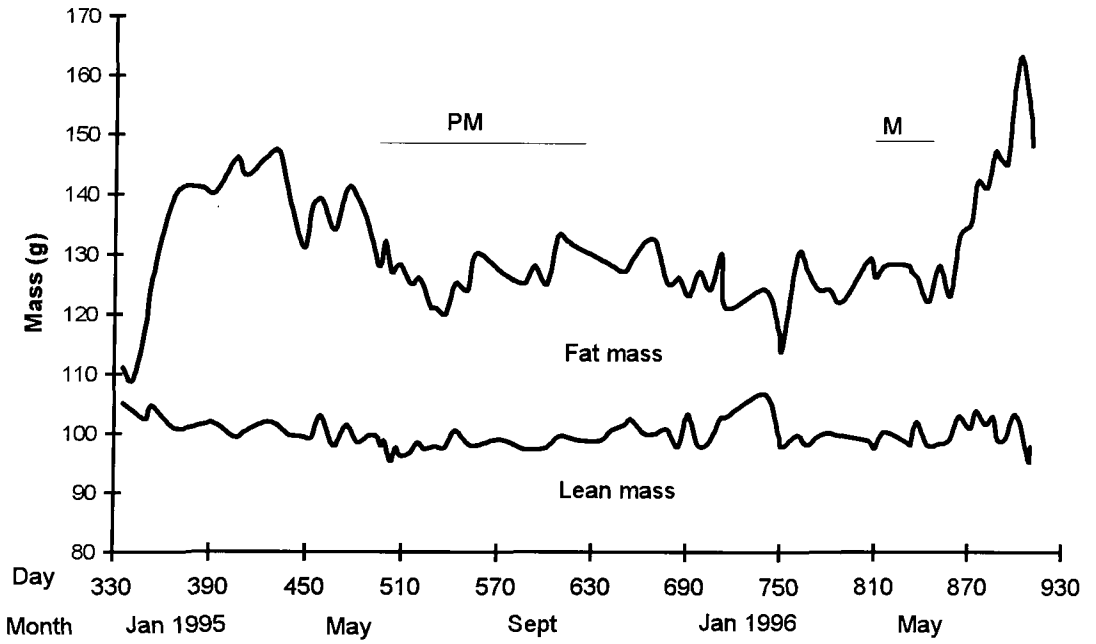


Figure 3.3.1.D: Knot GO -typical juvenile

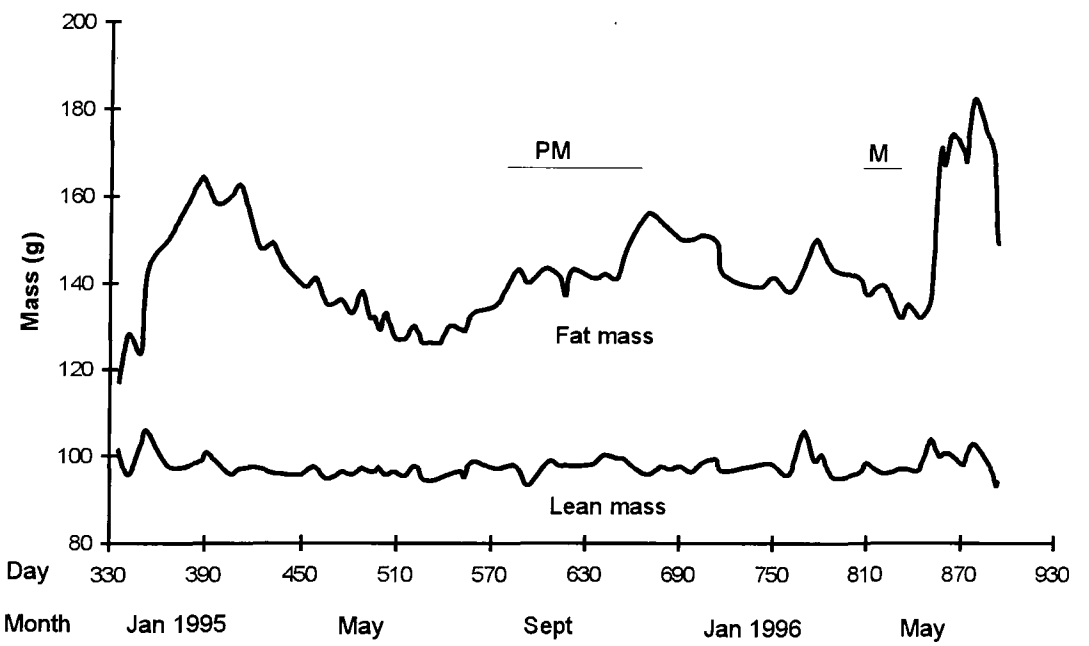


Figure 3.3.1.E: Knot GF -atypical juvenile

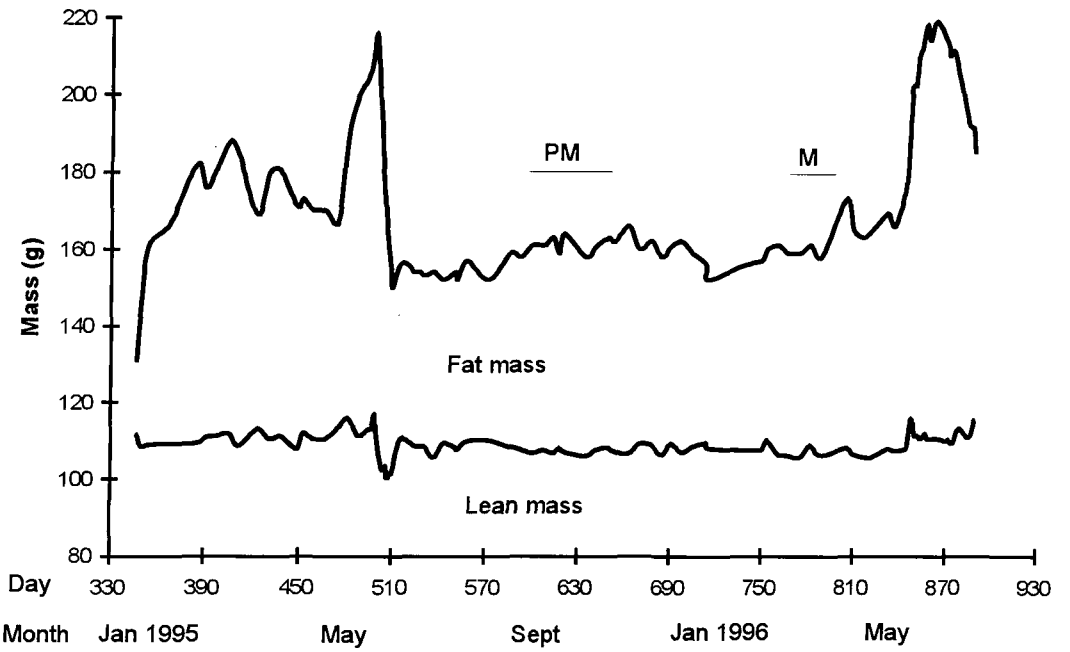
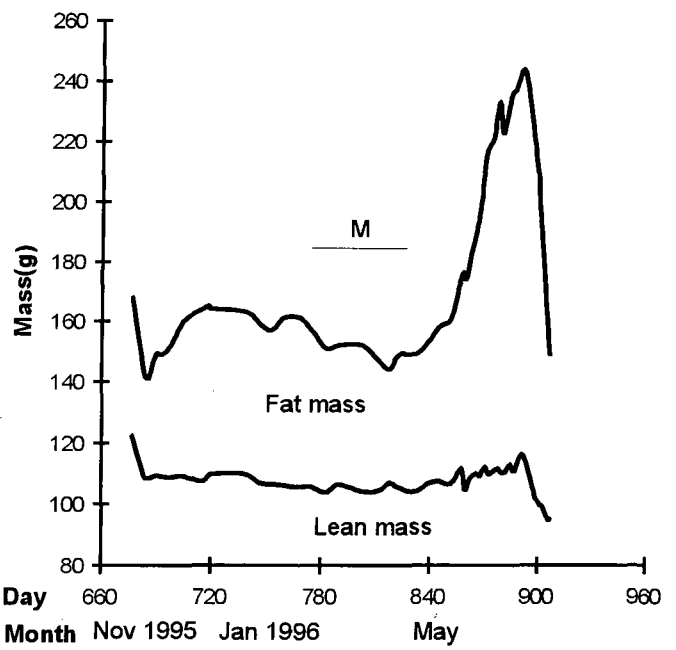


Figure 3.3.1.F: Knot WGL -atypical juvenile



individuals GF and WGL also showing slight peaks in PTLM during this period. These two individuals achieved the highest body masses of any Knot in any age-class, with Knot GF attaining a spring peak of 216g in its first and 219g in its second year of captivity and Knot WGL reaching 243g. Fat accounted for 46% and 50% of peak body mass in years one and two respectively in Knot GF and 52% of peak body mass in Knot WGL. Interestingly, Knot WGL moulted into partial breeding plumage during its first year in captivity, unlike Knot GF. Knot GF showed the fastest rate of fat deposition in spring of any captive Knot of 8g/day (24g/3 days) and WGL showed the fastest rate of fat loss immediately after peak body mass (8g/day). Captive Knot of all age classes ceased pre-nuptial moult before any increases were observed in body mass.

3.3.2 Diurnal variation in BMR

To determine whether diurnal variation in BMR occurred in captive Knot, the BMR of six individual Knot were measured during day-time and again during the next night. Although the mean level of BMR (see Table 3.3.2.1) was slightly lower during the night, this reduction was not statistically significant and did not occur in all individuals. This reduction in BMR paralleled a decrease in total body mass and fat mass. Other workers (Scott, 1991, Mansour, in prep) have also found no significant change in BMR of individual shorebirds measured during day and again at night.

Table 3.3.2.1: Comparison between the values obtained for BMR and body mass components between day and night. Mean value(SE)

	Day	Night	paired-t	P
BMR(kJ/day)	126(7.6)	118(5.2)	1.24	>0.05
Body mass	128(5.3)	123(5.3)	3.32	<0.01
PTLM	99(1.8)	99(1.3)	0.13	>0.05
PFAT	29(4.8)	24(4.4)	2.66	<0.05

3.3.3 Seasonal variation in Basal Metabolic Rate (BMR) within individual Knot

From the previous section it can be seen that captive Knot did exhibit seasonal variation in body mass, particularly in the fat component of body mass. Captive Knot also showed pronounced seasonal variation in BMR, with graphs 3.3.3.A-F showing the seasonal variation in BM and BMR (Watts) in both adult and juvenile Knot. Seasonal peaks in BMR can clearly be seen during the period of high BM in spring. Table 3.3.3.1 shows the magnitude of the increase (difference between level **before** the pre-migratory period and maximum level, as a % of **before** level) in BM, PTLM, PFAT, BMR (Watts) and lean-mass-specific BMR (mWatts g^{-1}) during the period of fat deposition and utilisation. (**before** level was calculated as the mean level of the last two measurements of BMR and body mass components immediately prior to BM increase in spring). The magnitude of the increase in BMR and lean-mass-specific BMR generally far exceeded any of the increases seen in BM and PTLM during this period.

Figures 3.3.3.A-B Seasonal variation in body mass and BMR in two typical adult Knot.

Figure 3.3.3.A: Knot GG

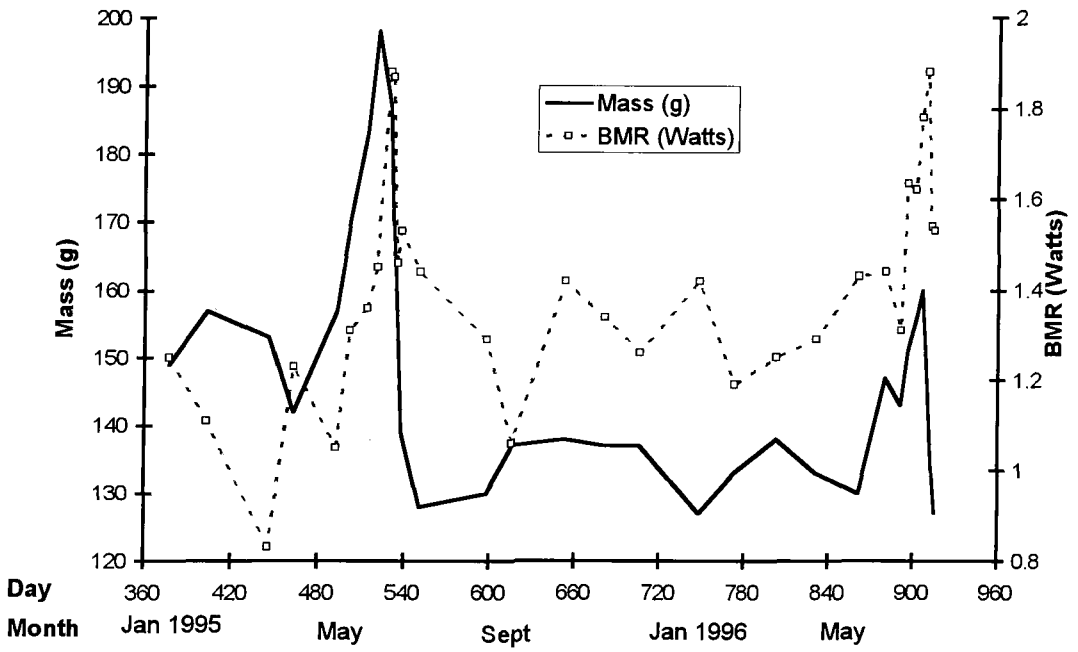
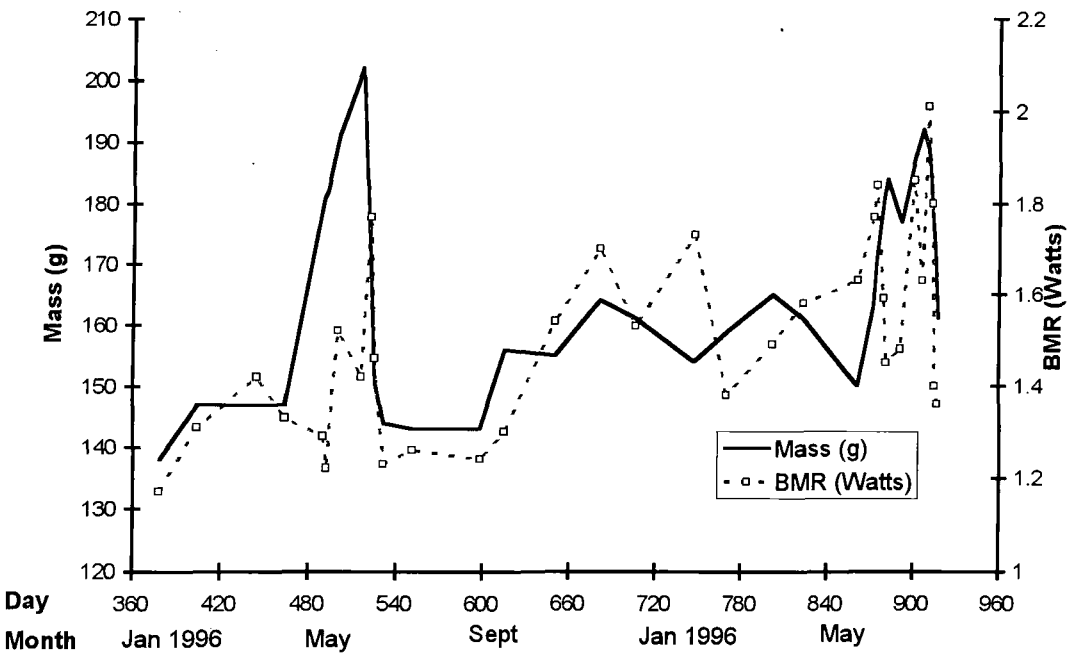


Figure 3.3.3.B: Knot OO



Figures 3.3.3.C-D Seasonal variation in body mass and BMR in two typical juvenile Knot.

Figure 3.3.3.C: Knot GL

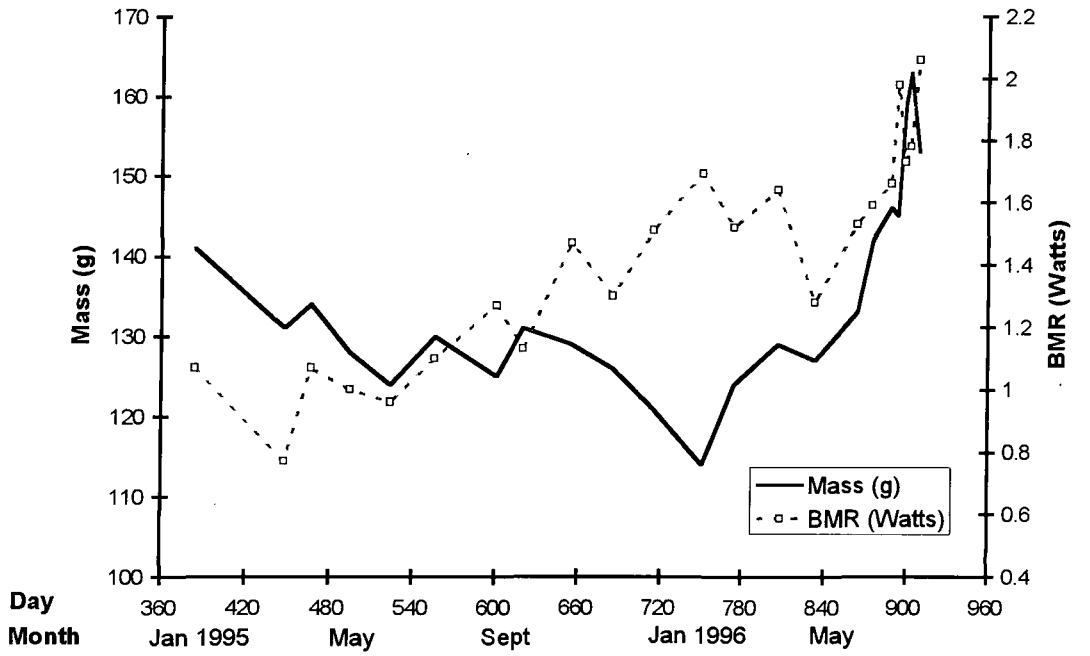
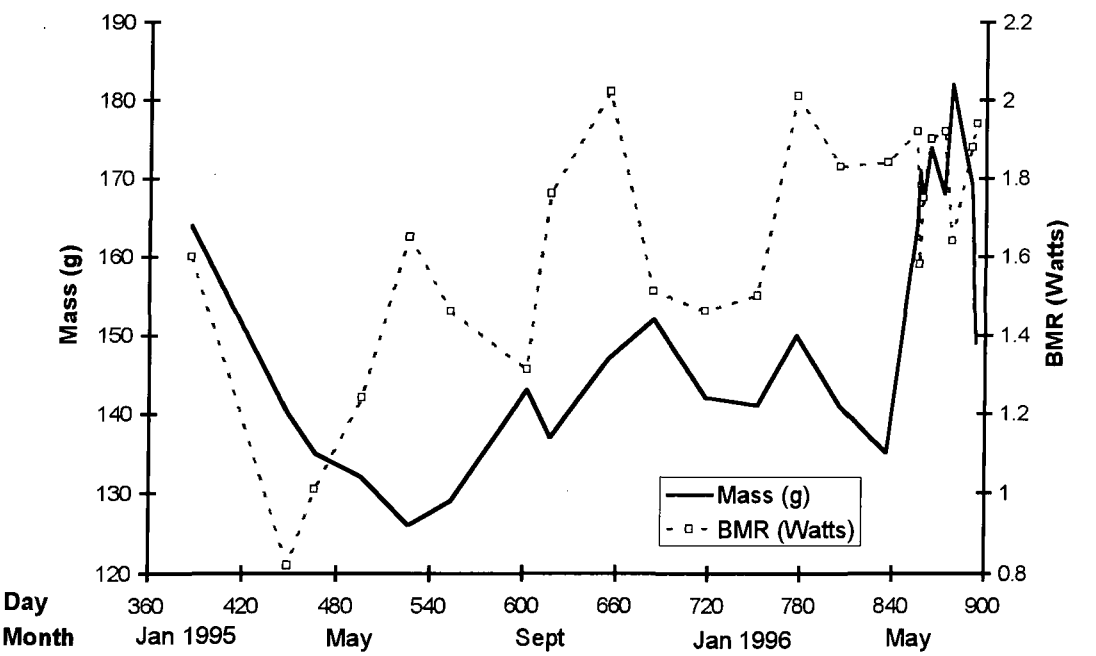


Figure 3.3.3.D: Knot GO



Figures 3.3.3.E-F Seasonal variation in body mass and BMR in two atypical juvenile Knot.

Figure 3.3.3E: Knot GF

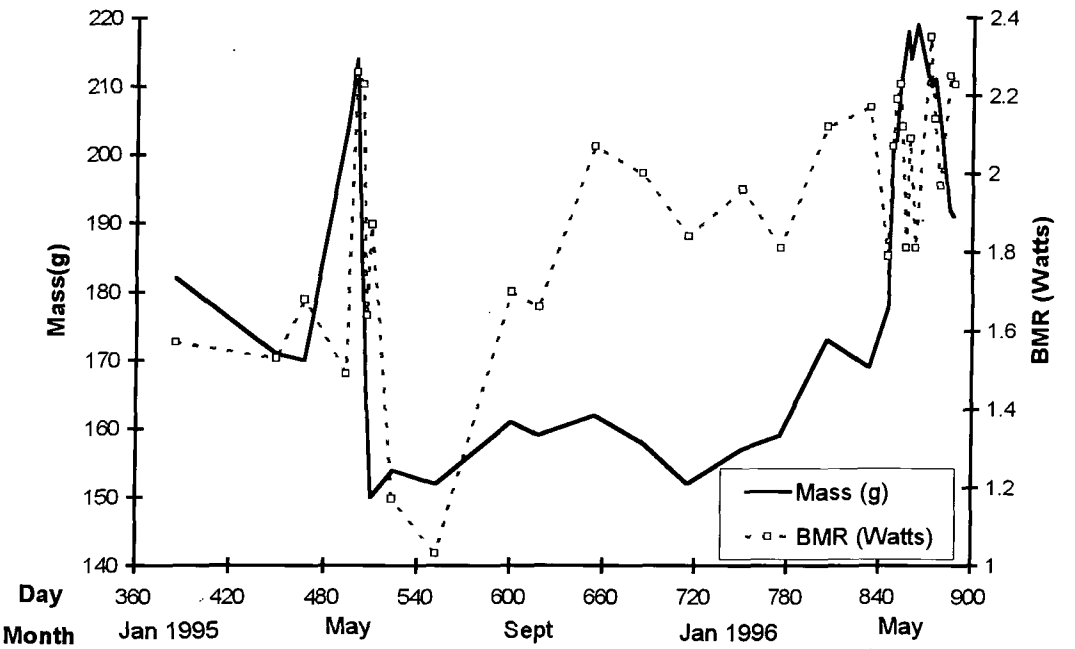


Figure 3.3.3.F: Knot WGL

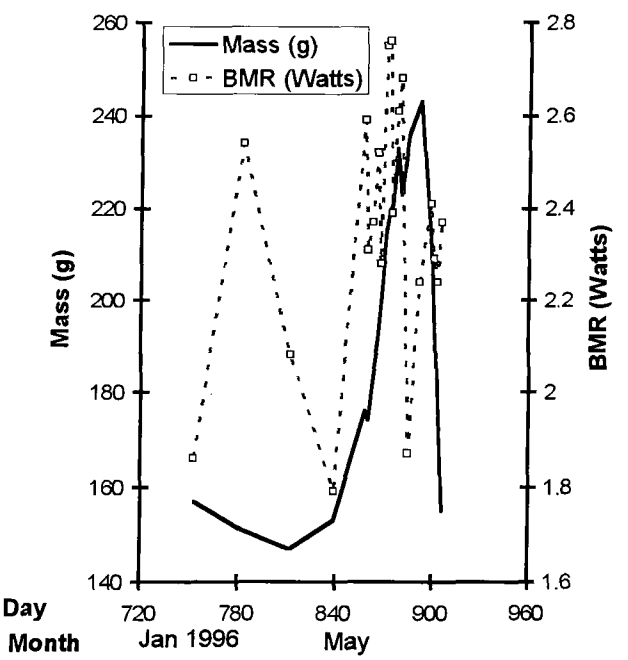


Table 3.3.3.1: Percentage increase in body mass components, BMR and lean-mass-specific BMR during the spring migratory period of captive Knot.

ID	BODY MASS	PTLM	PFAT	BMR	LMSBMR	% FAT
GF[1] *	26	2	26	53	66	46
GF[2]	30	7	30	31	31	50
GG[1]	28	6	26	78	82	43
GG[2]	9	4	23	46	43	36
OO[1]	37	8	37	46	51	44
OO[2]	28	4	28	39	38	43
LL[1]	27	9	27	107	105	45
WW[1]	27	5	27	70	42	34
YY[1]	20	5	20	45	39	29
YE[1]	21	5	21	13	20	41
GL[2]	15	4	23	25	46	37
GO[2]	11	4	35	22	30	43
GW[2]	40	5	40	45	61	48
GY[2]	13	5	15	13	12	28
WGL[1] *	59	4	59	55	50	52
WYY[1] *	16	2	16	17	26	48
MEAN (SE)	25(3.1)	5(0.5)	28(2.6)	44(6.2)	46(5.7)	42(1.7)

Figures in parentheses are standard errors

BM	**Body mass**
PTLM	**Predicted total lean mass**
PFAT	**Predicted fat mass**
BMR	**Basal metabolic rate**
LMSBMR	**Lean mass specific BMR (mWatts/g lean tissue)**
% FAT	**(PFAT/BM)*100, when BM at peak in spring**

1	**Indicates year one of captivity**
2	**Indicates year two of captivity**
*	**Indicates juvenile Knot**

Seasonal peaks in BMR generally did not occur on the same date as seasonal peaks in body mass but slightly later as body mass fell (see Table 3.3.3.2). The only exceptions were noted in Knot GO, Knot GF (year 1 only) and juvenile Knot WGL (Graphs 3.3.3.D-F). Indeed BMR on the date at which individual Knot reached peak body mass was consistently lower than on other dates during the spring migratory period. Table 3.3.3.2 confirms that the peak BMR during the period of fat deposition rarely coincided with the period of peak BM, peak PTLM or peak PFAT but, on average, occurred 5 days after peak body mass, 4 days after peak fat mass and 11 days after the peak in PTLM. (The peak in PTLM was taken to be the highest level of PTLM recorded during the period of body mass increase in spring). As mentioned earlier this was generally fairly small (3-4g of lean mass). It is interesting to note that in two of the three atypical juvenile Knot (Knot WGL and WYY) the peak in BMR occurred between two and three weeks before peak BM and PTLM. These data indicate that the metabolic intensity of the lean tissue was altering on a seasonal basis. This seasonal variation in metabolic intensity is clearly shown in the graphs 3.3.3.G-L, in which the highest levels of PTLM clearly preceded the peaks in lean-mass-specific BMR. This establishes that the average metabolic output per gram of the various lean tissues that make up overall lean mass in captive Knot alter on a seasonal basis.

No difference in the mean respiratory quotient (RQ) of 8 individual Knot was found between the three seasonal periods (Arcsine transformed ANOVA, $F_{2,21}$ = 0.984, $P>0.05$) The mean(SE) RQ measured as BM was falling in spring was slightly lower at 0.77(0.02), than that during the period of body mass increase in spring at 0.81(0.03) and the RQ calculated during the period outside the spring migratory period of 0.80(0.02). The lower RQ during the period of BM decrease in spring probably indicates that almost exclusively fat is being catabolized during this time.

Table 3.3.3.2: Timing of peak BMR in relation to timing of peak BM, PTLM and PFAT during the spring migratory period.

- **days before peak in body mass components**

+ **days after peak in body mass components**

0 **peak in BMR occurs on same day as peak in body mass components**

ID	BODY MASS	PTLM	PFAT
GF[1] *	0	+6	0
GF[2]	+9	+24	+9
GG[1]	+9	+9	+9
GG[2]	+4	+30	+4
OO[1]	+8	+8	+8
OO[2]	+4	+10	+4
LL[1]	+17	+17	+8
WW[1]	+6	+15	+6
YY[1]	0	0	0
YE[1]	+11	+11	+11
GL[2]	+6	+30	+6
GO[2]	+16	+16	+16
GW	+18	+20	+18
GY[2]	+7	+7	0
WGL[1] *	-19	-19	-19
WYY[1] *	-13	-13	-13
Mean (±SE)	+5(±2.3)	+11(±2.3)	+4(±2.3)

1 **Indicates year one of captivity**

2 **Indicates year two of captivity**

* **Indicates juvenile Knot**

Figure 3.3.3.G-L: Seasonal variations in total lean mass (predicted using TOBEC) and lean-mass-specific BMR in adult and both typical and atypical juvenile Knot.

Figure 3.3.1.G: Adult Knot GG

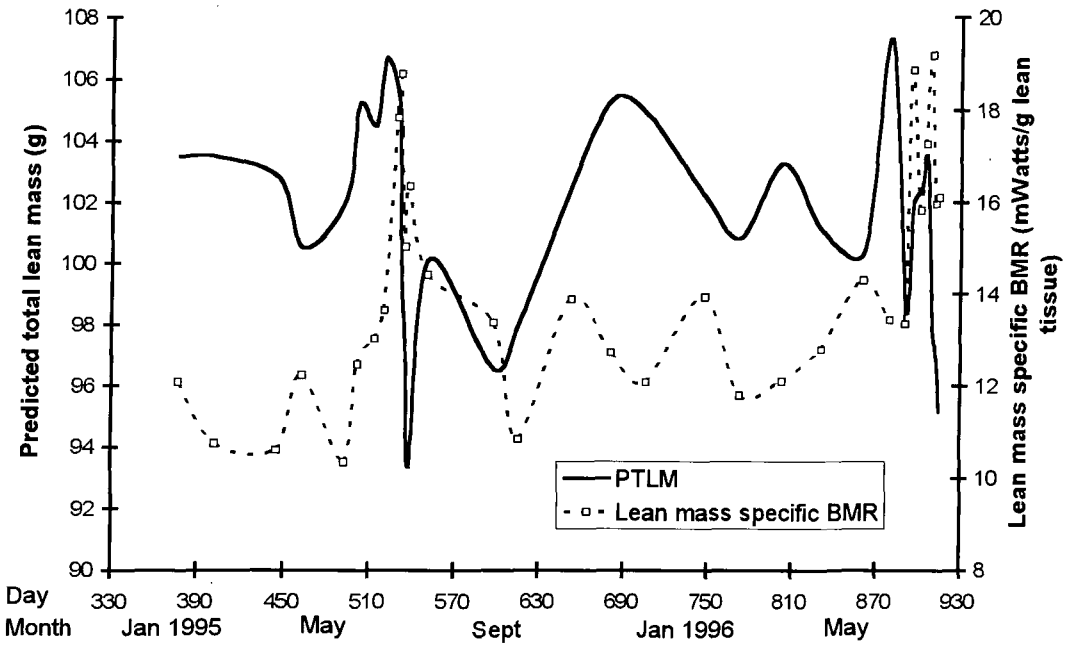


Figure 3.3.3.H: Adult Knot OO

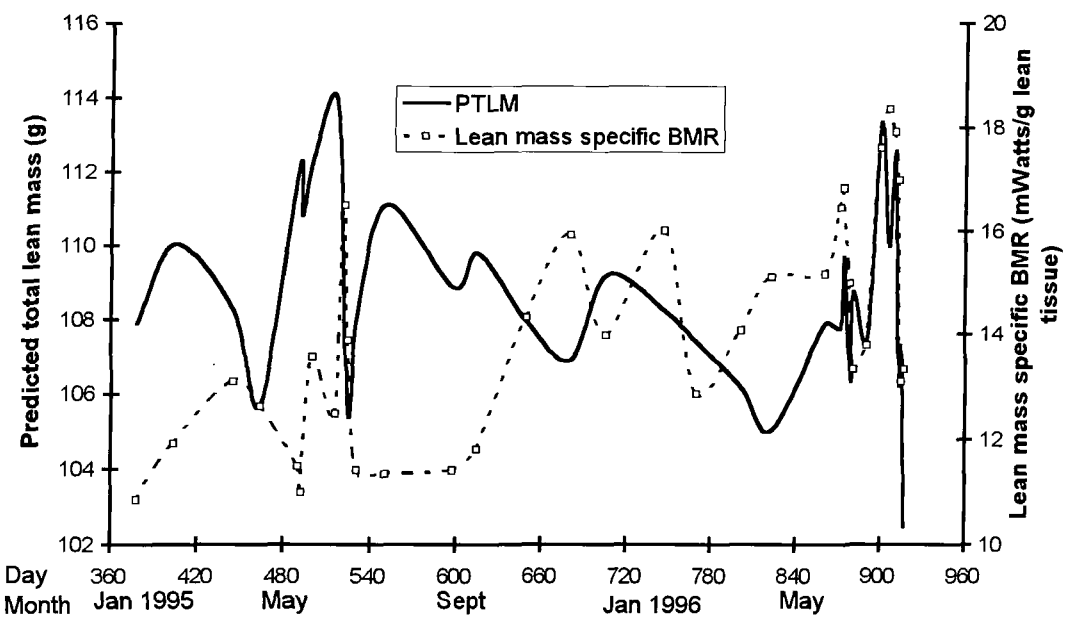


Figure 3.3.3.I: Juvenile Knot GL

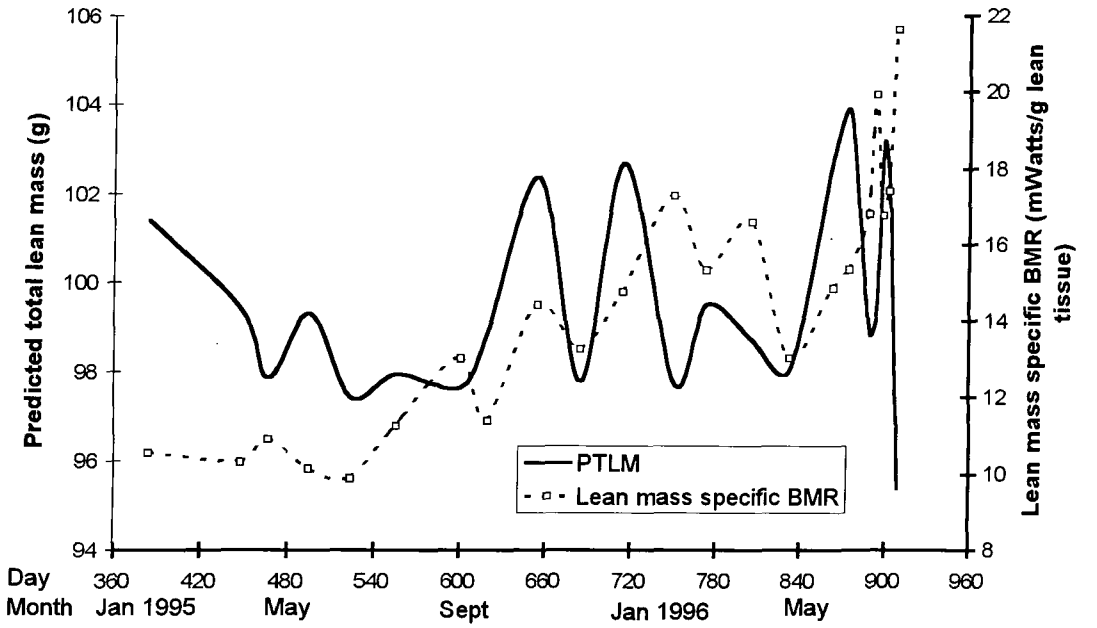


Figure 3.3.3.J: Juvenile Knot GO

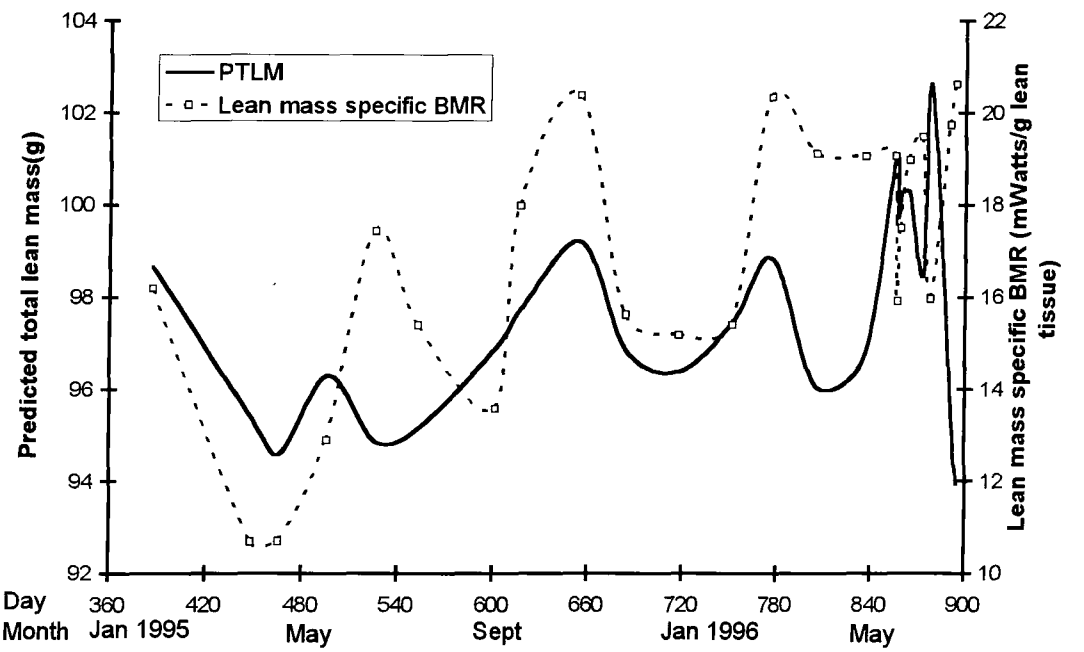


Figure 3.3.3.K: Juvenile Knot GF

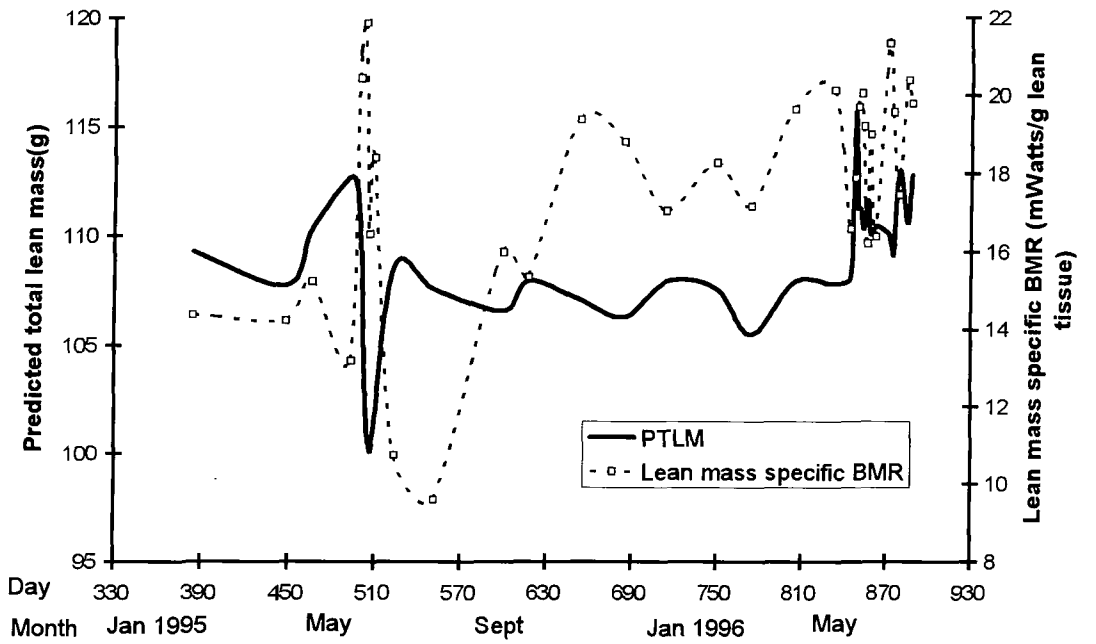


Figure 3.3.3.L: Juvenile Knot WGL

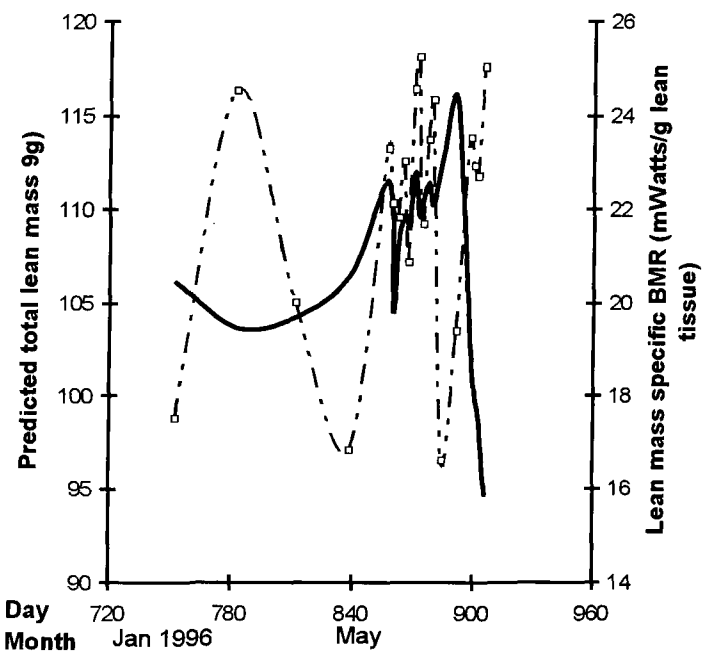


Table 3.3.3.3: Mean levels of BMR in individual Knot outside the migratory period and during the moult.

The range of BMR measurements recorded during the periods of BM increase and BM decrease in spring are also shown.

(Values are kJoules/day, with coefficient of variation in parentheses. Numbers in bold indicate sample size)

ID	WINTER		BM INCREASING IN SPRING		BM DECREASING		MOULT	
GF[1]*	139(19)	6	128-197(21)	2	142-193(13)	3	164 (9)	4
GF[2]	170 (6)	4	155-193 (9)	8	170-203 (6)	5	-	
GG[1]	101(17)	6	91-126(11)	4	125-162(11)	4	110 (9)	4
GG[2]	114 (7)	6	124-166(11)	4	114-162(13)	3	-	
OO[1]	114 (8)	9	105-132(26)	3	107-153(15)	4	130(10)	3
OO[2]	135 (8)	3	125-174(12)	6	118-174(16)	5	-	
LL[1]	102 (4)	4	71-139(26)	3	94-147(14)	6	-	
WW[1]	110(24)	22	108-127 (8)	3	80-138(22)	2	161(10)	3
YY[1]	140 (2)	7	121-132 (1)	2	97(0)	1	103 (1)	2
YY[2]	147 (4)	4	187 (0)	1	-		-	
GL[1]*	99 (22)	7	-		-		105(16)	3
GL[2]	140 (4)	6	137-154 (4)	4	171-178 (2)	2	-	
GO[1]*	116(21)	7	-		-		162 (4)	3
GO[2]	156(10)	5	137-164 (9)	4	152-167 (4)	4	-	
GW[1]*	138(17)	7	-		-		146(9)	3
GW[2]	160(13)	10	145-197(10)	7	149-210(10)	6	-	
GY[1]*	96 (14)	7	-		-		114(11)	3
GY[2]	124(15)	6	150 (0)	1	121-162(11)	4	-	
WGL[1]*	190(16)	5	162-238(10)	10	193-232 (6)	5	-	
WYY[1]*	200(11)	4	169-225(14)	3	175-197 (4)	6	-	

1 **Indicates year one of captivity**

2 **Indicates year two of captivity**

* **Indicates juvenile Knot**

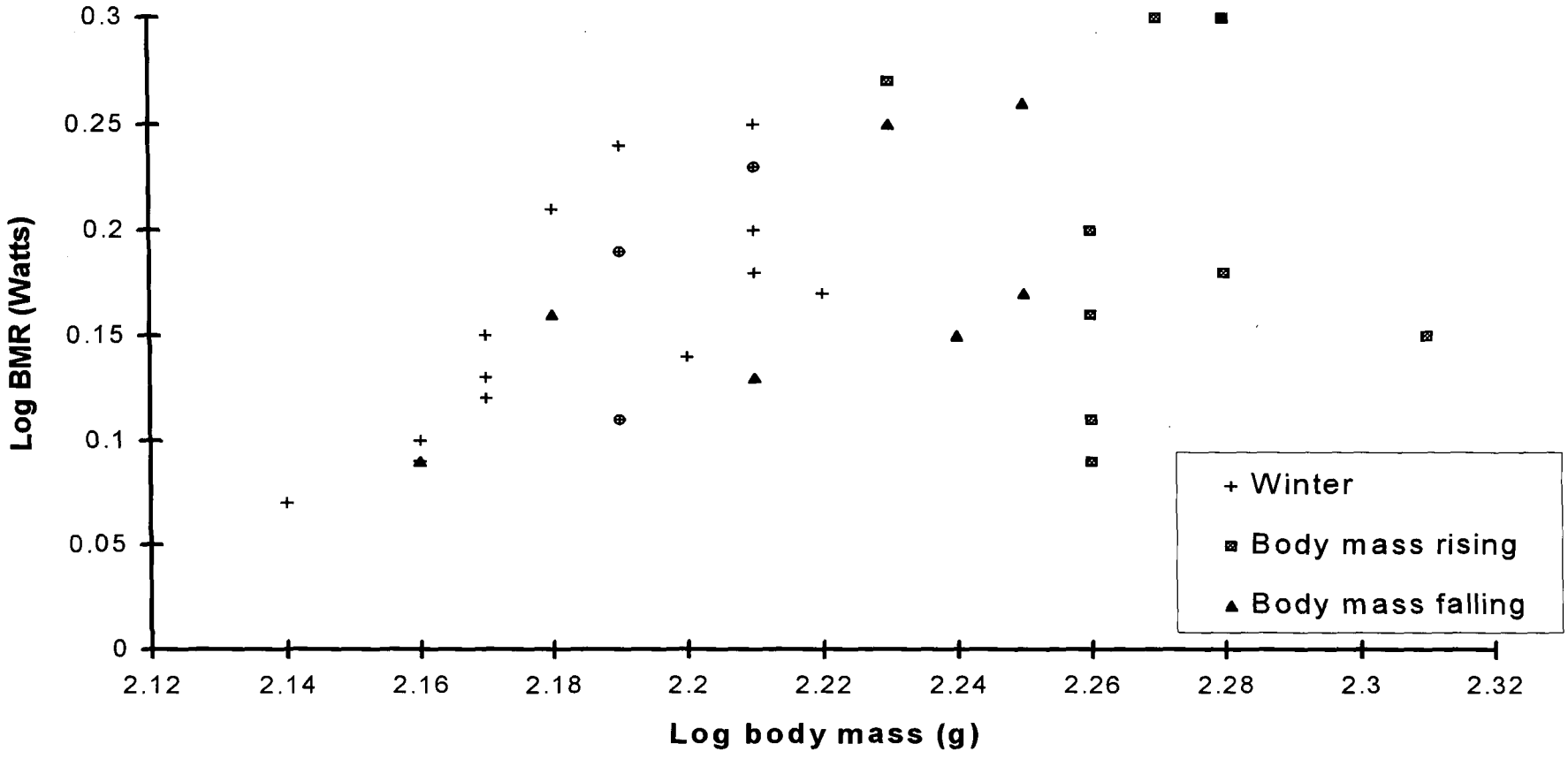
3.3.4 Relationship between BM, body composition and BMR within individual Knot

From the work discussed above, it can be clearly be seen that captive Knot exhibit seasonal variation in BMR, but with peaks in BMR during the spring generally not coinciding with the seasonal peaks seen in body mass or body composition. Therefore, mass-independent changes in BMR within captive Knot appeared to be taking place during this time. To investigate the relationship between BMR and BM, and between BMR and the components of BM (PTLM & PFAT) that occur within-individual Knot, regression analyses of Log BMR on Log BM (Tables 3.3.4.1-2), Log PFAT (Table 3.3.4.3) and Log PTLM (Table 3.3.4.4) were performed on 19 captive Knot, employing all data points collected for that individual during its time in captivity. Surprisingly only 9 Knot showed significant regressions between BMR and BM (Table 3.3.4.1), while 10 individuals showed non-significant regression equations (Table 3.3.4.2). Graph 3.3.4 A shows this relationship in a single adult Knot (Individual OO), with different symbols denoting different physiological states (Graphs for other individuals are presented in Appendix I). It can be seen that from this graph that BMR at peak mass tended to be below that expected allometrically, as was the case in most Knot that showed spring peaks in body mass (see Appendix 1).

Graphs 3.3.4.B-G (Knot OO, LL, GL, GO, GF, GW) show the residuals produced from individual regression lines of BMR on BM during different seasons (outside the migratory period, during wing and body moult, body mass rising to peak in spring and body mass falling after peak). It can be seen that in general, the BMR observed at peak body mass tended to be less than that expected from the intra-individual regression lines. Only for these 6 individuals were sufficient BMR measurements available during all season classes to enable analysis to be carried out on the residuals, and only in Knot GW (ANOVA $F_{3,22}=3.21$, $P<0.05$) was there a significant difference in residuals with season. The residuals in Knot GW

Graph 3.3.4.A- Scatterplot showing relationship between BMR and BM in Adult Knot OO (n=33).

Different symbols denote different physiological states (see key)



were significantly higher, i.e. BMR was higher than predicted for a given body mass, as body mass was falling after peak mass (Student-Neuman-Keuls Test, $P<0.05$).

Table 3.3.4.1: Significant relationships between Log BMR (Watt) and Log BM (g) within individual Knot. (LBM= Log body mass)

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -1.317(0.51)+0.704(0.23)LBM	32	0.243	<0.01
GL	LogBMR= -2.185(1.11)+1.095(0.52)LBM	22	0.181	<0.05
GO	LogBMR=-1.557(0.81)+0.811(0.37)LBM	23	0.184	<0.05
GW	LogBMR= -0.528(0.29)+0.368(0.14)LBM	33	0.193	<0.05
GY	LogBMR= -2.946(1.45)+1.446(0.68)LBM	21	0.191	<0.05
LL	LogBMR= -1.469(0.50)+0.720(0.23)LBM	13	0.475	<0.01
OO	LogBMR= -1.448(0.53)+0.733(0.24)LBM	33	0.233	<0.01
WG*	LogBMR= -2.400(0.95)+1.230(0.46)LBM	15	0.359	<0.05
WGY*	LogBMR= -1.839(0.96)+0.972(0.45)LBM	16	0.248	<0.05

* Individual did not undergo pre-migratory fattening

Figures 3.3.4.B-G: Residuals of the relationship between Log BMR and Log BM with different symbols denoting different physiological states.

Figure 3.3.4.B: Knot OO

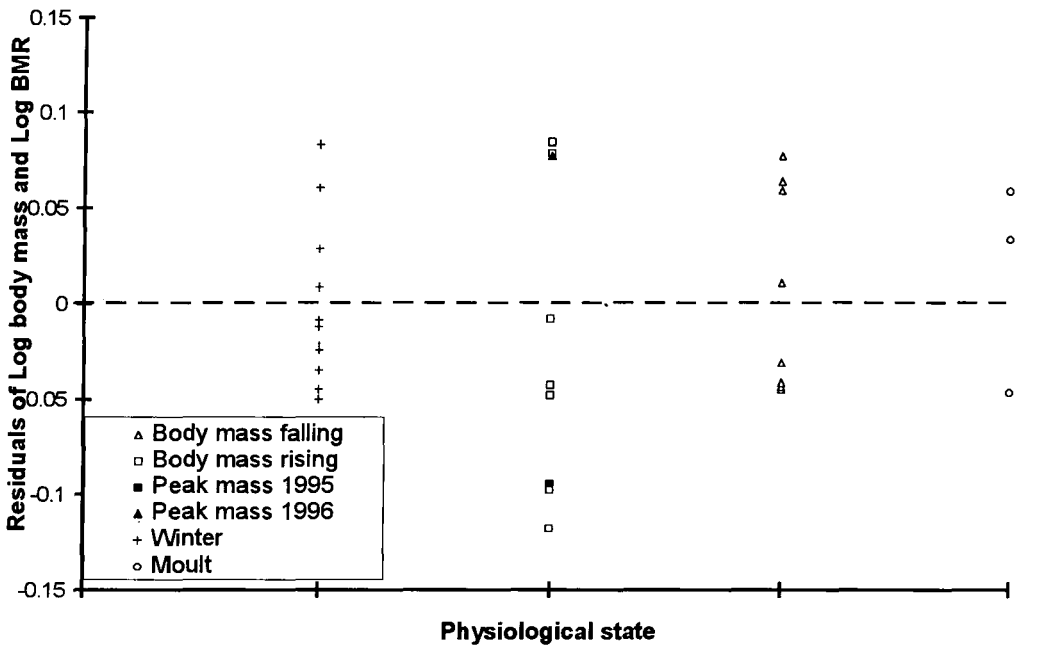


Figure 3.3.4.C: Knot LL

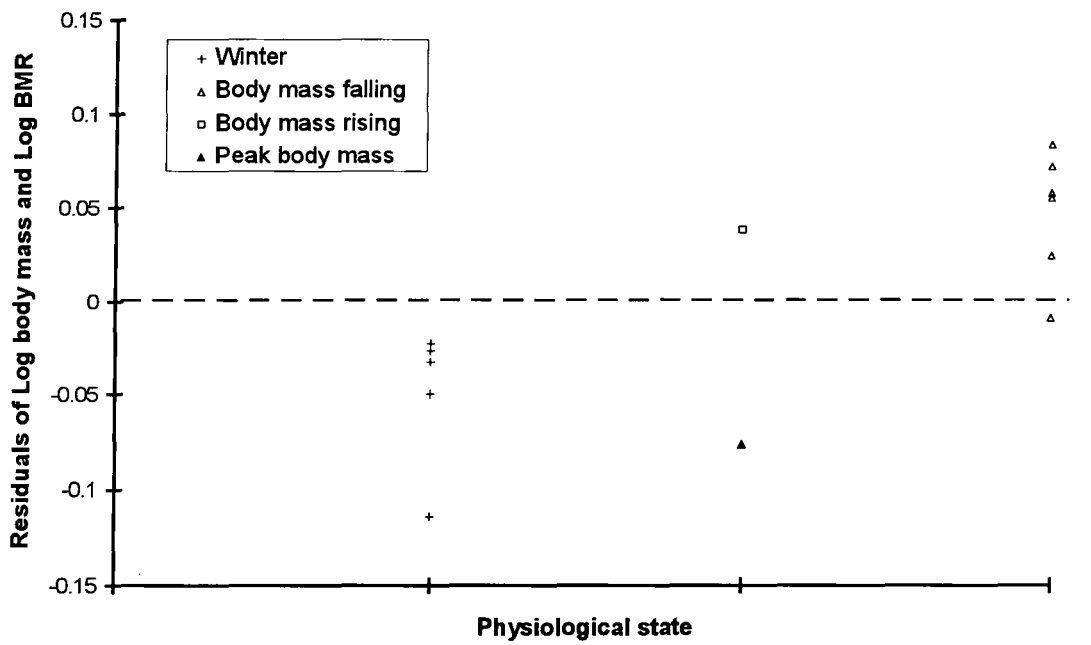


Figure 3.3.4.D: Knot GL

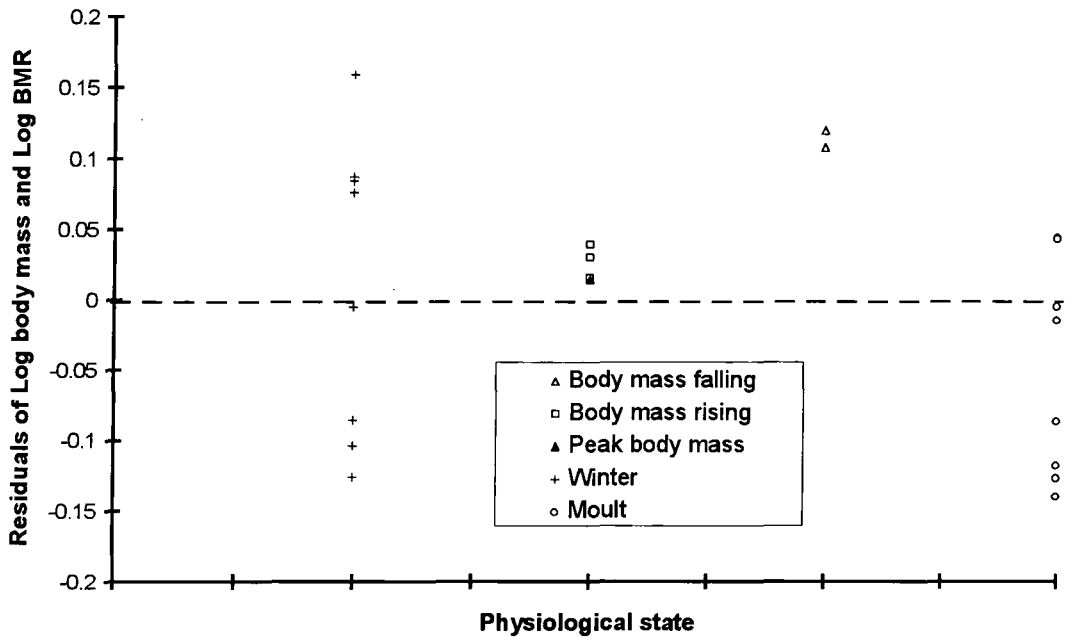


Figure 3.3.4.E: Knot GO

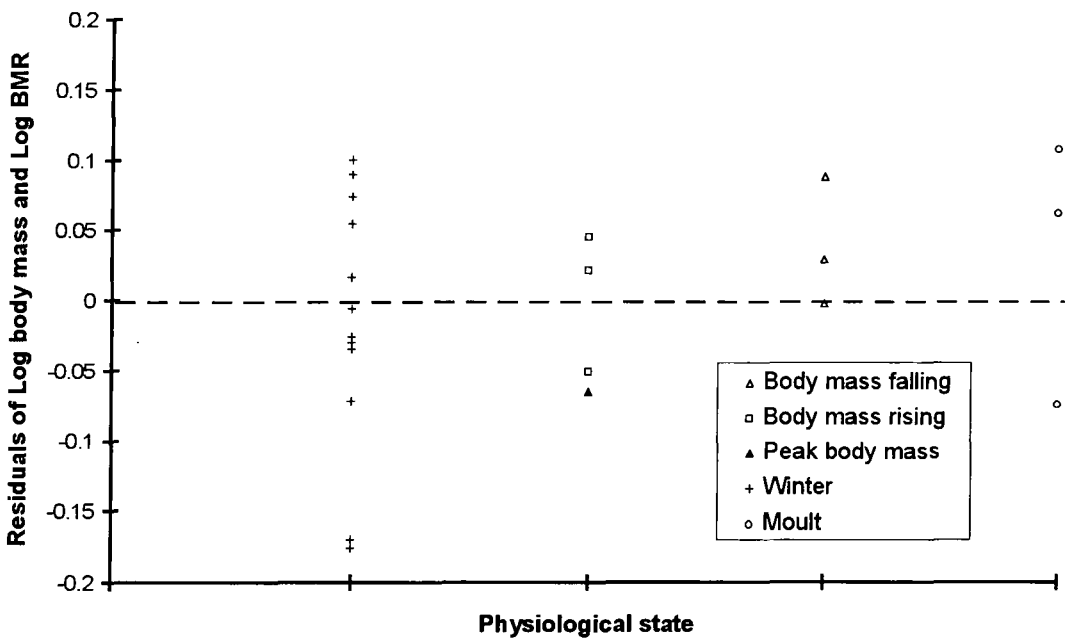


Figure 3.3.4.F: Knot GW

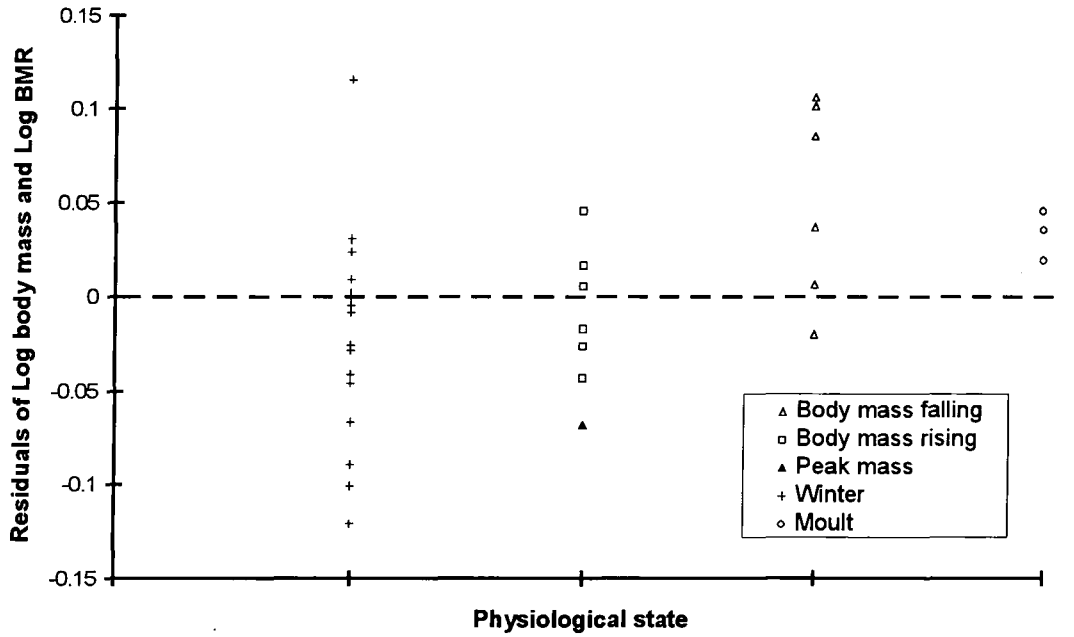


Figure 3.3.4.G: Knot GF

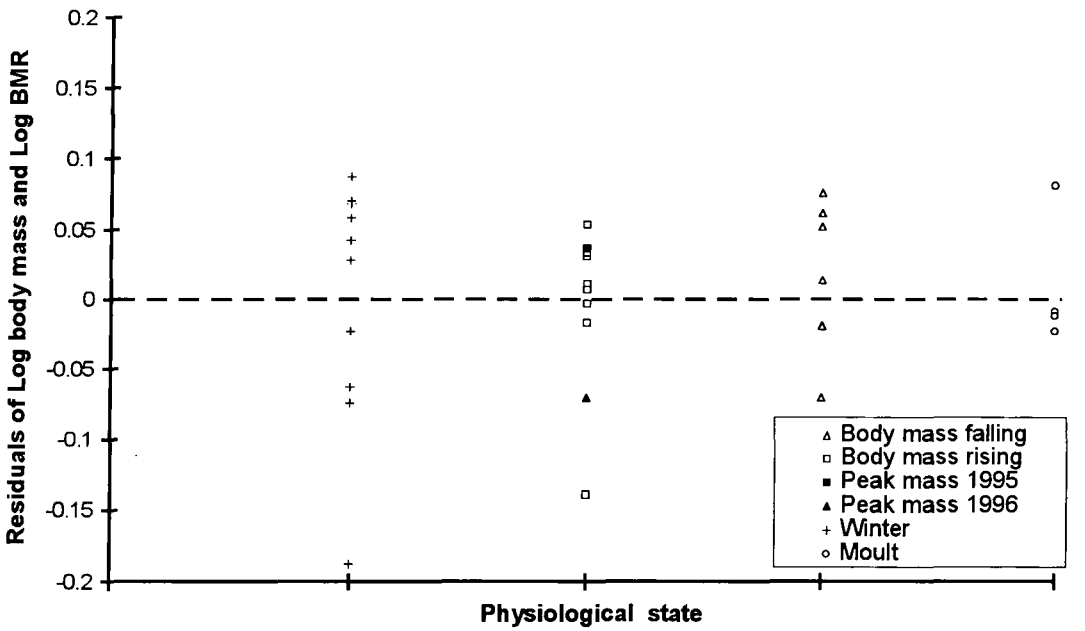


Table 3.3.4.2: The non-significant relationships between Log BMR (Watt) and Log BM (g) within individual Knot

ID	Regression equation	n	r^2	Significance
GG	LogBMR= -0.532(0.54)+0.313(0.25)LBM	31	0.051	>0.05
WW	LogBMR= -1.277(1.00)+0.690(0.47)LBM	30	0.070	>0.05
YY	LogBMR= -2.333(1.46)+1.158(0.68)LBM	18	0.154	>0.05
WGG	LogBMR= 0.158(0.51)+0.028(0.24)LBM	16	0.001	>0.05
WGL	LogBMR= -0.280(0.39)+0.286(0.17)LBM	20	0.133	>0.05
WLG	LogBMR= 0.126(0.71)+0.010(0.34)LBM	15	0.000	>0.05
WLL	LogBMR= -0.773(0.74)+0.427(0.34)LBM	17	0.100	>0.05
WWW*	LogBMR= 0.319(0.94)-0.107(0.45)LBM	11	0.006	>0.05
WYG*	LogBMR= -0.528(0.65)+0.311(0.30)LBM	18	0.060	>0.05
WYY	LogBMR= 0.164(0.49)+0.080(0.22)LBM	13	0.010	>0.05

* Individual did not undergo pre-migratory fattening

There were no significant difference between the slopes of the Log BMR against Log BM regressions in Table 3.3.4.1 (MANOVA, $F_{8,190} = 0.92$, $P>0.05$), although there was a significant difference between the elevations (MANOVA, $F_{8,198} = 12.76$, $P<0.001$). The mean slope for the relationship between Log BMR and Log BM within individuals was 0.898 (0.102SE), i.e. not significantly different to 1.0 but significantly different to 0.67. The differences in elevation that are seen between individual Knot could have resulted because certain individuals had a higher mass of metabolically active lean tissues at a given BM than other individuals, or because they had higher average metabolic outputs per gram of lean tissue.

Table 3.3.4.3 shows that only 7 individual Knot out of the 19 examined showed a significant allometric relationship between Log BMR and Log fat mass, so that as

fat mass increased, BMR also increased. The mean slope of 0.273(0.04SE) of this relationship however was far less steep than that seen between Log BMR and Log BM within the same 7 individuals of 0.882(0.127).

Table3.3.4.3: The relationship between Log BMR (Watt) and Log PFAT (g) within individual Knot. (LFM= Log Fat mass)

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -3.17(0.18)+0.319(0.10)LFM	32	0.270	<0.01
GW	LogBMR= 0.10(0.05)+0.110(0.03)LFM	33	0.240	<0.01
GY	LogBMR=-0.568(0.25)+0.468(0.17)LFM	21	0.285	<0.05
LL	LogBMR=-0.318(0.14)+0.252(0.08)LFM	13	0.481	<0.01
OO	LogBMR=-0.299(0.14)+0.258(0.13)LFM	33	0.285	<0.01
WG	LogBMR=-0.194(0.15)+0.269(0.12)LFM	15	0.309	<0.05
WGY	LogBMR=-0.124(0.16)+0.235(0.11)LFM	16	0.255	<0.05

In only one Knot (GW), which was brought into captivity as a juvenile, was there a significant relationship between Log BMR and Log PTLM; interestingly the mass exponent was negative, so as PTLM increased BMR decreased, showing that metabolic output per gram was altering. This individual exhibited a rather late "spring" peak in body mass, in mid-August-early September (see Appendix I). This individual showed a significant yet negative correlation between PTLM and PFAT (r_{33}= -0.357, P<0.05), that is as lean mass increased the mass of fat decreased. Therefore, although a stepwise multiple-regression with Log PTLM and Log PFAT of Knot GW as independent variables of Log BMR indicated that Log PTLM (T_{30}=2.740, P<0.05) rather than PFAT (T_{30}=1.358, P>0.05) explained more of the variation in BMR in this individual, it is arguable whether PTLM and PFAT in this individual are truly independent of each other.

Table3.3.4.4: The relationship between Log BMR (Watts) and Log PTLM (g) within individual Knot. (LLM= Log Lean mass)

ID	Regression equation	n	r^2	Significance
GW	LogBMR= 4.473(1.16)-2.096(0.58)LLM	33	0.296	<0.05

Table3.3.4.5: The relationship between Log BMR (Watts) and Log BM (g) in individual Knot with only measurements included that were measured outside the spring migratory period but including moult.

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -2.736(2.33)+1.343(1.05)LBM	14	0.119	>0.05
GG	LogBMR= 1.992(0.71)- 0.885(0.33)LBM	16	0.375	<0.05
GL	LogBMR= 4.390(1.84)- 2.030(0.87)LBM	16	0.280	<0.05
GO	LogBMR= -1.662(1.86)+0.857(0.87)LBM	16	0.070	>0.05
GW	LogBMR= 0.631(0.95)- 0.189(0.45)LBM	20	0.010	>0.05
GY	LogBMR= -0.663(2.14)+0.362(1.01)LBM	16	0.009	>0.05
OO	LogBMR= -3.700(0.97)+1.765(0.45)LBM	15	0.529	<0.01
WW	LogBMR= -2.123(1.18)+1.100(0.56)LBM	25	0.145	=0.06
YY	LogBMR= -1.190(2.38)+ 0.621(1.12)LBM	14	0.014	>0.05
WGG	LogBMR= 0.638(0.57)- 0.198(0.27)LBM	14	0.040	>0.05
WLG	LogBMR= 0.950(0.62)- 0.405(0.30)LBM	12	0.157	>0.05
WLL	LogBMR= -0.179(0.92)+ 0.154(0.42)LBM	15	0.010	>0.05

The hypothesis to be tested next was whether these seasonal mass-independent variations seen within individuals in BMR only occurred during a particular period in the annual cycle, i.e. during the spring migratory period. That is if the data

collected during the period of body mass increase and decrease of an individual Knot in spring were removed from the analysis, a significant allometric relationship would be present between Log BMR and Log BM and Log BMR and Log PTLM and PFAT.

This was not the case, as only 3 Knot (Individuals GG, GL and OO) showed a significant relationship between Log BMR and Log BM when measurements taken outside the spring migratory period were included in the regression analyses (see Knot WG and WGY also in Table 3.3.4.1). BMR measurements taken during moult were included because moult appeared to have little effect on BMR (see graphs 3.5A-F). The individuals LL, WGL and WYY did not have enough data points outside the spring migratory period to allow useful analysis, and the 4 individuals (WG, WGY, WWW and WYG) which did not show any pre-migratory fattening and are included in tables 3.3.4.1 and 3.3.4.2. Knot GL and GG exhibited a significant yet negative relationship between Log BMR and Log BM, that is when BM was higher than average the BMR measured was lower. However, Knot GL showed this relationship to be positive when all points are included in the analysis, as seen in table 3.3.4.1. Knot GG exhibited a significant relationship only when the data points from the spring migratory period were excluded from the analysis (Table 3.3.4.6).

Table3.3.4.6.: The relationship between Log BMR (Watts) and Log PFAT (g) in individual Knot outside the spring migratory period (including moult)

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -0.682(0.65)+0.527(0.37)LFM	14	0.143	>0.05
GG	LogBMR= 0.581(0.14)-0.310(0.09)LFM	16	0.504	<0.01
GL	LogBMR= 0.841(0.25)-0.511(0.17)LFM	16	0.389	<0.01
GO	LogBMR= -0.115(0.50)+0.180(0.31)LFM	16	0.026	>0.05
GW	LogBMR= 0.224(0.12)+0.008(0.09)LFM	20	0.001	>0.05
GY	LogBMR= -0.208(0.35)+0.214(0.24)LFM	15	0.053	>0.05
OO	LogBMR= -0.573(0.18)+0.446(0.11)LFM	15	0.549	<0.01
WW	LogBMR= -0.322(0.22)+0.348(0.15)LFM	25	0.190	<0.05
YY	LogBMR= -0.212(0.51)+ 0.230(0.33)LFM	14	0.038	>0.05
WGG	LogBMR= 0.316(0.10)- 0.066(0.07)LFM	14	0.075	>0.05
WLG	LogBMR= 0.275(0.09)- 0.119(0.06)LFM	12	0.254	>0.05
WLL	LogBMR= -0.063(0.28)+ 0.057(0.17)LFM	15	0.008	>0.05

Table 3.3.4.6 shows that only four individual Knot showed a significant relationship between Log BMR and Log PFAT, when only the points outside the pre-migratory period were included in the regression analysis. As with the BMR and BM relationship during this time, Knot GG and GL show a significant yet negative relationship between BMR and fat mass. Surprisingly there was not one significant relationship found between Log BMR and Log PTLM in any of the individual Knot when the data points during this period outside the spring migratory period were only included in the analysis (Table 3.3.4.7).

Table3.3.4.7: The relationship between Log BMR (Watts) and Log PTLM (g) in individual Knot outside the spring migratory period (including moult)

ID	Regression equation	n	r^2	Significance
GF	LogBMR= 2.036(0.04)-0.021(0.02)LLM	14	0.128	>0.05
GG	LogBMR= -1.744(1.68)+0.922(0.84)LLM	16	0.080	> 0.05
GL	LogBMR= -2.395(4.98)+1.250(2.49)LLM	16	0.018	>0.05
GO	LogBMR= -17.411(6.57)+8.857(3.31)LLM	16	0.355	>0.05
GW	LogBMR= 3.101(2.48)- 1.422(1.23)LLM	20	0.070	>0.05
GY	LogBMR= 0.428(4.88)-0.161(4.43)LLM	15	0.003	>0.05
OO	LogBMR= 5.858(4.62)-2.802(2.27)LLM	15	0.105	>0.05
WG	LogBMR= -3.554(2.62)+1.868(1.31)LLM	14	0.144	>0.05
WW	LogBMR= -0.100(3.40)+0.146(1.72)LLM	25	0.003	>0.05
YY	LogBMR= -1.432(3.87)+ 0.780(1.92)LLM	14	0.014	>0.05
WGG	LogBMR= -3.818(2.37)+ 2.000(1.18)LLM	14	0.195	>0.05
WLG	LogBMR= -3.972(2.24)+ 2.061(1.13)LLM	12	0.174	>0.05
WLL	LogBMR= -1.624(2.39)+ 0.885(1.89)LLM	15	0.041	>0.05
WWW	LogBMR= 3.063(2.39)- 1.492(1.20)LLM	11	0.146	>0.05
WYG	LogBMR= -2.211(1.88)+ 1.175(0.94)LLM	18	0.089	>0.05
WGY	LogBMR= -4.258 (3.11)+ 2.237(1.55)LLM	16	0.130	>0.05

The relationship between Log BMR and Log BM and Log BMR and Log PTLM/PFAT were then examined within-individual Knot to try and identify if these relationships changed during the period of body mass increase in spring and also as BMR fell from peak in spring. Only one of 7 Knot (Knot GL) showed a significant relationship between Log BMR and Log BM (Table 3.3.4.8) and Log BMR and Log PFAT (3.3.4.9) as body mass increased in spring to peak mass, an increase due (primarily) to fat deposition. Although some captive Knot exhibited

peaks in PTLM (estimated using TOBEC) during the period of BM increase in spring , no individual Knot showed sufficient directional increases in PTLM during this time to enable regression analysis to be carried out. As BMR fell rapidly after the spring migratory peak some highly significant relationships between Log BMR and Log BM (Table 3.3.4.10), Log BMR and Log PFAT (Table 3.3.4.11) and Log BMR and Log PTLM (Table 3.3.4.12) were revealed. Knot GF and Knot LL showed significant relationships, at the 5% and 1% significance level respectively, between Log BMR and Log PTLM during this time. The relationship between Log BMR and Log PTLM during this time was significant at the 10% level in Knot GW and Knot OO, with the mass exponent being negative in Knot GW.

Table3.3.4.8: The relationship between Log BMR (Watts) and Log BM (g) within individual Knot, as body mass increased to peak in spring

ID	Regression equation	n	r^2	Significance
GF	LogBMR= 0.144(1.39)+0.066(0.60)LBM	10	0.002	>0.05
GG	LogBMR= 1.738(1.07)- 0.699(0.48)LBM	7	0.295	>0.05
GL	LogBMR= -1.366(0.28)+0.731(0.13)LBM	4	0.941	<0.01
GO	LogBMR= 2.563(2.87)-1.038(1.28)LBM	4	0.247	>0.05
GW	LogBMR= -1.055(0.71)+0.596(0.32)LBM	7	0.416	>0.05
OO	LogBMR= 1.713(2.16)-0.669(0.95)LBM	10	0.048	>0.05
WGL	LogBMR= 1.021(0.83)-0.274(0.36)LBM	10	0.068	>0.05

Table3.3.4.9: The relationship between Log BMR (Watts) and Log PFAT (g) within individual Knot, as body mass increased to peak in spring

ID	Regression equation	n	r^2	Significance
GF	LogBMR= 0.259(0.534)+0.020(0.27)LFM	10	0.001	>0.05
GG	LogBMR= 0.579(0.36)- 0.221(0.20)LFM	7	0.195	>0.05
GL	LogBMR= -0.191(0.02)+0.247(0.01)LFM	4	0.996	<0.01
GO	LogBMR= 1.205(1.18)-0.519(0.63)LFM	4	0.251	>0.05
GW	LogBMR= -0.186(0.24)+0.252(0.13)LFM	7	0.443	>0.05
OO	LogBMR= 0.658(0.84)-0.246(0.45)LFM	10	0.036	>0.05
WGL	LogBMR= 0.624(0.34)-0.121(0.17)LFM	10	0.057	>0.05

Table3.3.4.10: The relationship between Log BMR (Watts) and Log BM (g) within individual Knot, as BMR decreased after migratory peak in BMR

ID	Regression equation	n	r^2	Significance
GF	LogBMR=Log -1.238(0.59)+0.685(0.26)LBM	9	0.504	<0.05
GG	LogBMR=Log -1.051(0.48)+0.583(0.22)LBM	8	0.583	<0.05
GW	LogBMR=Log -1.722(0.47)+0.953(0.22)LBM	7	0.791	<0.01
LL	LogBMR=Log -1.585(0.25)+0.800(0.11)LBM	5	0.942	<0.01
OO	LogBMR=Log -3.274(0.81)+1.560(0.34)LBM	8	0.752	<0.01
WGL	LogBMR=Log 0.246(0.34)+0.053(0.15)LBM	4	0.058	>0.05
WYY	LogBMR=Log -0.592(1.00)+0.429(0.47)LBM	4	0.294	>0.05

Table3.3.4.11: The relationship between Log BMR (Watts) and Log PFAT (g) within individual Knot as BMR decreases after migratory peak in BMR

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -1.106(0.31)+0.740(0.17)LFM	9	0.711	<0.01
GG	LogBMR= -0.131(0.15)+0.205(0.09)LFM	8	0.484	<0.05
GW	LogBMR= 0.009(0.07)+0.203(0.04)LFM	7	0.846	<0.01
LL	LogBMR= -0.285(0.09)+0.265(0.05)LFM	5	0.896	<0.05
OO	LogBMR= -0.606(0.23)+0.455(0.13)LFM	8	0.664	<0.01
WGL	LogBMR= 0.322(0.15)+0.022(0.08)LFM	4	0.040	>0.05
WYY	LogBMR= 5.088(1.71) - 2.42(0.87)LFM	4	0.007	>0.05

Table3.3.4.12: The relationship between Log BMR (Watts) and Log PTLM (g) within individual Knot as BMR decreases after migratory peak in BMR

ID	Regression equation	n	r^2	Significance
GF	LogBMR= -1.238(0.58)+0.685(0.26)LLM	9	0.504	<0.05
GG	LogBMR= -3.356(2.27)+1.790(1.14)LLM	8	0.291	>0.05
GW	LogBMR= 4.690(1.68)- 2.020(0.85)LLM	7	0.629	=0.06
LL	LogBMR= -5.768(0.54)+2.976(0.27)LLM	5	0.968	<0.01
OO	LogBMR= -9.539(4.06)+4.800(2.00)LLM	8	0.490	=0.05
WGL	LogBMR= 1.849(0.24)+0.400(0.65)LLM	4	0.160	>0.05
WYY	LogBMR= -0.363(4.89)+0.346(2.46)LLM	4	0.070	>0.05

3.3.5 Effect of captivity on metabolic output of the lean tissues

To determine whether changes occurred in the BMR of an individual Knot during time in captivity, when controlling for body mass, a comparison of lean-mass-specific BMR (see Section 3.2.1) and body composition predicted by TOBEC in individual Knot were compared between year 1 and year 2 of captivity was carried out with the results shown in Table 3.3.5.1. All BMR measurements taken were outside the pre-migratory period and did not include measurements taken during the moult because the mean levels calculated for each year only included measurements taken in comparable months of year 1 and year 2 (Jan-March). It can be seen that body mass tended to vary little between year 1 and year 2 within individual Knot, except in the individuals OO (increase) and GW (decrease). PTLM remained stable in all Knot between year 1 and 2 except in Knot WW, where there was a significant decrease of 3g in mean PTLM between year 1 and year 2. The individual WW, brought into captivity as an adult, only showed a clear spring peak in BM in year 1 of captivity and not in year 2. This alongside the loss in PTLM may indicate that this individual was suffering from some pathological condition, although no indication of this was seen when this individual was examined after being sacrificed. PFAT was slightly more variable between years, with Knot OO showing a significant increase in fat mass between year one and year two and a significant decrease in PFAT occurring in Knot GG, GL and GW between year one and year two.

Lean-mass-specific BMR increased significantly in all captive Knot between year 1 and year 2, with all but the increases seen in individuals GF and GO being significant. Therefore, it is clear that the average metabolic output per gram of the lean tissues was increasing to a significant degree between year 1 and year 2 of captivity. Analysis of the residuals comparing the relationship between BMR and PTLM during year one and year two could not be carried out satisfactorily

Table 3.3.5.1: Changes in body mass, body composition and lean-mass-specific BMR in individual Knot between year one and year two of captivity. All BMR measurements taken outside spring migratory period (Jan-March). All tests are t-tests *(Bonferroni correction in all cases)*

All BMR measurements were taken outside the spring migratory period and do not include periods of moult

1= Year one in captivity 2= Year two in captivity

LMSBMR= (BMR/PTLM$^{1.011}$)*1000--------(See Section 3.2)

ID	BM(g)			PTLM(g)			PFAT(g)			LMSBMR(mWatts/g)		
	1	2	P	1	2	P	1	2	P	1	2	P
GF	163	162	>0.05	108	107	>0.05	55	55	>0.05	14.9	18.4	<0.05
GG	143	133	<0.05	102	102	>0.05	41	31	<0.01	12.1	12.8	>0.05
OO	149	158	<0.05	109	107	>0.05	40	51	<0.05	12.5	14.5	<0.01
WW	130	126	>0.05	98	95	<0.01	32	31	>0.05	14.2	18.1	<0.01
YY	136	136	>0.05	103	103	>0.05	33	33	>0.05	12.7	14.7	=0.05
GL	130	125	<0.05	99	100	>0.05	31	25	<0.05	11.5	15.3	<0.001
GO	141	142	>0.05	97	97	>0.05	44	45	>0.05	15.1	17.8	=0.06
GW	131	122	<0.01	104	103	>0.05	27	19	<0.01	15.4	17.9	<0.05
GY	132	132	>0.05	103	102	>0.05	29	31	>0.05	11.6	14.1	<0.05

because no individual Knot exhibited a significant relationship between BMR and PTLM during this time (see Table 3.3.4.7).

3.3.6 Variation in the ratio and mass of different organs in captive Knot sacrificed in different physiological states

From the work reported in section 3.3.1, it is known that the total lean mass, as predicted with TOBEC, did not tend to vary significantly within individual Knot seasonally, but the actual contribution of the organs that make total lean mass may well alter on a seasonal basis. To try and identify whether the relative masses of the organs involved in digestion (liver, gut and stomach) and exercise (pectoralis major and heart) were changing during the period of BM increase and decrease in spring when compared to those outside the migratory period, two simple equations were employed (Adapted from Piersma, 1993 & Piersma, *pers com.*). Only individual birds used for enzyme assays were used in this analyses (All data Arcsine transformed).

(Wet mass in grams) (See also Appendix III)

Equation 1: $\dfrac{\text{Liver mass}}{\text{Single pectoralis major muscle}}$

Equation 2: $\dfrac{\text{Stomach mass + gut mass + liver mass}}{\text{heart mass + single pectoralis major mass}}$

* Single pectoral muscle mass used simply because this followed the protocol of Piersma (*pers. com*).

The ratios produced by equation 1 differed significantly with physiological state (ANOVA, $F_{2,20}$ =5.080, P <0.05). The mean (SE) liver to PM ratio was significantly lower in birds sacrificed as body mass was decreasing in spring (0.135±0.014, SNK P<0.05) than in birds during body mass increase in spring 0.213(0.023) and birds outside the spring migratory period 0.203(0.019) respectively. There was no relationship in spring between days since peak BMR and ratio of liver to PM mass in individual birds (r_7 = -0.129, P>0.05). Equation 2 did not produce significantly different ratios with season (ANOVA, $F_{2,18}$ =1.412, P >0.05), although the ratio calculated in birds decreasing in body mass were once again lower at 0.663(0.046), than in birds increasing body mass in spring (0.783±0.057) or in birds sacrificed outside the spring migratory period (0.696±0.041). There was also no relationship in spring between days since peak BMR and the ratio between the mass of the digestive organs and the exercise organs within individual birds (r_7 = -0.129, P>0.05). When an index of muscle mass, the standard muscle index (SMI), which takes into consideration body (skeletal) size (Evans & Smith, 1975) was calculated (Mean±SE), no significant difference was found (Arcsine transformed ANOVA, $F_{2,19}$ =1.507, P>0.05) between the periods of BM increase in spring (0.240±0.05), BM decrease in spring (0.218±0.06) and outside the spring migratory period (0.222±0.05).

Another method employed to determine whether certain organs did alter in mass on a seasonal basis, was to examine the residuals produced from log-log regressions of organ mass (wet mass) on total lean mass (TLM, wet mass), as derived from carcass analysis (see Appendix II). The residuals produced from regression of Log liver mass on Log TLM (no data available on two individuals sacrificed during the period outside the spring migratory period) were significantly more positive (ANOVA, $F_{2,16}$= 3.247, P=0.05), i.e. individuals had a higher liver mass for a given total lean mass, at the 5% significance level when birds were depositing fat in spring than during the period of body mass loss in spring and outside the spring migratory period (SNK, P<0.05). The residuals produced when regressing log stomach + gut mass against Log TLM were also only positive, i.e.

Table 3.3.6.1: Ratios obtained when comparing organ masses of individual Knot in different physiological states.

ID	SEASON	EQUATION 1	EQUATION 2
WYY	3	0.218	0.769
LY	3	0.124	0.834
BW	3	0.188	0.611
WYG	3	0.259	0.545
WGY	3	0.189	0.610
WG	3	0.280	0.653
WWW	3	0.165	0.833
WGG	1	0.295	0.922
WLL	1	0.221	0.916
WLG	1	0.254	0.860
GW	1	0.231	0.927
YY	1	0.152	0.694
AA	1	0.127	0.625
YG	1	0.124	0.535
GO	2	0.173	0.647
GG	2	0.140	0.721
OO	2	0.130	0.770
GY	2	0.091	0.461
WGL	2	0.138	0.748
GL	2	0.086	0.505
GF	2	0.198	0.790

Equation 1 **Liver/PM (wet mass)**

Equation 2 **Liver+stomach+intestine/PM+heart (wet mass)**

All Knot sacrificed had been in captivity for over 1 year (see Appendix III)

1. Period of body mass (BM) increase to peak in spring- BM increasing

2. Period of BM decrease, after spring peak in mass- BM decreasing

3. Any period outside time period 1 and 2- Outside-

higher than expected stomach and gut mass for a given TLM, during the period of fat deposition in spring than during the other two periods but the difference was not significant (ANOVA, $F_{2,16}$= 2.167, P>0.05), as were the residuals produced between the pectoral muscle mass (single PM + single supracoracoideus) and TLM (ANOVA, $F_{2,16}$= 1.094, P>0.05).When log-log regression of heart mass and TLM was carried out the residuals were also more positive during the period of body mass increase in spring than during body mass decrease in spring (ANOVA, $F_{1,12}$= 2.395, P>0.05), although data on heart mass was not available for Knot sacrificed during the period outside the spring migratory period. A similar story was seen when the lean dry mass of the liver and PM were regressed against the total lean dry mass (as determined using solvent analysis, see Chapter 2). In those individuals sacrificed as they deposited fat in spring, the residuals produced were significantly more positive, i.e. they had significantly larger lean dry liver masses for a given total lean dry mass (ANOVA, $F_{2,16}$= 3.987, P<0.05, SNK<0.05). Although the residuals produced in the birds depositing fat in spring were more positive than during the other two periods, the difference was not significant at the 10% level (ANOVA, $F_{2,16}$= 1.657, P>0.05).

3.3.7 Seasonal variation in the percentage of mitochondria (by volume) measured in superficial and deep pectoral muscle and in the liver of captive Knot.

From the regression analyses carried out in section 3.3.4 it can be clearly seen that seasonal variations in BMR in most individual Knot are not simply due to seasonal variations in body mass or in body mass components but that some mass-independent factor must be affecting an individual's BMR.

Two procedures were employed to try and identify what and where these seasonal changes in metabolic output per gram occurred. These were:

1. Measurement of percentage composition (by volume) of mitochondria in the liver and PM of captive Knot under different physiological states.

2. Measurement of two aerobic enzymes, succinate dehydrogenase and citrate synthase in the liver, PM, gut and heart of captive Knot. (see Section 3.3.8).

From the summary table 3.3.7.1 (also Tables 3.3.7.2 to 3.3.7.4), it can be seen that the mean percentage of mitochondria (by volume) in the liver of captive Knot remained fairly constant between seasons, with the highest mean percentage being measured as body mass was increasing during spring. There was no significant seasonal difference in mean percentage volume of mitochondria between any of the three groups: (Mann Whitney U-tests)

BM increasing and BM decreasing in spring, $U= 7.5$, $P>0.05$,

BM increasing and outside migratory period, $U=8.0$, $P>0.05$,

BM decreasing and outside migratory period, $U=7.5$, $P>0.05$.

Table 3.3.7.1: Mean (±SE) seasonal percentage by volume of mitochondria in liver and superficial and deep aspects of pectoralis major (PM) muscle. (n=4 in all cases). BM= Body mass

Season	Liver	Superficial PM	Deep PM
Outside migratory period	21(0.01)	24(0.02)	25(0.01)
BM rising	23(0.02)	24(0.01)	25(0.01)
BM falling	22(0.02)	22(0.01)	25(0.01)

Table 3.3.7.1 also shows that the mean percentage of mitochondria by volume was slightly lower in the superficial aspect of the PM when Knot were losing mass

Table.3.3.7.2: Percentage of mitochondria (by volume) in liver of captive Knot, where each count denotes a different electron micrograph.

1. Period of body mass (BM) increase to peak in spring- BM increasing

2. Period of BM decrease, after spring peak in mass- BM decreasing

3. Any period outside time period 1 and 2- Outside-

ID	SEASON	COUNT 1(%)	2	3	4	5	6	7	8	9	10	MEAN(SE)
WL	**3**	20	15	24	18	17	14	21	19	19	18	19(1.0)
GN	**3**	26	27	18	19	18	17	19	21	17	21	20(1.1)
OR	**3**	28	20	21	24	20	23	19	19	24	28	23(1.1)
YL	**3**	27	21	26	22	19	28	25	19	26	20	23(1.1)
GW	**1**	20	20	21	19	16	22	21	25	27	17	21(0.9)
WLL	**1**	32	27	26	24	26	29	20	30	24	32	27(1.3)
WGG	**1**	19	16	20	23	20	16	17	15	19	14	18(0.6)
WLG	**1**	23	23	19	22	22	20	24	19	22	21	21(0.6)
YE	**2**	29	23	20	25	29	22	30	29	30	24	26(1.1)
WH	**2**	29	23	22	24	31	30	21	32	32	30	28(1.4)
GO	**2**	16	18	17	23	17	18	21	22	16	21	19(0.9)
GL	**2**	20	20	20	23	15	15	19	17	14	18	18(0.9)

Table.3.3.7.3: Percentage of mitochondria (by volume) in superficial aspect of pectoralis major muscle of captive Knot.

ID	SEASON	COUNT 1(%)	2	3	4	5	6	7	8	9	10	MEAN(SE)
WL	**3**	18	18	17	24	25	16	22	20	25	18	20(1.1)
GN	**3**	22	19	30	23	29	24	30	21	30	23	25(1.3)
OR	**3**	17	24	20	24	25	21	23	18	26	23	22(1.0)
YL	**3**	25	32	27	26	26	33	34	26	31	26	29(1.1)
GW	**1**	17	17	15	16	15	23	23	23	22	16	18(0.9)
WLL	**1**	26	22	26	25	26	20	24	25	26	24	24(0.6)
WGG	**1**	27	23	21	27	24	25	24	21	28	23	24(0.6)
WLG	**1**	20	23	20	29	17	26	24	22	23	20	22(0.9)
YE	**2**	22	23	25	23	25	20	24	25	25	24	24(0.6)
WH	**2**	27	22	30	31	22	31	26	20	26	24	26(1.2)
GO	**2**	20	19	20	21	21	23	21	18	26	22	21(2.1)
GL	**2**	19	21	23	23	26	27	23	26	23	25	24(0.6)

Table.3.3.7.4: Percentage of mitochondria (by volume) in deep aspect of pectoralis major muscle of captive Knot.

ID	SEASON	COUNT 1(%)	2	3	4	5	6	7	8	9	10	MEAN(SE)
WL	**3**	23	26	21	24	24	24	22	24	23	25	24(0.5)
GN	**3**	21	26	30	23	21	18	23	24	21	26	23(1.0)
OR	**3**	21	25	21	21	21	22	23	20	23	19	22(0.5)
YL	**3**	33	31	26	28	31	26	27	28	27	21	28(1.0)
GW	**1**	24	24	28	25	29	30	32	29	20	26	27(0.9)
WLL	**1**	24	22	20	23	28	26	30	27	25	25	25(0.9)
WGG	**1**	19	19	24	24	19	22	22	23	23	23	22(0.6)
WLG	**1**	25	26	26	25	29	22	20	21	20	24	24(1.1)
YE	**2**	21	28	31	25	29	25	30	23	25	23	26(1.0)
WH	**2**	21	25	26	24	23	24	27	27	26	20	24(0.7)
GO	**2**	21	21	26	24	23	29	23	23	26	31	22(0.9)
GL	**2**	24	23	29	21	26	29	26	25	31	32	27(0.9)

after peak body mass in spring (22%), than during the other two periods (24%), but this difference was not significant. (BM increasing and BM decreasing, U= 5.0, P>0.05, BM increasing and outside, U=5.0, P>0.05, and BM decreasing and outside U=8.0, P>0.05). The mean percentage of mitochondria by volume in the deep (dorsal) portion of the PM did not vary significantly between seasons. There was no significant difference in mean mitochondrial volume when the values calculated for the superficial and deep aspects of the PM were compared (Arcsine transformed Paired T-test, where T_{11} =1.796, P<0.05).

3.3.8 Seasonal variation in the activity per gram (wet mass) of the aerobic enzymes succinate dehydrogenase and citrate synthase in various lean tissues of captive Knot.

No seasonal variation in the volume composition (percentage) was shown during the three distinct physiological periods, so the actual activity of mitochondrial enzymes in various lean tissues was then carried out to see if they changed on a seasonal basis. The graphs 3.3.8.A-D and the Table 3.3.8.1 show that no significant seasonal variation in the mean activity per gram (wet mass) of succinate dehydrogenase (SDH) was found in neither the liver nor heart tissue of captive Knot (One-way ANOVA, $F_{2,20}$ = 0.242, P>0.05 and $F_{2,16}$ = 1.039, P>0.05 respectively). Captive Knot that were undergoing fat deposition in spring did however show significantly lower mean levels of SDH activity in the pectoralis major (PM) muscle and significantly higher mean activity of the enzyme in the gut (small intestine) compared to the other two seasons, (ANOVA, $F_{2,20}$ = 5.058, P<0.05, $F_{2,20}$ = 3.799, P<0.05 respectively, where Student-Neuman-Keuls Test was significant if P<0.05).

Table 3.3.8.1: Mean(SE) activity of succinate dehydrogenase (μmol min^{-1} g^{-1}) in various lean tissues of captive and wild Knot.

Tissue	Outside migratory period n=8	BM rising n=7	BM falling n=7	Wild Knot in late winter n=5
Liver	13.5(2.3)	16.4(3.5)	14.5(2.9)	17.7(1.7)
Pectoral muscle	7.6(0.7)	2.8(0.3)	8.4(2.1)	16.8(2.3)
Gut	7.8(0.8)	14.1(2.2)	11.7(1.7)	-
Heart	29.4(2.2)	21.4(2.3)	21.1(4.9)	-

Citrate synthase (see Tables 3.3.8.2 and 3.3.8.4) did not show any significant variation with season. Liver (One-way ANOVA, $_{F2,20}$ = 0.710, P>0.05), PM ($F_{2,20}$ = 2.297, P>0.05) heart ($F_{2,16}$ = 0.544, P>0.05) and gut ($F_{2,20}$ = 2.194, P>0.05).

Table 3.3.8.2: Mean(SE) activity of citrate synthase (μmol min^{-1} g^{-1}) in various lean tissues of captive and wild Knot.

Tissue	Outside migratory period n=8	BM rising n=7	BM falling n=7
Liver	10.0(2.0)	9.5(0.2)	8.7(0.6)
Pectoral muscle	131.4(6.1)	107.9(6.8)	104.0(12.8)
Gut	7.2(0.6)	9.5(0.2)	8.7(0.6)
Heart	85.8(7.9)	81.4(3.2)	87.6(7.7)

Significant and positive correlations may have been expected between the activity of the two aerobic enzymes in the various lean tissues. However, when

correlations were carried out on the pooled data there was a significant and positive correlation between the activity of SDH and CS in the gut (Pearson product-moment correlation $r_{22} = 0.510$, $P<0.05$), but while the relationships between the two enzymes in the other tissues were positive, they were not significant: liver ($r_{22} = 0.295$, $P>0.05$), PM ($r_{22} = 0.319$, $P>0.05$) and heart ($r_{18} = 0.136$, $P>0.05$).

From table 3.3.8.1, it is clear that SDH activity in the gut was significantly higher during the period of body mass increase in spring. To establish if SDH activity decreased gradually or rapidly in the gut following from the significantly higher levels measured during fat deposition, a correlation was carried out between SDH activity and days since BMR peak. A highly significant negative correlation ($r_7 = -0.879$, $P<0.01$) existed between SDH activity in the gut and days since peak BMR and SDH activity in the liver decreased significantly at the 10% level with days since peak BMR ($r_7 = -0.663$, $P=0.078$). No significant correlations were found between SDH activity and days since peak BMR in the other 2 lean tissues (See Graphs 3.3.8.E-H). In these 7 individuals, peak lean-mass-specific BMR occurred on the same date as peak BMR, therefore there was a highly significant negative correlation between SDH activity in the gut and days since peak lean-mass-specific BMR also. Therefore at peak BMR it would appear that the levels of SDH in the gut and liver, i.e. the metabolic activity of these tissues were still high. The activity of SDH in the pectoral muscle of individuals sacrificed as BM was falling in spring was close to the mean value calculated during the period of BM loss, which was significantly higher than the level measured in the PM of individuals sacrificed as BM was increasing in spring. That is that the level of SDH in the PM was high during the period of peak BMR, as BM fell, at the same time as SDH activity in the gut and liver was decreasing but still relatively high.

There were no significant relationships between days since peak body mass and activity of SDH in liver ($r_7 = 0.103$, $P>0.05$), PM ($r_7 = 0.327$, $P>0.05$), heart ($r_7 =$

-0.231, P>0.05) and gut (r_7 = -0.273, P>0.05).This would tend to suggest that the increase in SDH that must occur in the pectoral muscle between the period of BM increase and then PM decrease must occur rapidly. No significant correlations were found between the activity of CS in the liver, PM, heart or gut and lean-mass-specific BMR, days since peak BMR, days since peak body mass or days since body mass increase in spring during any of the three distinct physiological periods (P>0.05 in all cases).

Figures 3.3.8.A-D: Show seasonal activity of succinate dehydrogenase in the liver, pectoralis major (PM), small intestine and the heart of captive Knot at different physiological states in their annual cycle.

Figure 3.3.8.A: Succinate dehydrogenase activity per gram in the liver

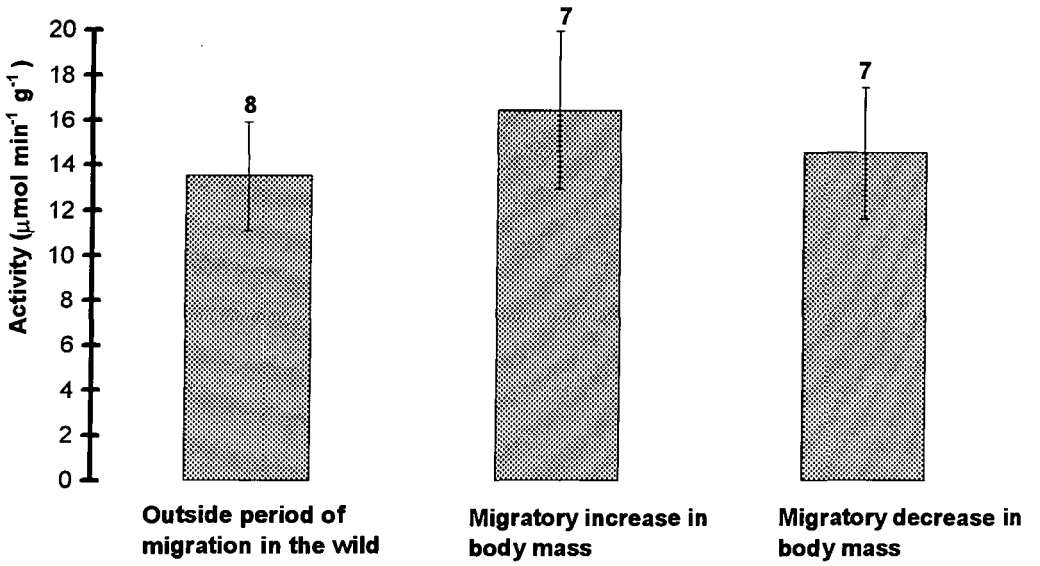


Figure 3.3.8.B: Succinate dehydrogenase activity per gram in the PM

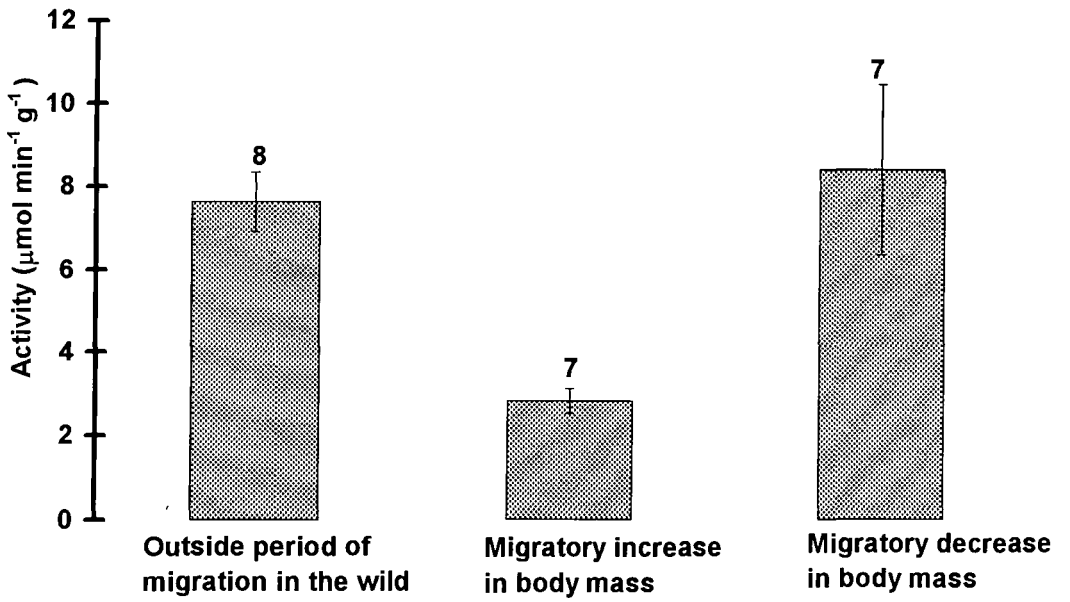


Figure 3.3.8.C: Succinate dehydrogenase activity per gram in the small intestine

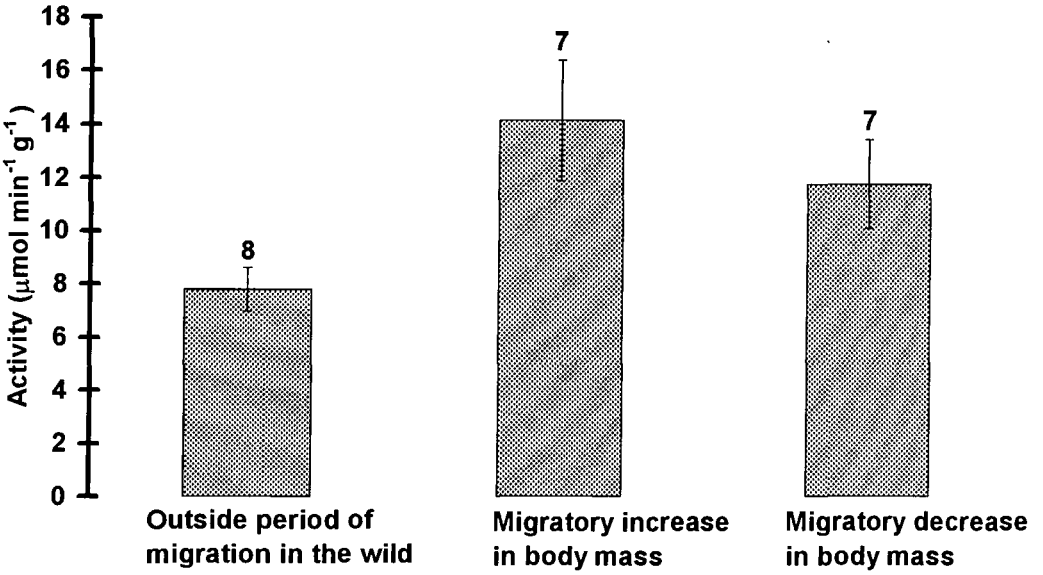


Figure 3.3.8.D: Succinate dehydrogenase activity per gram in the heart

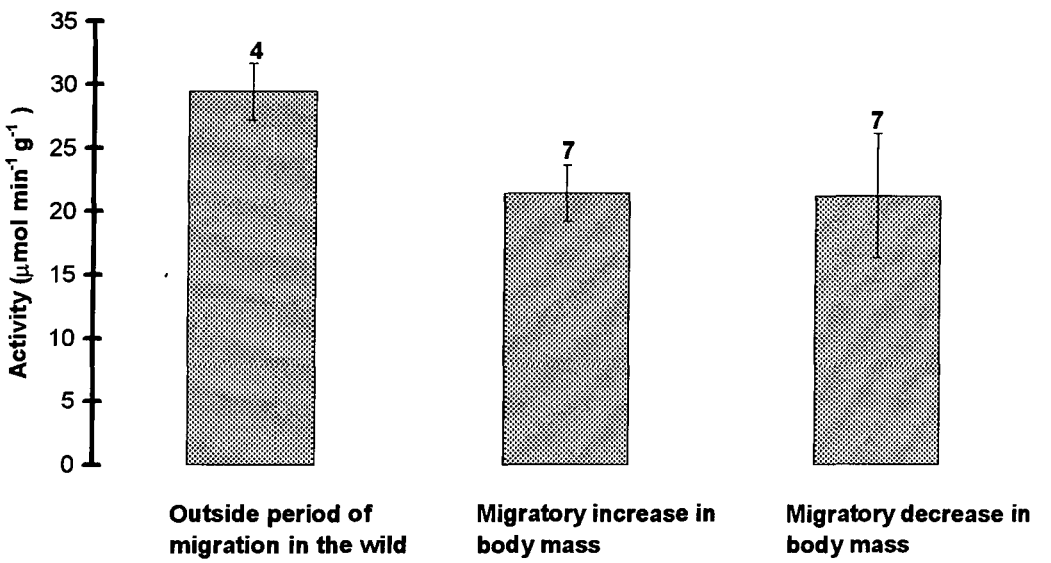


Figure 3.3.8.E: Relationship between succinate dehydrogenase activity (μmol min^{-1} g^{-1}) in liver and time since peak BMR, in captive Knot

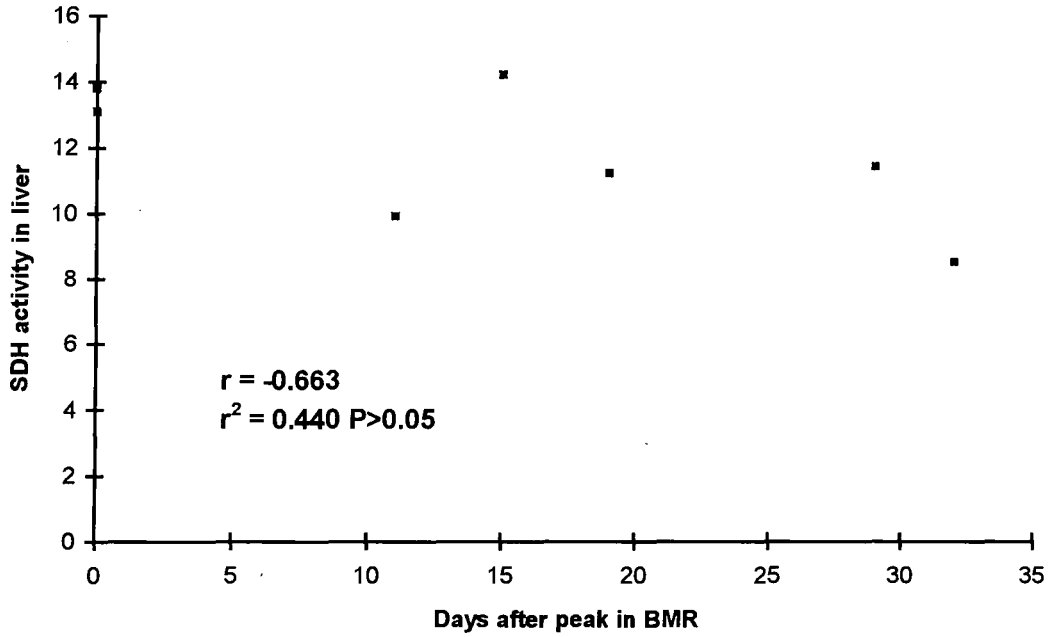


Figure 3.3.8.F: Relationship between succinate dehydrogenase activity (μmol min^{-1} g^{-1}) in PM and time since peak BMR

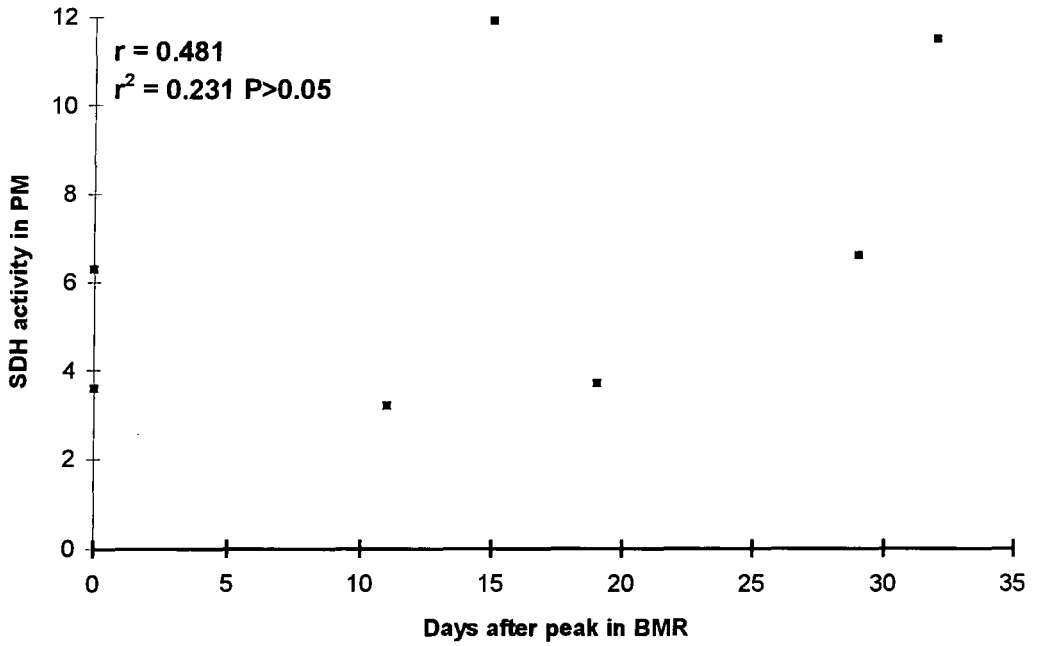


Figure 3.3.8.G: Relationship between succinate dehydrogenase activity (μmol min^{-1} g^{-1}) in heart and time since peak BMR

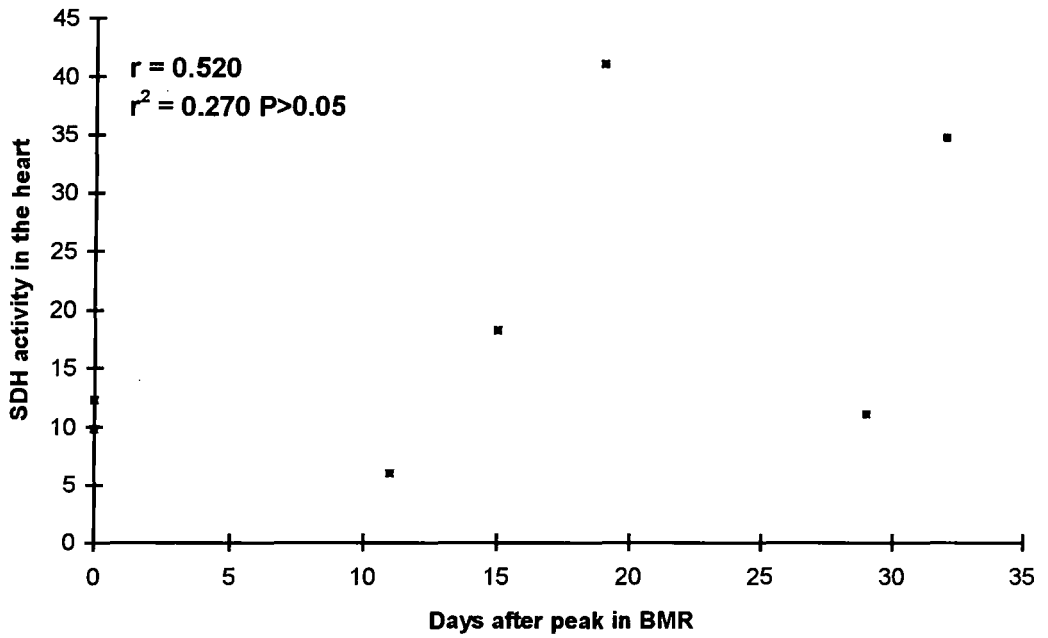


Figure 3.3.8.H: Relationship between succinate dehydrogenase activity (μmol min^{-1} g^{-1}) in gut and time since peak BMR

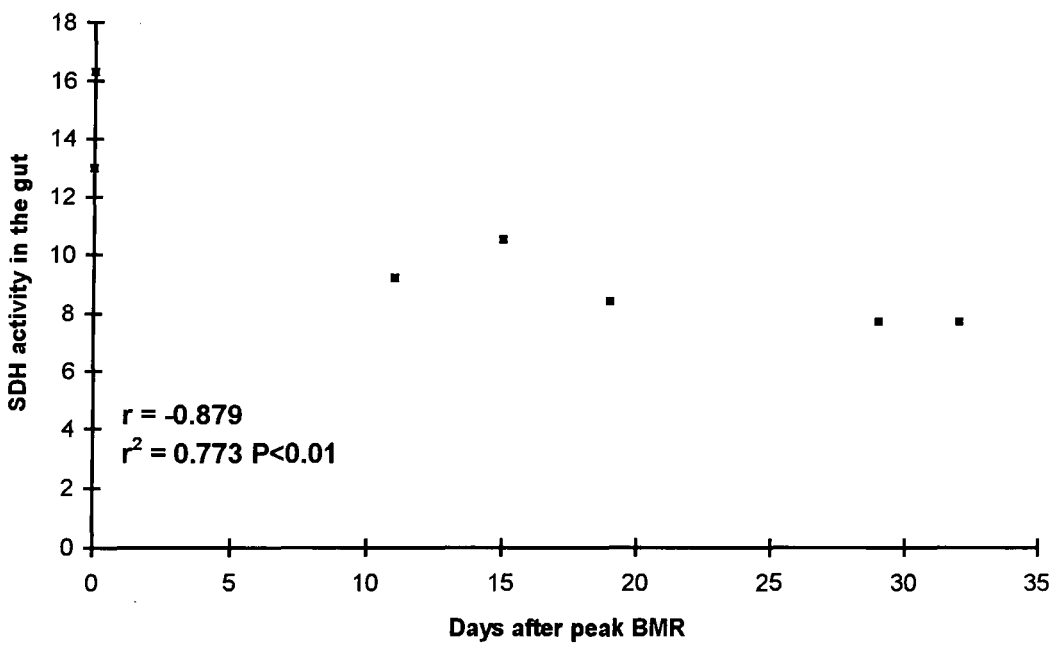


Table 3.3.8.3: Activity (μmol min^{-1} g^{-1}_{wet}) of succinate dehydrogenase in various lean tissues of individual captive Knot. (Seasons follow protocol set out in Section 3.2)

ID	SEASON	LIVER	PM	HEART	GUT
WYY	3	16.367	6.631	29.248	6.960
LY	3	8.215	9.121	25.551	7.149
YW	3	23.003	9.910	-	6.033
LW	3	17.068	10.491	-	5.862
WO	3	8.193	7.813	-	6.165
YYY	3	20.930	5.170	-	12.793
BW	3	5.412	6.131	27.024	8.331
LG	3	8.906	5.800	35.729	9.053
YY	1	8.312	1.882	22.624	10.537
AA	1	6.534	2.055	23.201	7.830
YG	1	18.237	3.617	30.170	20.088
WLL	1	8.673	2.739	17.737	8.216
WLG	1	28.662	2.140	21.748	11.911
WGG	1	15.859	3.427	11.206	22.505
GW	1	28.393	3.600	22.843	17.588
GO	2	13.114	3.617	12.275	16.334
GG	2	11.186	3.745	41.038	8.415
OO	2	9.915	3.243	5.972	9.239
GY	2	11.360	6.639	11.069	7.745
WGL	2	8.451	11.532	34.688	7.636
GL	2	13.766	6.345	9.773	12.989
GF	2	14.177	11.897	18.219	10.516

Table 3.3.8.4: Activity (μmol min^{-1} g^{-1}_{wet}) of citrate synthase in various lean tissues of individual captive Knot.

ID	SEASON	LIVER	PM	HEART	GUT
WYY	3	14.424	129.413	68.233	9.421
LY	3	6.787	129.413	105.594	8.686
YW	3	6.940	121.325	-	7.652
LW	3	11.041	129.413	-	7.802
WO	3	7.118	121.325	-	8.095
YYY	3	11.225	169.855	-	6.247
BW	3	10.966	113.237	79.669	4.328
LG	3	11.332	137.501	89.564	5.741
YY	1	9.706	129.413	68.233	6.477
AA	1	9.334	126.889	82.475	9.569
YG	1	9.153	121.325	89.780	10.599
WLL	1	9.995	88.885	90.257	9.656
WLG	1	10.022	86.225	76.389	8.654
WGG	1	8.965	98.556	74.556	8.998
GW	1	9.003	103.669	86.289	9.669
GO	2	10.784	97.060	62.846	12.807
GG	2	8.974	88.972	71.824	5.300
OO	2	10.180	105.148	71.824	6.916
GY	2	8.007	80.883	103.464	7.655
WGL	2	7.610	80.883	93.372	6.036
GL	2	6.201	97.060	121.276	7.066
GF	2	9.453	177.943	107.736	5.303

3.4 Discussion

3.4.1 Seasonal changes in body mass and body composition in captive Knot

Knot in captivity exhibited and maintained annual cycles in body mass (BM) very similar in timing, duration and intensity to those seen during the non-breeding seasons in wild conspecifics (Piersma & Davidson, 1992) and in other studies that used captive Knot (Cadee, 1992, Piersma, 1994). These seasonal changes in BM of captives, that had access to food *ad libitum*, suggest that seasonal variations in BM may be under some pre-programmed endogenous control (Gwinner, 1990) and are not due directly to seasonal fluctuations in food availability. The environmental factors in this study under manual control were photoperiod and temperature (set to mimic the external daylength and follow the ambient temperature as closely as possible in Durham), and it seems likely that photoperiod is involved in the synchronisation and timing of the clear annual cycles in body mass seen in captive Knot. Significant increases in BM generally occurred during April-late May only in adult captive Knot. This is the period in the wild when fat deposition occurs to fuel long-distance migration to the breeding grounds.

Adult Knot did not show body mass peaks in autumn in this study, mirroring the lack of an autumnal peak in mass seen by Piersma *et al* (1995), although some juveniles did show slight autumnal increases in BM, during their first autumn in captivity. The probable explanation for this general lack of an autumnal peak in the BM of captive Knot, was that the photoperiod that wild birds experience on the breeding grounds at this time, was not replicated in captivity. Captive Knot in this study followed the light regime of Durham, NE England and therefore the photoperiod encountered by Knot just before southward migration from the

breeding grounds was absent in captivity. It may also be feasible that some environmental cue other than decreasing day-length is required to stimulate fat deposition in autumn and that this cue is absent in captivity.

Only three juvenile Knot showed clear pre-migratory increases in BM during their first spring in captivity but in two individuals these increases exceeded the levels measured in any other captive Knot. It is generally thought that few juvenile Knot migrate to the breeding grounds in their first spring. If in the wild juvenile Knot are constrained through an inability to compete for food as successfully as adults or forage as efficiently as adults, this may affect their ability to deposit sufficient fat stores, both in terms of volume and in timing, to fuel long-distance migratory flight. Possibly the availability in captivity of food *ad lib* forgoes the necessity for juveniles to 'compete' for food, thus allowing them to lay down sufficient fat to undergo 'migration'. However, if this was the case then most, if not all juveniles would exhibit migratory increases in their first year in captivity and this is certainly not the case unless spring migratory fattening is under both genetic and environmental control. It is possible that in the wild certain juvenile Knot can successfully compete for food with adults or are highly efficient foragers and are able to deposit sufficient fat stores in their first spring to fuel migration. Such individuals will then surely be at a selective advantage if they can breed in their first year. There appeared to be no sexual bias to whether a juvenile exhibited a spring peak in BM during year 1 of captivity or not, as two of the three 'atypical' juveniles were female (Knot GF and WGL) and one was male (Knot WYY).

The application of Total Body Electrical Conductivity (TOBEC) in this study enabled changes in body composition, i.e. predicted total lean mass (PTLM) and predicted total fat mass (PFAT), to be followed seasonally within individual Knot. As with wild Knot, where fat mass accounted for 30-35% of peak total body mass at the staging post of Balsfjord (Evans, 1992), the largest proportion of the spring increase in BM in captivity was due to the deposition of fat, although fat mass

accounted for a greater percentage of total body mass in captivity (up to 52% in one individual, see also Appendix III).

Increases in lean mass have been shown to occur in wild Knot during the period of fat deposition in spring, due to both flight muscle hypertrophy and the hypertrophy of other components of the lean tissues (Davidson & Evans, 1989; Evans, 1992). Seasonal peaks in PTLM occurred in some captive individuals during the period of fat deposition in year one of captivity, although these increases in lean mass of around 2-3g were considerably less than the 12g increase in lean mass reported by Evans (1992) seen between a wintering population of Knot and a population en route to the breeding grounds (but also see Lindstrom & Piersma, 1993). In my study, the seasonal peak in PTLM measured within an individual during the spring migratory period tended to occur well in advance of the seasonal peak of BM and PFAT. This implies that if flight muscle hypertrophy (which forms only a part of the increase in PTLM) occurs to increase muscle power to carry large fat stores, i.e. the 'power training effect' described by Marsh (1984), this phenomenon does not appear to occur in captive Knot. Although captive Knot do not undergo long-distance flight, it is known that the amount of exercise that the PM muscles experience in captivity is sufficient to maintain the mass of these muscles (see Appendix III) but not enough to lead to muscle hypertrophy during the period of fat deposition in spring.

The slight increase in PTLM, as BM is rising in spring, may indicate a period of gut hypertrophy which may aid food uptake and assimilation (Heitmeyer, 1987). Wild Knot of the subspecies *islandica* staging in the Wadden Sea in early spring had significantly greater stomach masses than individuals sampled later in the spring on Iceland, even though the former birds had considerably lower body masses (135±13g) than the latter (208 ±11g) individuals (Piersma, 1994). TOBEC, unfortunately cannot differentiate between the different tissues and organs that contribute to PTLM. Therefore it is possible that the flight muscle

may actually hypertrophy in captive Knot at peak BM (see Weber & Piersma, 1996) with possibly a compensatory decrease in gut mass. Gut mass is thought to decrease in certain wader species prior to long-distance flight (Piersma, 1994, Piersma & Gill Jr, 1998), possibly as an adaptation to decrease mass and hence wing loading. Seasonal changes in the mass of various lean organs will be discussed later in section 3.4.8.

Most adults showed an extensive if not a complete moult into breeding plumage in spring but juveniles tended not to show breeding plumage until their second year in captivity, as found also in the wild. All Knot in this study ceased pre-nuptial moult before they began fat deposition in spring. Piersma *et al* (1996) reported that 'any potential effect of moult on BMR was obscured by the large seasonal mass-associated variations'. This would imply that in their study, birds continued with pre-nuptial moult during fat deposition in spring.

3.4.2 Diurnal variation in BMR

Captive Knot in my study did not show any significant diurnal variation in BMR. In particular they did not show significantly lower levels of BMR at night, unlike those seen in certain passerines (Aschoff & Pohl, 1970). Feeding in the wild by Knot is likely to be governed by the tide and not by the light intensity (see Kersten & Visser, 1996), therefore there is no reason that BMR will be depressed in waders at night. No evidence of diurnal variation in BMR was found by Scott (1991) in captive Grey Plover, Redshank and Sanderling; by Scheiffarth *(pers. com)* in Bar-tailed Godwits *Limosa lapponica*; or by Mansour (in prep) in Dunlin and nocturnal foraging by waders has been recorded in the wild in many species (McCurdy *et al*, 1997).

3.4.3 Effects of captivity on BMR

The effect of captivity on BMR has been studied primarily by comparing wild and captive birds of the same species and of similar mass. In some, no difference has been reported (Dawson & Carey, 1976; Weathers *et al*, 1983); in others captives had an increased BMR (Warkentin & West, 1990) and in yet others, captives had a decreased BMR compared to wild conspecifics (Piersma *et al,* 1996). It would appear from the literature that no study, before this one, has looked intensively (see Cadee, 1992) at how BMR changes within an individual bird over time in captivity.

Table 3.3.5.1 shows that BMR, hence average metabolic rate per gram of the lean tissues (lean-mass-specific BMR) in Knot, brought into captivity either as adults or juveniles, increased between the first and second year of captivity, although lean body mass remained stable. As reported earlier (Section 3.4.1) Knot did not moult fully into breeding plumage in captivity and may not have achieved full winter plumage in captivity either. Thermal conductance is a function of plumage state so that a decrease in the mass of contour feathers is mirrored by an increase in thermal conductance (Piersma, 1994). If captive Knot did not moult fully into winter plumage, so that some worn old feathers were retained, their thermal conductance may have been higher that normal. This would have led to a decrease in core T_B unless the metabolic output per gram of the lean tissues increased or other insulating mechanisms increased. It is not known whether the captive Merlins in Warkentin & West's (1990) study were undergoing moult or not during the period of measurement. As the mass of fat carried by a captive individual outside the spring migration period was similar in its first and second years, both in terms of absolute fat carried and relative to BM, any insulating effect of the adipose tissue (if any) will have been similar in the two years. In retrospect, it may have been useful to have measured the core body temperatures of individual captive Knot during the two years of captivity, and to have

monitored any changes which occurred in T_B within individual birds in different physiological states.

Warkentin & West (1990) postulated that the captive Merlins in their study had higher T_B and BMR than wild conspecifics because significant atrophy of the pectoral muscle (PM) mass had occurred in captivity, although they did not measure this and total body masses were similar in captive and wild birds. They argued muscle atrophy had been compensated by growth of other components of lean mass, mainly highly metabolically active organs such as the liver, in captives, leading to a higher T_B and BMR. If a loss in PM mass did occur, and it was not actually measured, this loss was probably due more to disuse atrophy because the birds had been injured and were incapable of flying, rather than anything else. The results in Appendix III of this study and those of Piersma (1994) throw doubt on Warkentin & West's explanation, because in captive Knot, the masses of the liver and gut are significantly smaller in captive Knot than in wild Knot. It may be possible in the future, to investigate changes in various organ masses with time in captivity through using a technique such as nuclear magnetic resonance (NMR) imaging. The application of this procedure was investigated during this project but was found to be prohibitively expensive.

3.4.4 Seasonal variation BMR within individual Knot

The main aim of work reported in this chapter was to investigate the mechanisms underlying seasonal variations in BMR within an individual Knot. While it is well established that captive Knot maintain lower BMR and lean masses (Piersma *et al*, 1996; this study) than wild conspecifics, the factors involved in seasonal changes in BMR are likely to be the same in both groups. The initial question being addressed was whether BMR varied seasonally simply in proportion to seasonal variation in the mass of the metabolically active lean tissues, as claimed by

Piersma *et al*, (1996) and Lindstrom, (1997) or whether the metabolic intensity of the lean tissues also changed with season.

The results of my study clearly show that seasonal peaks in BMR in captive Knot did not coincide with and therefore are not necessarily a consequence of seasonal peaks in body mass (BM), or of PTLM but that the average metabolic output per gram of the lean tissues alters on a seasonal basis. These findings are in direct contrast to those of Cadee (1992), Piersma (1994) and Piersma *et al* (1995). The seasonal maxima in both metabolic rate and lean mass specific metabolic rate tended to occur soon after body mass began to fall in spring. The rapid rate at which body mass is lost during this time may be due in part to the elevated BMR but it also requires a voluntary reduction in food intake (cf. Kersten & Piersma, 1987). The 3-4 day duration for which BMR is at a peak is approximately around the same time that it takes a wild Knot to fly from the final staging post to the breeding grounds, if an airspeed of $10m^{-1}\ s^{-1}$ (Piersma, 1994) is assumed and the distance covered to enable a Knot to fly from west Iceland over the Greenland inlandice to the breeding grounds at Ellesmere Island is approx. 2300km (Alerstam *et al*, 1986; Davidson & Wilson, 1992). The peak in BMR at this time may allow Knot to increase their metabolic output when most needed, such as during migratory flight, without placing undue strain on the support systems, assuming a relationship exists between the maximal sustainable metabolic rate and BMR (see Ricklefs *et al*, 1996; Ricklefs, 1996). A lower BMR at other times of year should lead to a lower energy intake rate and hence lower food requirements. The peaks in BMR may have been missed by other authors (Cadee, 1992, Piersma, 1994) because they did not measure BMR in individual birds as frequently as in this study; Piersma (1994), for example, used measurements at 6-week intervals.

3.4.5 The relationship between BMR, body mass and body composition within individual Knot

The results in this study show that, within an individual, changes in metabolic rate are correlated significantly with changes in body mass in some but not all captive Knot and that in birds that showed a significant relationship between BMR and BM, fat mass was a better predictor of BMR than lean mass, when all data points are included in the analysis. The supposition from this is that more of the variation in BMR within an individual bird is associated with, though not necessarily caused by, changes in the mass of fat rather than in the mass of lean tissues. These findings agree with those reported to occur in captive Redshank by Scott *et al* (1996). Captive Knot exhibited only up to 9 % variation in PTLM (Knot WGL) on a seasonal basis (see 3.4.1), with most body mass change (up to 59 %) being accounted for by variation in PFAT. This contrasts markedly to the suggestion of Piersma (1994) and Piersma *et al* (1996) that the 'mass of the metabolically highly active tissue varies more than BM, in the course of the annual cycle of an individual Knot', although it is not made clear whether the metabolically active tissues vary more in absolute terms or in percentage terms.

In those Knot in which a significant relationship existed between log BMR and log BM (all data points included in the analysis), the mean mass exponent of 0.90(±0.10SE, where n=9) was considerably less steep than those calculated by Daan *et al* (1989) for Kestrel of 1.67 (±0.12), Scott (1991) for Redshank of 1.23 (±0.23) and Piersma (1994) for Knot of 1.38(0.02). The mean value of the mass exponent in my study was however very similar to that calculated by Scott (1991) for Grey plover 0.92(±0.13). There was no significant difference found between the mass exponents of the lines of best fit between Log BMR and Log BM within the individual Knot. In my Knot, the mass exponent between individual birds varied between 0.368 (±0.14), which is less than the slope for homomorphic change (0.667), to 1.446(0.68) which is greater than the slope for mass

proportionality (1.0). The significant variation found between individuals in their intercepts, i.e. where the line of best fit bisects the y-axis, reflects that some individuals had a higher BMR for a given BM than others, possible reflecting a higher mass of lean tissues and/or a higher average mass specific metabolic output of these lean tissues. Therefore, even between individuals of the same species under exactly the same environmental conditions, the within-individual relationship between BMR and BM can differ significantly. This variation between individuals will be investigated in the next chapter.

That a rise in BMR within an individual Knot occurs primarily in association with fat deposition agrees with the findings of Scott *et al* (1996) on Redshank. The majority of the metabolic heat produced by an animal at rest is generated by the organs of the thoracic cavity and the brain and during activity by the skeletal muscles (Schmidt-Nielsen, 1984). These tissues all possess moderate to high metabolic activity per gram. Previous workers have assumed that fat deposition could increase BMR within an individual only in an indirect manner because avian adipose tissue is known to have a low rate of oxygen consumption per gram (Scott & Evans, 1992). However, although the rate of oxygen consumption is low, the overall mass of fat deposited by captive Knot in spring can be considerable. From the values of oxygen consumption rate (ml $O_2 g^{-1} h^{-1}$) quoted by Scott & Evans (1992) for Dunlin (0.06 for adipose tissue, 0.65 for skeletal muscle (PM muscle) and 0.84 for liver), the overall O_2 consumption by each tissue per hour can be estimated for Knot, if these values are multiplied by estimates of the mean masses of these particular tissues in Knot. For a Knot of mass 150g (100g of lean mass and 50g of fat mass, *pers. obs*), with a pectoral muscle mass of 30g and a liver mass of 3g (see Appendix III), the basal O_2 consumption of each tissue will be 3 ml $O_2 g^{-1} h^{-1}$, 19.5 ml $O_2 g^{-1} h^{-1}$ and 2.5 ml $O_2 g^{-1} h^{-1}$ for the adipose tissue, pectoral muscle and the liver respectively. Thus fat would contribute to about 10% of total metabolic activity per gram in these three tissues and more overall than the liver. As the maximum spring peak mass of fat recorded in an individual Knot was 127g (this study), the direct contribution of fat mass to

overall metabolic rate may be considerable, although later work in chapter 4 tends not to support this.

Although the direct contribution of adipose tissue to BMR may be greater than previously realised in species that deposit large amounts of fat in spring, the main effect of fat deposition on BMR is likely to be indirect. To support these large fat deposits may require skeletal muscle tone to increase to maintain posture at rest and so lead to a higher BMR. Increased work levels required during locomotion will lead to a higher daily energy expenditure (DEE) but this will affect BMR only if BMR parallels DEE (Kersten & Piersma, 1987), which is known to be debatable in birds, although such a relationship appears to exist in mammals (Ricklefs *et al*, 1996). The cost of heating the fat stores has been put forward as another reason why BMR increases in fat (Heldmaier & Steinlechner, 1981, Witter & Cuthill, 1993), but as argued by Scott et al (1996), this increase would not account for 'increases in the BMR/BM exponent above unity'. BMR may also increase as fat mass increases, within an individual, due to an increase in thermal conductivity, particularly if the feather mass remains constant. This decrease in insulation could be compensated by any insulatory effects of the fat deposits (Mortensen & Blix, 1986), although an insulatory capacity has not been shown experimentally in adult birds (see Scott *et al*, 1996).

The residuals from the log-log regression of BMR on BM of 6 captive Knot consistently showed BMR at peak BM in spring to be less than that expected allometrically. It has generally been thought that the fatter a bird becomes the higher its BMR. Scott (1991) referred to this phenomenon of increasing BMR with increasing fat mass as the "law of diminishing returns". Each gram of fat laid down increases BMR, therefore every subsequent gram becomes energetically more expensive and difficult to obtain, although Scott (unpublished data) calculated that the additional increase in BMR at a maximum fat deposition rate of 9g/day in captive Knot to be only 0.4% per day of mean daily BMR. The law of

diminishing returns predicts that migrating birds should use several migratory staging posts and avoid the costs associated with maximum fat loading, but only if migration is not time constrained and that there are no benefits to the bird arriving on the breeding grounds with additional energy stores. But if some mechanism exists during fat deposition that could 'dampen' this allometric relationship, a bird may be able to achieve peak mass by a steady rate of gain in fat mass from a constant rate of food intake, without ever-increasing metabolic costs. Certainly during the period of fat deposition in spring there was, with the exception of one individual, generally a very poor relationship (very low r^2 values) between log BMR and log BM (Table 3.3.4.8), and between log BMR and log PFAT (Table 3.3.4.9). Daan *et al* (1989) suggested that Kestrels on low maintenance food regimes reduced energy metabolism below that of homomorphic change through a disproportionate reduction in the heart and kidney lean mass. They however did not discuss whether a reduction in metabolic activity of these tissues could also have taken place during this time. This is conceivably what may be happening in captive Knot at this time, when energy saving may aid fat deposition through a reduction in highly metabolically active organs such as the heart and the kidneys and/or a reduction in the metabolic activity per gram of these lean tissues.

A relationship between BMR/BM and BMR/PFAT tended to occur only in individuals that had undergone pre-migratory fattening and loss (exceptions being individuals WG and WGY), but these significant relationships generally disappeared if only the measurements of BMR outside the spring migratory period were included in the analysis. When taking only the BMR measurements recorded outside the spring migratory period, as done by Piersma (1994), only five Knot in my study showed a significant relationship between BMR and BM. Two of these individuals showed negative mass exponents, where BMR actually decreased steeply as BM increased. The number of Knot that showed a significant relationship between BMR and PFAT outside the migratory period were very few. There were no significant relationships between BMR and lean mass in any of the 19 Knot studied during this period. During the 2 week period as BMR

decreased from peak some Knot showed highly significant relationships between log BMR and BM, log BMR and PFAT and also between log BMR and PTLM. This was the only period that any Knot (exception being GW) showed any significant relationship between BMR and lean mass. Only 7 individuals had sufficient BMR measurements taken during this time to enable regression analysis to be undertaken. No Knot showed any significant relationship between BMR and PTLM, as PTLM fell during spring. This indicates that the average metabolic intensity of the lean tissues was changing, unless other sources of change in BMR were over-riding. As mentioned earlier not all captive Knot showed clear peaks in PTLM during the period of body mass increase in spring and those that did often showed periods of fluctuating but not directional increases or decreases in PTLM during this time.

The consistent lack of a significant relationship between BMR and BM within individual captive Knot that did not undergo pre-migratory fattening, and within Knot that did but when outside the period of spring fat deposition and loss is puzzling. It was not however, simply individuals that showed small scale variation in BM that showed no relationship between BMR and BM (see Scott, 1991). It would appear that a factor or factors other than the mass of the body tissues is involved in determining BMR of an individual at any one time. These data would strongly suggest that the average metabolic output per gram of the lean tissues is clearly changing within individual Knot with time and that BMR is not simply a consequence of the body mass, fat mass or lean mass carried by an individual. It may be possible that the contribution of various metabolically active tissues are changing on a seasonal basis both in mass and metabolic intensity, although PTLM is remaining constant. This possibility will be discussed later (Section 3.4.8).

3.4.6 Seasonal changes in the proportion of various organs contributing to total lean mass in captive Knot

From the results section, it can be seen that there tends to be a clear peak in BMR and the average lean mass-specific BMR in individual captive Knot that occurs as their total body mass and total lean mass is decreasing in spring. Therefore, the average mass-specific metabolic output of these lean tissues is changing on a seasonal basis (see section 3.4.4). Is there any evidence in this study that the mass of the metabolically active lean tissues are changing on a seasonal basis, as reported in wild conspecifics (Kersten & Piersma, 1987, Piersma *et al*, 1996, Piersma & Lindstrom, 1997)?

Table 3.4.6.1: Oxygen consumption in various lean tissues of the rat (Adapted from Field *et al*, 1939)

ORGAN	O_2 consumption (ml O_2 hr^{-1} g^{-1}_{wet})	Whole organ (ml O_2 hr^{-1})
KIDNEYS	4.120	5.76
LIVER	2.010	16.48
HEART	1.930	1.35
BRAIN	1.840	4.23
SPLEEN	1.330	0.53
ALIMENTARY CANAL	1.010	8.08
SKELETAL MUSCLE	0.875	53.72

Various organs that make up total lean mass of an individual bird or mammal contribute disproportionately to the overall BMR, due to their high mass-specific oxygen consumption per gram (Field *et al*, 1939; Krebs, 1950). The organs with

the highest rates of O_2 consumption (ml g^{-1}_{wet} hr^{-1}) in the rat, calculated by Field *et al* (1939) are given in Table 3.4.8.1 and there is good evidence that BMR correlates significantly with the masses of vital organs, such as the kidney, brain and liver (For review see Piersma & Lindstrom, 1997). The energy consumption of these organs (at rest) make up a significant part of an animals BMR.

The equations used in section 3.3.8 were employed to try and identify whether changes in the relative proportions of various lean tissues involved primarily in:

1. Digestion (liver, gut and stomach)
2. Exercise (pectoralis major muscle and heart);

change with physiological state (see also Appendix III).

Equation 1. $$\frac{\text{liver mass}}{\text{single pectoralis major muscle}}$$

Equation 2. $$\frac{\text{stomach mass + gut mass + liver mass}}{\text{heart mass + single pectoralis major mass}}$$

When equation 1 was employed, significantly lower mean ratios were calculated during the period of BM decrease in spring than during the other two periods, with the highest mean ratios being produced in birds depositing fat in spring. This significantly lower ratio, as BM was decreasing in spring implies that liver mass was relatively smaller during this time or that the mass of the PM, due to hypertrophy, was relatively larger or both, i.e. the use of proportions can be ambiguous (see Packard & Boardman, 1988). It is perfectly feasible that liver mass decreases after the period of fat synthesis and deposition in spring, as the liver is the major site of fat synthesis in birds (see Ramenofsky, 1990). Equation 2 (digestive organs/exercise organs) produced ratios that were lower during the period of body mass loss in spring, when compared to the other two seasons but these differences were not significant at the 5% level. The mean ratio for equation 2 was highest during the period of fat deposition in spring.

Arguably a better method to investigate whether seasonal changes do occur in the masses of various organs with season is by using log-log regression of various organ masses with total lean mass (TLM, wet weight), with subsequent analysis of the residuals produced or by using calculations that take into account some measure of body size. The first method was employed to see whether seasonal differences occurred in the relative masses of liver, PM, gut mass (intestine mass + stomach mass) during the three physiological states and heart mass (only for individuals increasing and decreasing in BM during spring). Birds sacrificed as they deposited fat in spring had significantly more positive residuals (5% level), i.e. higher liver masses for a given TLM and higher total lean dry mass for a given TLDM, than during the other two periods. The residuals produced when regressing gut mass, PM mass and heart mass against TLM produced more positive residuals, (but not significantly so) in individuals were increasing in mass in spring. When employing an index of muscle mass, the standard muscle index (SMI), there was no significant difference between the three periods in SMI, although the highest mean SMI was recorded during the period of fat deposition in spring.

Of the individuals sacrificed as BM was falling in spring, two individuals were sacrificed at their seasonal maximum in BMR, with the others being sacrificed between 5 and 33 days after peak BMR. The two individuals sacrificed at their seasonal highs in overall BMR had the lowest ratios between liver-PM and digestive organs-exercise organs of any Knot sampled. This would suggest that during peak BMR the absolute contribution of the exercise organs to total lean mass is relatively greater and/or the relative contribution of the digestive organs is relatively less or both. We know from captives in this study, that there is firm evidence that the mass of the digestive organs is higher during the period of fat deposition, with also higher than average masses of the exercise organs occurring during this time. There is evidence that PM mass and heart mass are highest in wild Knot around one week before spring migration (Piersma, *unpublished data*),

with reduced stomach mass being recorded in heavy premigratory individuals (Piersma, 1994).

The digestive organs are likely to be non-functional, therefore relatively costly to maintain during this time of peak BMR because, it is likely that captive Knot when losing mass in spring are reducing food intake or have ceased it all together and therefore have little need for a large gut mass and the reduction of gut may also take place in captivity. A large gut (stomach + intestine) mass may not even be necessary on arrival at the breeding grounds as change of diet occurs between the breeding grounds and the wintering grounds (Piersma, 1994), although much of the work involving wild birds during this migratory period has concentrated solely on mass changes occurring in the stomach and not in the intestine (Piersma, 1994, but see also Piersma & Gill Jr, 1998). A large gut mass may also increase wing loading and decrease flight speed during migratory flight in the wild (Jehl, 1997). A reduction in gut mass, before migratory flight may lead to the maintenance of small but highly metabolically activity gut, rather than a large but moderately metabolically active gut, if the costs due to wing-loading of carrying a large gut exceed costs of maintaining metabolically active gut mass. On arrival on the breeding grounds if an individual bird has to wait for the gut mass to increase, albeit fairly rapidly, the additional advantage of maintaining high gut metabolic activity may enable the maintenance of a high gut assimilation rate, if gut aerobic activity is an indication of high gut assimilation rate. Recent work has shown that food intake level in a Thrush nightingale *Luscinia luscinia* was 60% higher on day two than day one after a 12 hour flight in a wind tunnel, with an apparent deposition of protein structures during refuelling. This may indicate a very rapid increase in the gut mass during this time (Klassen, Kvist & Lindstrom, *unpublished data*). It is also likely that the metabolic costs of maintaining an active gut during this time is likely to be minuscule compared to the metabolic costs of flight.

From the work in my study it is also known that the level of SDH activity in the gut decreases linearly with time after peak BMR. The period of peak BMR as BM is falling in spring is short-lived and so any changes in metabolic activity and organ mass that may occur during this time, leading to a clear increase in the average lean-mass-specific BMR within individual Knot, may have been missed because most Knot were sacrificed many days after their seasonal peak in BMR. Of the two individuals sacrificed immediately as BM was falling in spring, it can not be certain that BMR was not simply rising to a peak and the BMR measured in these two individual was not going to increase yet higher.

The aerobic capacity of the gut, which is known to decrease linearly with days since peak BMR (see 3.3.7), was at a high level (similar to mean value as BM increasing in spring) in the two Knot sacrificed as they lost BM in spring. Therefore, the peak BMR seen in spring may be due to an increase in the metabolically active exercise organs, which while having a small mass have a high mass-specific metabolic rate and therefore probably contribute a large percentage to overall BMR. But at this time there is also a high metabolic output per gram of the gut, although it is unlikely that the birds rapidly losing mass during this period are actually feeding. The high aerobic activity of the gut (SDH activity) seen in these two birds, when it is unlikely that active digestion is taking place, suggests a rapid turnover of gut tissue during this time. This may be a factor involved in the elevated BMR generally seen during the period of BM decrease in spring. Thus, this period of peak BMR in spring, as BM is falling, may simply reflect a energetically highly costly window of organ reorganisation, with certain organs decreasing in mass and others increasing in mass. It is also likely that organs such as the kidney and to a lesser extent the liver will be involved in the waste removal and metabolism of tissues during this time. Both are organs with relatively small masses but very high mass-specific metabolic rate.

The degree and speed at which organ flexibility occurs has been examined in various species of bird (Heitmeyer, 1987; Piersma, 1993; Jehl Jr, 1997) and also in reptiles (Secor *et al*, 1994). Eared-grebes are known to double their PM mass within two weeks (Gaunt *et al*, 1990) and Burmese pythons (Secor *et al* 1994) can increase small intestinal mass by 2-fold and increase their liver and kidney mass by 45% only 24 hours after ingesting prey, well before the prey has reached the small intestine. The masses of the stomach, lungs and heart also increase during this time, with a 7-fold increase in resting metabolic rate. It is also known that wild Bar-tailed godwit increase their stomach mass by around 30% (from 8g - 11g) during the first half of their three week stay at a migratory stopover site in spring, gut mass then decreases by around 20% (11g - 9g) in the second week (Piersma *et al*, 1993; Piersma & Lindstrom, 1997). It is therefore well known that these changes in organ mass can occur rapidly within individuals.

Of the other metabolically active lean tissues of the body that contribute to overall BMR, it is highly unlikely that the mass of the brain alters on a seasonal basis. Spleen mass has been shown to be increased during moult in Mallards (Heitmeyer, 1987), although spleen mass in these birds is only around 4% of liver mass. The spleen will be important during migration as it is involved in the production and storing of the erythrocytes that supply oxygen to actively respiring tissues. The concentration of haemoglobin is known to be higher in Bar-tailed godwits just before leaving on a long migratory flight, when compared with non-migrating conspecifics (Piersma *et al*, 1996). It is possible that the spleen enlarges in captivity, probably in conjunction with the other exercise organs (including the heart and lungs), thus increasing the BMR of an individual. It is also possible that the high BMR seen in captive Knot as BM is decreasing in spring is simply a result of an increase in the metabolic activity of the skeletal muscle through shivering thermogenesis. This may act as a mechanism to remove the large fat stores deposited in spring, that are obviously not catabolised as fuel during flight. If the mass of skeletal muscle is active through shivering, even within the thermoneutral zone during a BMR measurement, this will drastically increase the

BMR measured, particularly if the muscle activity is triggered not by ambient temperature but by another mechanism that acts independently of ambient temperature. Measurements of body temperature in conjunction with BMR measurements during this time may indicate this.

3.4.7 Seasonal variation in the volume composition of mitochondria in various lean tissues of captive Knot

The volume composition (percentage) of mitochondria in the liver and pectoral muscle (PM) did not alter significantly with season (Table 3.3.7.1), although a slightly higher mean percentage volume of mitochondria did occur in the liver during the period of fat deposition in spring, which may indicate an increase in the metabolic activity of the liver during this period of intense fat synthesis and fat deposition. The liver is known to be the main site of fat, protein and carbohydrate metabolism within the body.

The mitochondrial volume in the deep aspect of the PM did not alter between seasons and the volume composition in the superficial aspect of the PM was only slightly lower at 22% during the period of BM loss in spring than during the other two periods (24%). It is known that the deep (dorsal) aspect of the PM in 43 species of carinate birds, including the Least sandpiper *Calidris minutilla* and the Pectoral sandpiper *Calidris melanotos*, contained a significantly higher proportion of red fibres than the superficial (ventral) aspect (Rosser & George, 1985). These red or fast-oxidative glycolytic (FOG) fibres are myoglobin rich, fat-loaded, contain high levels of succinate dehydrogenase and are adapted primarily for rapid fatigue resistant aerobic activity, such as long-distance migratory flight. In the superficial area of the PM a greater proportion of fibres will be of the white or intermediate variety that are generally anaerobic, glycolytic and adapted for brief, powerful bursts of activity (George & Berger, 1966, Butler, 1991).

Wild Knot had a slightly lower mean percentage by volume of mitochondria in the PM (23%, Evans *et al*, 1992) than that measured in captive Knot (25%, in this study). This difference is difficult to explain. Although the muscle blocks in the work reported by Evans *et al* (1992) were taken from the deep aspect of the muscle, the heterogeneity in fibre type seen between the deep and superficial aspects of pectoral muscles within individual birds (for review see Rosser & George, 1985) may affect results, particularly if different researchers collect and analyse tissues from different birds of the same species. It is also known that both the distribution and the density of mitochondria in muscle fibres are very heterogeneous (Hoppeler *et al*, 1981). Seasonal variation in the percentage composition by volume of mitochondria in the PM between winter and spring has been reported in wild Dunlin (increase from 28-34%) and Sanderling (27-35%) by Evans *et al* (1992). This increase in mitochondrial volume composition in the PM will increase the mechanical power output of the PM, if we assume that the volume of mitochondria is directly proportional to the mechanical power output of the muscle (Pennycuick & Rezende, 1984), when most needed, i.e. at the beginning of long-distance flight when carrying large fuel stores.

These seasonal differences seen in the wild may have not been seen in captivity because the periods when the percentage composition of mitochondria increase in the wild may have been missed. Most of the captive Knot undergoing fat deposition in spring were sacrificed at a fairly early stage of fat deposition and it is possible that the mitochondrial volume increase occurs later, as the bird reaches its seasonal maximum in BM. The mitochondrial percentage composition in the PM of Knot arriving and just before departure at a spring migratory staging post in Norway were not significantly different from each other (Evans *et al*, 1992). Changes in mitochondrial volume might, of course, have occurred earlier in the migratory season, as with Dunlin and Sanderling (i.e. just before departure from the wintering sites). Evans *et al* (1992) actually reported that the mean mitochondrial volume in the PM of Dunlin on arrival (34%) and just before departure (34%) from an autumnal staging post did not significantly differ and

that these values were closer to those seen in spring just before migration (34%) to the breeding sites than those seen in mid-winter (28%). If this phenomena occurs in Knot also, then it would seem that the mitochondrial percentage volume in the PM of captive Knot is considerably higher, particularly during winter, than would be expected in wild Knot wintering on Teesmouth.

It is possible that although PM mass is maintained in captivity (see Appendix III), the fibre type composition alters. Metabolic power input available to working muscle can be provided by either sustainable aerobic power (red fibres) or by aerobic, short burst activity provided by the white, anaerobic fibres (Bishop *et al*, 1995). In captivity, Knot were restricted in their opportunities to fly. Therefore one may expect an increase to occur in the white, anaerobic fibres as most 'flight' is going to be of very short duration, short-burst and anaerobic. But this is unlikely to cause an increase in the volume composition of mitochondria because white fibres tend to contain less and smaller mitochondria. The constancy seen, both between seasons and between individual captive Knot, in the volume percentage of mitochondria in the deep aspect of the PM may simply be because the endurance muscular activity known to affect aerobic capacity may not occur to such a degree in captivity. It is known that endurance muscle activity causes an increase in the enzymes involved in the tri-carboxylic acid (TCA) cycle, an increase in the enzymes involved in the oxidation of succinate and an increase in both the size, number and volume density of mitochondria (Hoppeler & Linstedt, 1985). As mentioned earlier in section 3.4.1, the required muscle activity may be absent or not present to a sufficient degree in captivity to cause mitochondrial volume to increase in spring, as seen in Dunlin and Sanderling in the wild (Evans *et al*, 1992). The higher than expected mitochondrial composition (by volume) in the PM of captive Knot, when compared to wild birds, may simply be because the need for shivering thermogenesis is increased in captivity, possibility as a result of the incomplete moult exhibited by captive birds. It is unlikely though that the costs of thermoregulation are higher in captivity than in the wild, although the amount of exercised-induced heat produced is likely to be less in captivity.

3.4.8 The seasonal variation in the activity of two aerobic enzymes; succinate dehydrogenase and citrate synthase in various lean tissues of captive Knot

Although seasonal variation in mitochondrial composition (by volume) was not seen in the liver and PM of captive Knot, the activity of the aerobic enzyme succinate dehydrogenase (SDH) in the PM and gut of captive Knot did alter significantly on a seasonal basis, although levels in the heart and liver did not. The activity of SDH measured in the above 4 tissues compared fairly favourably to the ranking of the same 4 tissues in terms of their oxygen consumption (see Table 3.4.8.1) in the rat (Field *et al*, 1939), although the activity of CS in my study did not compare well to Field *et al's* (1939) results.

Table 3.4.8.1

Study	**Field *et al*, 1939**	**This study**	**This study**
Technique n=Mean value	O_2 consumption in tissue slices (ml O_2 h^{-1} g^{-1})	Succinate dehydrogenase assay (μmol min^{-1} g^{-1}) Outside migratory period	Citrate synthase assay (μmol min^{-1} g^{-1}) Outside migratory period
Study species	Laboratory rat	Knot	Knot
Liver	2.0	13.5	10.0
Heart	1.9	29.4	85.8
Gut	1.0	7.8	7.2
PM	0.8	7.6	131.4

The reduced activity of SDH in the flight muscle of Knot undergoing fat deposition indicates that the aerobic capacity of this muscle per gram (wet mass) was lower during this time. This reduction in SDH activity during fat deposition

may indicate a reduction in locomotor activity during this time, aiding fat deposition due to a decrease in DEE but steady food intake, as reported by Stokkan (1992) in Ptarmigan *Lagopus spp.* Kersten & Visser (1996) have reported the existence of a digestive bottleneck in free-living Oystercatchers, where they collect food quicker than they can process it. This forces them to interrupt their feeding at regular intervals, with periods of feeding being replaced with periods of inactivity. The reduction in aerobic enzyme activity seen during the period of fattening may be due to a greater reliance on glycolytic metabolism during this time, although this was not seen by Lundgren & Kiessling (1986) in pre-migratory and migratory Reed warblers *Acrocephalus scirpaceus.* If DEE is reduced during this time of fat deposition, there would then be no need for an increase in glycolysis. It is generally accepted that the enzymes involved in glycolysis occur in inverse proportions to enzymes of the citric acid cycle (Yacoe *et al,* 1992). Marsh (1981) reported that the mass specific levels of phosphofructokinase, a key glycolytic enzyme, did not alter between the period of pre-migratory fattening and migration in Grey-catbirds. Nor did the mass specific levels of citrate synthase alter during this time.

A decrease in aerobic capacity has also been reported in skeletal muscle that undergoes hypertrophy due to mechanical overload (Kreiger *et al,* 1980), although this is unlikely to be occurring in captive Knot. The low levels of SDH activity in the PM during the period of fat deposition in spring may have considerable bearing on the BMR, but only if the energy consumption of skeletal muscle contributes considerably to BMR. During a BMR measurement, the contribution of skeletal muscle to overall BMR will primarily be from muscle respiration to maintain muscle tone and to support the fat mass. The study by Field *et al* (1939) showed that although skeletal muscle had a relatively low rate of O_2 consumption per gram, due to the mass of muscle in the rat it actually accounted for around 50% of the total body O_2 consumption. From the work by Scott & Evans (1992) it can be calculated that the O_2 consumption per gram of the PM in Dunlin was approx. 75% of that measured in the liver, but with a

considerably greater mass. Therefore, even small changes in the metabolic output of the skeletal muscle may affect BMR, certainly in birds, to a considerable degree.

Similar findings to my results were reported in the paper by Dreidzic *et al* (1993), who measured the activity of citrate synthase in the PM and heart of Semipalmated sandpipers *Calidris pusilla* at a stop-over site. Citrate synthase activity in both the PM and in the heart was significantly lower in 'heavy' birds than 'light' birds'. Heavy birds were termed so because they were in the advanced stages of migratory fattening. These interesting results were not discussed in the above paper, other than to say that the decrease in the activity of citrate synthase in the PM was influenced by the large increase in the tissue lipid content. Therefore, captive Knot appear to exhibit seasonal adjustments in the aerobic capacity of their flight muscle, with significantly lower mass-specific levels of succinate dehydrogenase occurring during the period of fat deposition in spring. The levels of SDH in the PM and liver of wild Knot (See Table 3.3.8.1) were considerably higher than those measured in captive birds. At first sight, these results do not tie in well with the difference in percentage composition of mitochondria in the PM of wild and captive Knot (see Section 3.4.7). However, because succinate dehydrogenase is incorporated into the inner-mitochondrial membrane, it is possible that in captive Knot, although the mitochondrial volume composition (percentage) of the deep aspect of the PM may remain constant with season, the mitochondrial cristae may increase in surface area. It is thought that the surface density of the inner mitochondrial membrane does not alter between species (Mathieu *et al*, 1981 but also see Bartels, 1982), although to my knowledge no study has investigated whether seasonal variation in the surface area of the inner mitochondrial membrane does occur in birds.

The levels of SDH were significantly higher in the gut during the time of fat deposition and thus the aerobic capacity per gram of the gut was higher during this time. This increased intensity may be a mechanism to facilitate migratory fattening by increasing the rate of food uptake by the gut. Evidence for an increase in gut uptake efficiency, expressed as food metabolised/gross food intake, has been reported by Bairlain (1985), but other studies (Gifford & Odum, 1965) reported little evidence of this. Biebach (1996), in reviewing the processes involved in migratory fattening wondered why, if birds could increase uptake from their gut at particular periods during the annual cycle, they did not maintain this higher level throughout the annual cycle. It is known that liver, heart, gut and skeletal muscle show moderate to high rates of oxygen consumption (Field *et al*, 1939) and it is known from this study that the aerobic capacity of various lean tissues alter on a seasonal basis. Therefore, if one component of total lean mass such as the gut is elevated during fat deposition, then another component such as the pectoral muscle may offset this by decreasing in activity, therefore BMR is not increased to the detriment of fat deposition.

The lack of any significant seasonal variation in the levels of CS and the absence of any significant correlation between the activity of SDH and CS, except in the gut, is difficult to explain. Perhaps the SDH assay was more sensitive to the seasonal changes in aerobic capacity of the lean tissues in captive Knot than the citrate synthase assay. However, significantly higher activities of citrate synthase have been reported between passerines of the same species, e.g. Sedge warblers *Acrocephalus shoenobaenus* and Blackbirds *Turdus merula* undergoing migration and during the breeding season, and between Reed warblers *Acrocephalus scripaceus* during the pre-migratory and migratory periods in the wild respectively (Lundgren & Keissling 1985; 86). Ludgren (1988) also reported significantly higher oxidative capacities (levels of citrate synthase) in the PM of migratory birds when compared to non-migratory birds of the same species, e.g. Great tits *Parus major* and Goldcrest *Regulus regulus*. The fact that activity levels of CS in the liver and gut of captive Knot in this study are only around 10% of those in the

PM would tend to suggest that the values of CS activity in the liver and gut obtained in this study were lower than expected. From table 3.4.7.1. it can be seen that O_2 consumption (at basal levels) is generally higher in the liver and the gut than skeletal muscle. Weber & Piersma (1996) found considerably higher activities of the aerobic enzyme cytochrome-c oxidase in the PM of captive *islandica* Knot when compared to activity measured in the liver. The activity of citrate synthase measured in the heart in my study were however, fairly similar (see Table 3.4.8.2) to those measured in wild Blue-winged teal by Saunders & Klemm (1994) but levels measured in the PM in my study were slightly higher. The CS activities measured by Dreidzic *et al* (1993) in the PM and heart of Semipalmated sandpipers sampled during a stop-over phase of their southward migration from the breeding grounds, were considerably higher than those measured in captive Knot in my study.

Table 3.4.8.2 The comparative activity of citrate synthase measured in various lean tissue, during 3 separate studies

Study	Species studied	Range of citrate synthase activity (μmol min $^{-1}$ g $^{-1}$) measured	n
This study	Knot	PM 104 - 131 Heart 81 - 88	22
Dreidzic *et al* (1993)	Semipalmated sandpiper	PM 231 - 300 Heart 154 - 209	12
Saunders & Klemm (1994)	Blue-winged teal	PM 52 - 95 Heart 86 - 97	34

A significant relationship occurred only between SDH activity in the gut and days since peak BMR. This would imply that the aerobic capacity of the gut is still high

even after fat deposition has ceased in spring, as peak BMR tended to occur as BM was dropping in spring. Therefore, during this period of BM loss in spring, when BMR is high, the metabolic activity of the gut is still high but then decreases in a steady fashion as days after peak BMR increase. During this period of BM loss and high BMR, it is likely that the bird has ceased eating or has reduced its food intake during this time, therefore it is unlikely that the gut is actively involved in the digestion and uptake of food. It is possible that the high aerobic capacity during this time is due to active resorption of intestinal material, as the Knot is not involved in active digestion of food. It is also known that some protein catabolism is necessary during periods of intense fat catabolism, because of the requirement to provide amino acids to supply glucogenic precursors and citrate cycle intermediates (Schwilch *et al*, 1996). If internal reorganisation of various organs does take place during this time, and assuming these changes are rapid, it is likely that cellular metabolic activity within these tissues will increase during the build up and loss of these organs. Two birds sacrificed during this period of BM loss in spring were actually sacrificed at their seasonal maxima in BMR and peak lean-mass-specific BMR. These two individuals exhibited the highest activity SDH activity in the gut of any of the birds sacrificed during this time of BM loss, which were closer to the significantly higher mean value obtained as birds deposited fat in spring. A similar, yet not significant, pattern also emerged in the liver, during the period of BM loss in the spring. In these two individuals the SDH activity in the PM was close to the mean measured in the PM of all birds sacrificed during the period of BM loss in spring. Therefore, it would appear that the SDH activity during the period immediately as body mass is dropping in spring, which generally coincides with the period of peak BMR, occurs at a time when the SDH activity is high in the gut, liver and PM.

In summary captive Knot exhibit variation in body mass and body mass components on a seasonal basis. BMR also varies within individual Knot on a seasonal basis, but these variations are not due solely to seasonal changes in BM and BM components. Therefore, mass-independent factors must also be involved.

During the period of body mass increase in spring, only one out of seven individuals showed a significant relationship between BMR and BM and between BMR and fat mass. At peak BM, BMR was often less than expected allometrically for that individual. However, as BM and PTLM dropped rapidly from peak mass, 13 out of 15 individuals exhibited a seasonal peak in BMR.

From investigation into whether the metabolic activity of various lean tissues changed on a seasonal basis, my study showed that the volume composition (percentage) of mitochondria did not alter on a seasonal basis in the liver or pectoral muscle of captive Knot but that the activity of an aerobic enzyme succinate dehydrogenase (SDH) did alter. The level of SDH activity in the gut was significantly higher in the small intestine as BM increased in spring, possibly as an adaptation to aid fat deposition and then decreases slowly during the period of BM loss. SDH activity in the liver follows a similar, yet non-significant pattern to that seen in the small intestine. The SDH activity in the pectoral muscle is considerably lower in Knot during the period of fat deposition in spring, possibly leading to the lower that predicted BMR in most individuals at peak BM. The level of SDH activity in the PM does not appear then to increase in a stepwise manner as BM is then decreasing, but appears to increase rapidly and occurs at the same time as a high yet decreasing level of SDH in the gut and the liver. As discussed earlier, skeletal muscle respiration may account for a large proportion of oxygen consumption in both birds and mammals, particularly if supporting a large fat mass. It must be remembered that during this time of BM decrease in spring and peak BMR, that absolute body mass is still very high.

Seasonal variation in overall lean mass, as predicted by TOBEC, does not tend to occur in captive Knot but the proportion of metabolically active organs that contribute to overall lean mass do vary on a seasonal basis. My work has shown that the mass of the liver is significantly higher during the period of fat deposition and that the mean mass of gut, heart and the PM were also higher, though not

significantly so, during fat deposition. The masses of these organs tend to be smaller that that expected for a given mass during the period of BM loss in spring, but are generally at a high metabolic activity. The period of peak BMR, as mentioned earlier in the chapter, is highly short-term, so it is not known whether the masses of these metabolically active organs are still high during the peak in BMR and then gradually decrease as BM falls. To investigate this, birds would need to be sacrificed immediately as BM is falling in spring and then the relative contribution of the various organs to total lean mass could be examined.

Chapter 4.0: Intraspecific variation in the Basal Metabolic Rate (BMR) of Knot

4.1.1 Introduction

The aim of the work presented in this chapter was to determine the factors that account for the variation seen in basal metabolic rate (BMR) amongst individual birds of the same species, which are in the same physiological state. As in Chapter 3, the species used to examine the causes of intraspecific variation in BMR was the Knot *Calidris canutus*, of the sub-species *islandica.* The original hypothesis tested in this chapter was that those factors which account for seasonal differences in BMR within individual Knot (see Chapter 3), would also account for differences in BMR amongst individuals that are in the same physiological condition.

Most work hitherto on intraspecific variation in BMR in birds has examined allometrically the relationship with body mass (BM); very few studies have actually examined correlations between BMR and body composition (For review see Chapter 1, General Introduction). In this study I examined initially, whether the differences in BMR seen amongst individual Knot could be explained simply by differences in BM or in the major components of BM (Total lean mass, total lean dry mass and total fat mass). However, differences in BMR seen amongst individuals of the same species might also result from intraspecific differences in the relative contributions of various metabolically active lean tissues/organs to overall lean mass, and/or by differences in the metabolic output per gram of these lean tissues/organs.

4.1.2 Intraspecific variation in BMR and its relationship with body mass and body composition

Variations in BMR, both amongst and within individuals of the same species, have become a topic of interest only recently in both birds (Daan *et al*, 1989; Scott 1991; Piersma, 1994; Piersma *et al*, 1996; Scott *et al*, 1996) and mammals (Konarzewski & Diamond, 1995; Meerlo *et al*, 1997). As shown in Chapter 3, BMR varies within individual captive Knot on a seasonal basis and this variation cannot be explained solely by seasonal fluctuations in the mass of the metabolically active lean tissues; as claimed by Kersten & Piersma (1987), Weber & Piersma (1996) and Piersma *et al* (1996). I found, within individual Knot, that the average metabolic output per gram of the lean tissues altered seasonally as a result of both the metabolic activity (measured using enzymatic assays) and the mass of various lean tissues altering on a seasonal basis.

Piersma *et al* (1996) correlated BMR with total body mass and with 'estimated' total lean mass in four distinct groups of Knot. The 4 groups consisted of wild Knot of the *islandica* (n=8) and *canutus* (n=13) sub-species, and captive birds of the same two subspecies (n=8 and n=12 respectively). All birds in their study were captured in mid-winter. Actual lean mass was not, however, measured by Piersma *et al* (1996) but was extrapolated from data on carcass composition, collected by Piersma (1994) from 4 similar categories of Knot (*islandica* and *canutus*, wild and captive). Lean mass was estimated by subtracting the average fat mass calculated from Piersma's (1994) data from actual body mass (minus estimated feather mass, between 6-8g). Piersma *et al* (1996) reported that measured differences in BMR amongst the 4 groups of Knot paralleled differences in total 'estimated' lean mass (minus feathers) amongst the 4 groups. Indeed, these reported differences in lean mass accounted for 99% of the variation seen in BMR amongst the 4 groups of Knot, whereas total BM only explained only some 24%

of the between-group variation in BMR. This means that the average BMR per group of Knot were strongly correlated with the group averages of lean mass. This led Piersma *et al* (1996) to suggest that lean mass alone determines BMR.

However, while the lean tissues undoubtedly produce the majority of the metabolic heat in birds, differences in fat mass may also account for some of the intraspecific differences seen amongst individuals in BMR. This may be particularly true when comparing those individuals of a species carrying a considerable mass of fat with fairly lean birds, as seen in Piersma *et al's* (1996) study where fat mass ranged between 7 to 30g amongst Knot. The energetic cost of a fat mass is likely to be indirect, related to supporting and carrying the mass (Witter & Cuthill, 1993), rather than to the direct consumption of oxygen by the adipocytes (see Scott & Evans, 1992). Piersma *et al* (1996) did admit that there was 'considerable variation in BMR' between individuals in each of the 4 groups, with much of this information being 'lost' when the data were pooled. Captive Knot in their study had significantly lower BMR than wild conspecifics and also had lighter lean dry masses; particularly of stomach, intestine, kidney and liver mass (see also Appendix III). This lead Piersma *et al* (1996) to suggest that these differences in organ mass between the wild and captive Knot (11-13% of total lean dry mass) were likely to be major influences on BMR amongst these groups and that variation seen in the components of lean mass was the vehicle for seasonal adjustments in metabolic physiology in Knot.

Scott *et al* (1996) also investigated the correlations between BMR and BM, and BMR and body composition (lean mass and fat mass, predicted using TOBEC), measured during the non-breeding season in a group of 21 captive Redshank, *Tringa totanus.* They found that, intraspecifically, mean log BMR increased significantly with mean log body mass (BM) in captive Redshank. It also increased significantly with mean log predicted total lean mass (PTLM), but not with mean log predicted fat mass (PFAT).

4.1.3 Intraspecific variation in BMR and its relationship with organ mass

The metabolic activities per gram of different organs and tissues that contribute to the total lean mass of an individual differ considerably in both mammals (Krebs, 1950; Schmidt-Nielsen, 1984) and birds (Scott & Evans, 1992). High metabolic activities per gram occur in those tissues involved in providing metabolic energy (e.g. liver), organs that excrete waste products (e.g. kidney) and organs that transport metabolic energy (e.g. heart) in the animal (Konarzewski & Diamond, 1995). These highly metabolically active organs tend to constitute only a small fraction of total lean mass, but may contribute a disproportionately large percentage to overall BMR. Therefore, even small changes in the mass of these organs may have considerable effects on an individual's BMR (see Ricklefs, 1996). Relatively low mass-specific metabolic activities are known to occur in tissues such as feathers, adipose tissue and in the skeletal mass (Scott & Evans, 1992; Meerlo *et al*, 1997). The masses of many of these tissues and organs are known to vary on a seasonal basis, even in captive birds (see Chapter 3 for review). Therefore it is feasible that differences in BMR seen amongst individuals of the same species may be due to differences in the relative contributions of these highly metabolically active organs to overall lean mass. Indeed, Kersten & Piersma (1987), Weber & Piersma (1996) and Piersma *et al* (1996) all suggest that BMR is simply a consequence of the masses of various metabolically active organs, i.e. those individuals with higher than average organ masses, for a given total lean mass, will have higher than average BMR.

Daan *et al* (1989) investigated some correlates of the intraspecific variation in BMR seen amongst Kestrels *Falco tinnunculus*, differing both in sex and in body mass. They found that Kestrels kept on low maintenance diets (low metabolizable energy intake) showed considerable reductions in their mass-specific BMRs that could not be explained simply by a reduction in body mass or in nocturnal core

body temperature. Carcass analysis of these Kestrels showed disproportionate reductions in their heart and kidney lean dry mass (as well as in fat mass), when compared to individuals kept on *ad libitum* high maintenance diets. The birds on low maintenance diets, however, showed significant relative increases during this time in both metabolically active lean tissues (brain mass, leg muscle mass) and in tissues with low or negligible metabolic outputs per gram (carcass water content and remainder of carcass, including skeleton). This lead Daan *et al* (1989) to suggest that the variation in BMR between individual Kestrels, fed on low and high maintenance diets, primarily reflected variation in the mass of highly metabolically active tissues, such as the heart and kidney, although these tissues mass contributed only 0.61% of total lean dry mass. Piersma *et al* (1996) also reported that starved Knot that exhibited reductions in BMR, exhibited reductions in the lean dry mass of the heart and PM.

In mammals, intraspecific variation in BMR and the factors behind it have been examined primarily in rodents. Konarzewski & Diamond (1995) found that strains of mice *Mus musculus* with exceptionally high (or low) BMR had disproportionally large (or small) organ masses. Variation in masses of heart, liver, kidney and small intestine, (all organs with high mass-specific-metabolic rates, Schmidt-Nielsen, 1984), accounted for 52% of the observed variation in BMR measured between the strains, although they accounted for 'no more' than 17% of total body mass. Meerlo *et al* (1997) carried out in-depth investigations into the relationship between organ masses and intraspecific variation in BMR in the Field vole, *Microtus agrestis*. They found that the residuals of lean dry mass of the heart (deviations from the allometrically predicted values) correlated positively with the residuals of BMR, but that no other residuals of body mass components (12 tissues in total) correlated with residual BMR. Meerlo *et al* (1997) suggested that 'variation between individuals of the same species in BMR can to some extent reflect variation in the size of the metabolically active organs'. None of the above authors however, with the exception of Weber & Piersma

(1996), investigated whether differences also occurred in the metabolic intensity per gram of particular lean tissues.

4.1.4 Intraspecific variation in BMR and its relationship with aerobic enzyme activity and mitochondrial volume

Within the literature, there is a paucity of reports of research that attempts to investigate whether intraspecific variations in BMR can be explained by differences amongst individuals in the metabolic activity per gram of various lean tissues, e.g. through aerobic enzymatic assays or through stereological analysis of mitochondrial volume in these lean tissues. Of the papers that do exist, with the exception of Weber & Piersma (1996), all tend to be concerned with the differences in enzyme activity or mitochondrial volume that are found between different orders of vertebrates or between different species of mammals (see Schmidt-Nielsen, 1984).

Else & Hulbert (1981) investigated the factors that may explain why mice had a 6-fold greater standard metabolic rate than a species of lizard *Amphibolurus nuchalis*, even though both species had the same overall body mass and body temperature. They found that the mice had relatively larger organ masses, had a greater volume proportion of mitochondria in these organs and that the mitochondria within these organs had relatively greater cristae surface area when compared to the lizards. Interspecifically, it has been shown in mammals that the total number of liver mitochondria per gram of body mass (Smith, 1956) and the activity of the aerobic enzyme cytochrome c-oxidase (CCO) in skeletal muscle both decrease with increase in BM, with an exponent very similar to the exponent relating BMR to body mass of the whole animal. As mentioned earlier, the single attempt to determine whether variability in BMR amongst birds of the same species can be explained by intraspecific variation in the masses of various lean

tissues, or by variations in the metabolic output per gram of these lean tissues, was carried out by Weber & Piersma (1996). They suggested that variation in BMR amongst captive Knot measured at different times during the 4 week period of body mass loss in spring, was explained better by variation in the lean dry mass of organs with a high metabolic scope, particularly the heart and PM (as indicated by high cytochrome c-oxidase activity), rather than by variation in the metabolic output per gram of these tissues.

4.2 Methods

The protocols that were employed in the studies summarised in this chapter are those described in Chapter 2 (see sections 2.1-2.7). All Knot used in this study were sacrificed under licence and had been held in captivity for at least 5 months. As in Chapter 3, the distinct physiological/seasonal periods used in this chapter are:

1. Period of body mass (BM) increase to peak in spring -BM increasing
2. Period of BM decrease, after spring peak in mass- BM decreasing
3. Any period outside time period 1 and 2- Outside

The group of 8 captive Knot used to examine whether variations amongst individuals in BMR could be explained by variations in their organ masses were all sacrificed outside the spring migratory period and thus were in the same physiological state. All 8 birds were captured on 7/1/97 and are the group termed 'B' in Appendix III. The Knot used to examine the relationships between (i) BMR and liver/PM mass, (ii) BMR and aerobic (succinate dehydrogenase/citrate synthase) enzyme activity in various lean tissues, and (iii) between BMR and mitochondrial enzyme activity, were the birds described in Chapter 3, which were sacrificed to identify the causes of seasonal variation in BMR.

In the analyses that follow an attempt has been made to estimate the contribution of basal metabolism from the lean parts of the body mass (BMR*) to overall BMR by the following method: The oxygen consumed directly by the fat mass of each individual Knot (value x), was estimated by assuming an O_2 uptake rate of 0.06ml g^{-1} hr^{-1} by adipose tissue and multiplying by the total fat mass of that individual (value y). (The value of 0.06ml hr^{-1} g^{-1} was the O_2 uptake by fat in Dunlin *Calidris alpina*, measured using an O_2 electrode by Scott & Evans (1992)). Value y was then multiplied by 4.7 (Kcals produced per litre O_2 when fat is fully metabolised),alue y was converted into joules (J) by multiplying by 4.184 and then finally converted into Watts (for conversion factors see Schmidt-Nielsen, 1984). Value x was then subtracted from actual BMR measurement, to give a value of BMR minus energy consumed directly by the adipose tissue.

4.3 Results

4.3.1 Intraspecific variation in BMR and its relationship with body mass and body composition

Table 4.3.1.1 summarises all body composition parameters, physiological state (denoted by season), age at death (adult or juvenile) and BMR measurement of the 40 birds used in this study. Body composition was measured directly using solvent analysis (see Section 2.4) and the BMR value quoted was calculated from the measurement recorded immediately before the individual was sacrificed (see Section 2.3). The hypothesis being tested was that the factors which accounted for seasonal change in BMR within an individual (see Chapter 3), would also account for differences in BMR amongst individuals (in the same physiological state, see Section 4.2). First, I examined whether the differences in BMR amongst individuals could be explained simply by differences in body mass (BM), total lean mass (TLM), total fat mass (TFAT) or total lean dry mass (TLDM), i.e. the mass of the metabolically active tissues. To achieve this, I employed least-square linear regression analysis with BMR as the dependent variable and BM, TLM, TFAT and TLDM as the independent variables.

The results from the least -squares regression analyses are shown in Table 4.3.1.2. where; LBMR = Log BMR (Watts), LBM = Log total body mass (g), LTLM = Log total lean mass (g), LTFAT = Log total fat mass (g) and LTLDM = Log total lean dry mass (g). No significant regression equations were produced for any of the relationships if birds were classified by physiological state. Particularly low coefficients of determination (r^2) were seen when LBMR was regressed against LTLDM. Therefore, these initial results show that differences in BMR seen amongst individual Knot, in each of the three distinct physiological states, cannot be explained

by the differences that exist in body mass or in any major component of body mass.

Table 4.3.1.1 Summary data of Knot used in allometric analysis of BMR and body composition. Body composition estimated using Soxhlet solvent analysis (see Section 2.4)

BM= Total body mass TLM= Total lean mass

TFAT= Total fat mass TLDM= Total lean dry mass

BMR = Basal metabolic rate Season = Physiological state

1. Period of BM increase to peak in spring- BM increasing
2. Period of BM decrease, after spring peak in mass- BM decreasing
3. Any period outside time period 1 and 2- Outside

ID	SEX	BM	TLM	TFAT	TLDM	BMR (Watts)	SEASON	AGE
JWW	M	99	88	11	32	1.38	3	2
JYG	M	112	92	20	33	0.92	3	2
JLL	M	116	94	22	33	1.17	3	2
JWG	M	106	88	18	32	0.80	3	2
JYY	M	120	96	24	32	1.13	3	2
BGG	M	112	92	21	33	1.38	3	2
JLW	F	119	94	25	33	1.42	3	2
RGG	M	144	92	52	32	1.27	3	2
AA	F	166	127	39	49	1.87	1	2
YG	F	160	105	55	35	1.47	1	2
WGG	F	158	109	49	39	1.90	1	2
WLL	F	182	119	62	44	1.78	1	2
WLG	F	145	102	43	38	1.40	1	2
GW	F	130	109	21	39	1.94	1	2

LG	F	135	99	51	36	1.57	3	2
YY	F	130	106	24	35	2.17	1	2
WGL	F	150	103	47	32	2.37	2	1
YEL	F	163	107	57	39	1.84	2	2
WH	M	153	100	53	35	2.05	2	2
GO	M	150	97	53	31	1.94	2	2
GG	F	120	84	36	31	1.53	2	2
OO	F	156	107	47	34	1.36	2	2
GY	M	124	100	24	42	1.72	2	2
GF	F	185	125	60	35	2.23	2	2
GL	M	153	99	55	32	2.06	2	2
WYY	M	127	85	41	28	1.90	3	1
LY	M	100	81	20	35	1.98	3	2
BW	F	121	84	37	35	1.32	3	2
YW	M	148	110	38	39	1.62	3	2
LW	M	155	104	51	37	1.12	3	2
WO	M	128	101	27	37	1.66	3	2
YYY	F	122	99	23	35	1.66	3	2
WWW	F	129	105	24	39	1.17	3	2
WYG	F	112	105	7	40	1.19	3	2
WGY	F	112	98	14	36	1.33	3	2
WG	F	109	98	11	36	1.42	3	2
WL	M	131	108	22	36	1.56	3	2
GN	F	120	92	29	31	1.14	3	2
OR	M	126	105	21	35	1.31	3	2
YL	M	114	101	12	34	1.46	3	2
Mean (SE)	-	**134(3.5)**	**100(1.7)**	**34(2.6)**	**36(0.6)**	**1.56(0.06)**	-	-

Table 4.3.1.2

Season	Regression equation	n	r^2	Significance
3	Log BMR= -0.157(0.87)+ 0.135(0.42)LBM	24	0.052	>0.05
3	Log BMR= 0.044 (0.57) + 0.038(1.13)LTLM	24	0.001	>0.05
3	Log BMR= 0.046(0.01) + 0.063(0.13)LTFAT	24	0.011	>0.05
3	Log BMR= 0.159(0.56) - 0.123(0.87)LTLDM	24	0.004	>0.05
1	Log BMR= 1.067(1.16) - 0.375(0.53)LBM	7	0.091	>0.05
1	Log BMR= -1.177(1.73)+ 0.697(0.85)LTLM	7	0.119	>0.05
1	Log BMR= 0.610(0.22) - 0.221(0.14)LTFAT	7	0.335	>0.05
1	Log BMR= 0.148(0.80) + 0.063(0.50)LTLDM	7	0.003	>0.05
2	Log BMR= -1.091(0.98)+ 0.628(0.45)LBM	9	0.216	>0.05
2	Log BMR= -1.018(1.19)+ 0.643(0.59)LTLM	9	0.143	>0.05
2	Log BMR= -0.172(0.35)+ 0.267(0.21)LTFAT	9	0.189	>0.05
2	Log BMR= 0.554(0.98) - 0.186(0.64)LTLDM	9	0.003	>0.05

In Knot sacrificed during period 3 (outside the spring migratory periods 1 and 2), both TLM and TFAT of an individual Knot increased significantly with an increase in body mass (Pearson product-moment correlation, $r_{24} = 0.542$, $P<0.01$ and $r_{24} = 0.770$, $P<0.001$ respectively). However, fat mass was not significantly correlated with lean mass ($r_{24} = -0.041$, $P>0.05$). In individuals sacrificed when depositing fat in spring (Season 1), only TFAT was significantly correlated with BM ($r_7 = 0.894$, $P<0.01$). Although TLM increased with an increase in BM in these individuals, this correlation ($r_7 = 0.600$) was not significant even at the 10% level, possibly due to the small sample size. The actual fat mass measured in these individuals did not correlate significantly with TLM ($r_7 = 0.186$, $P>0.05$), showing that the fattest individuals did not necessarily have the largest lean masses. In those birds sacrificed as they lost mass after the spring migratory peak (season 2), both TLM and TFAT decreased

significantly with a decrease in BM ($r_9 = 0.855$, $P<0.01$ and $r_9 = 0.851$, $P<0.01$), although TLM and TFAT were once again not significantly correlated even at the 10% level ($r_9 = 0.457$).

That differences in BMR amongst individuals in the same physiological state could not be directly related to differences in the total mass of the metabolically active lean tissues (TLDM) indicates that the average metabolic output per gram of the lean tissues differed amongst individual Knot in the same physiological state. Differences in the average metabolic output per gram of these lean tissues also explained some of the seasonal variation seen in BMR (see Chapter 3). These differences in BMR seen amongst individual Knot could be explained by differences in the relative masses of various metabolically active organs that contribute to total lean mass and/or by differences occurring in the metabolic output per gram of these lean tissues.

4.3.2 Does variation in the mass of the metabolically active lean tissues cause intraspecific variation in BMR?

To examine whether the differences seen in BMR amongst captive Knot in the same physiological state, could be explained by differences in the relative masses of various metabolically active organs that make up total lean dry mass (TLDM), 8 captive Knot were sacrificed outside the spring migratory period, after each undergoing a BMR measurement. The lean dry mass of various organs was then determined using Soxhlet apparatus with petroleum ether as the solvent (see table 4.3.2.1). All 8 individuals (ID- JWW, JYG, JLL, JWG, JYY, BGG, JLW, RGG) were adult and ,with the exception of individual JLW, were all male. From the Tables 4.3.1.1 it can be seen that while the total lean dry masses of these 8 individual Knot were very similar (32-33g), their BMR were not. Before analysis, these BMR were modified by deducting the contributions to total metabolic rate of the metabolic activity of the fat

Table 4.3.2.1 Lean dry mass (g) of various organs in 8 captive adult Knot. Residual BMR is the BMR* measured for each individual (minus oxygen directly consume by fat mass) minus mean BMR* for those 8 individuals.

ID	LIVER	HEART	KIDNEY	SMALL INTESTINE	STOMACH	PECTORAL MUSCLE	REST	TOTAL	RESIDUAL BMR
JWW	0.838	0.373	0.038	0.811	0.581	3.287	25.911	31.839	0.187
JYG	0.995	0.355	0.029	0.750	0.882	2.267	27.080	33.358	-0.273
JLL	0.660	0.364	0.047	0.573	0.573	3.328	27.896	33.441	-0.033
JWG	0.693	0.353	0.015	0.746	0.445	3.284	26.327	31.863	-0.263
JYY	0.882	0.366	0.039	0.833	0.612	3.619	25.843	32.194	-0.073
BGG	0.616	0.362	0.051	0.724	0.552	3.308	26.471	32.084	0.177
JLWϕ	0.517	0.316	0.036	0.571	0.478	3.486	27.284	32.688	0.217
RGG	0.479	0.316	0.058	0.546	0.446	3.091	26.673	31.609	0.057

ϕ Female Knot

the birds carried (see Section 4.2). These modified metabolic rates, BMR*, were estimated from oxygen consumption rates/g of fat measured in Dunlin *Calidris alpina* (Scott & Evans, 1992).

The direct O_2 consumption of avian adipose tissue is low (Scott & Evans, 1992), and even in a bird undergoing pre-migratory fattening in spring, (e.g. Knot WGL, predicted fat mass 127g and BM of 243g), the direct metabolic rate contribution of fat was calculated at only 0.042 Watts or 1.9% of total BMR. Of the 8 individual wintering Knot used in the following study, one individual (RGG) had a fairly high total fat mass of 52g, but the other 7 had fat masses ranging between only 11-25g (see Table 4.3.1.1).

In the following analyses, I assumed that the TLDM was the same for each individual. I calculated a residual BMR* for each individual by subtracting the mean group BMR* from the actual BMR* measured. These residuals of BMR* were then correlated with the lean dry masses of various organs (see Table 4.3.2.1 and figures 4.3.2.1-8), to determine whether differences seen in BMR amongst these birds were associated with differences in the relative lean dry masses of particular metabolically active tissues/organs that make up total lean dry mass, or whether differences in BMR were due to differences in the metabolic activities per gram of these lean tissues.

From figures 4.3.2.1-8, it is clear that the differences in BMR amongst these 8 individuals cannot be explained simply by differences in the lean dry masses of various metabolically active organs that make up TLDM. Indeed individuals with larger lean dry livers ($r_8 = -0.534$), hearts ($r_8 = -0.219$), small intestines ($r_8 = -0.276$), and stomachs ($r_8 = -0.434$), tended to have lower than average BMR*, although none of

Figure 4.3.2.1. The relationship between residual BMR* and lean dry mass of liver

F denotes female (ID=JLW)
Large square identifies individual RGG (Larger fat mass)

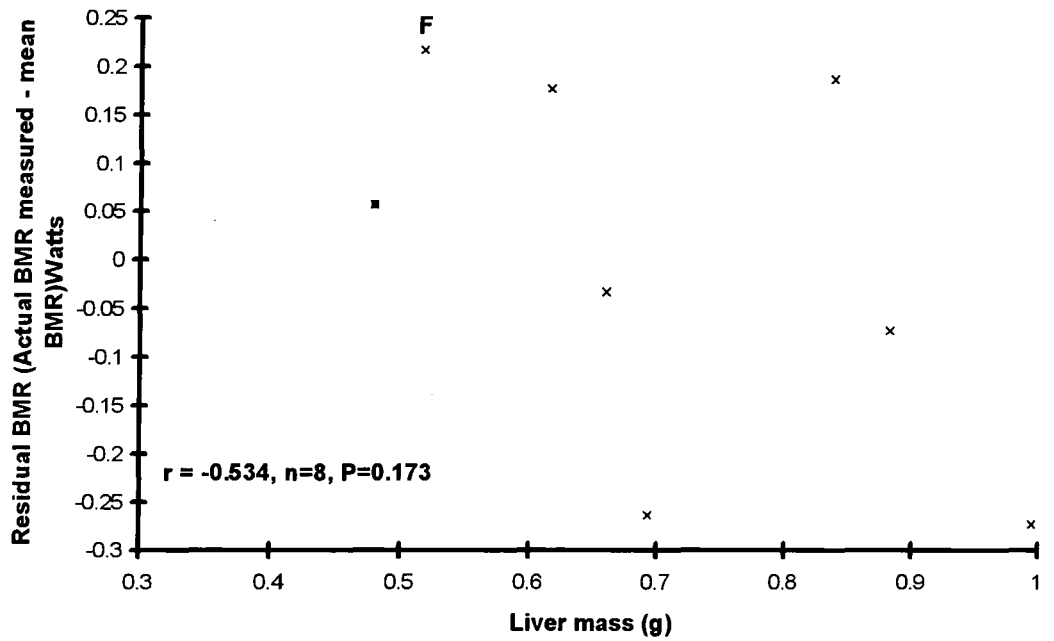


Figure 4.3.2.2. The relationship between residual BMR* and lean dry mass of heart

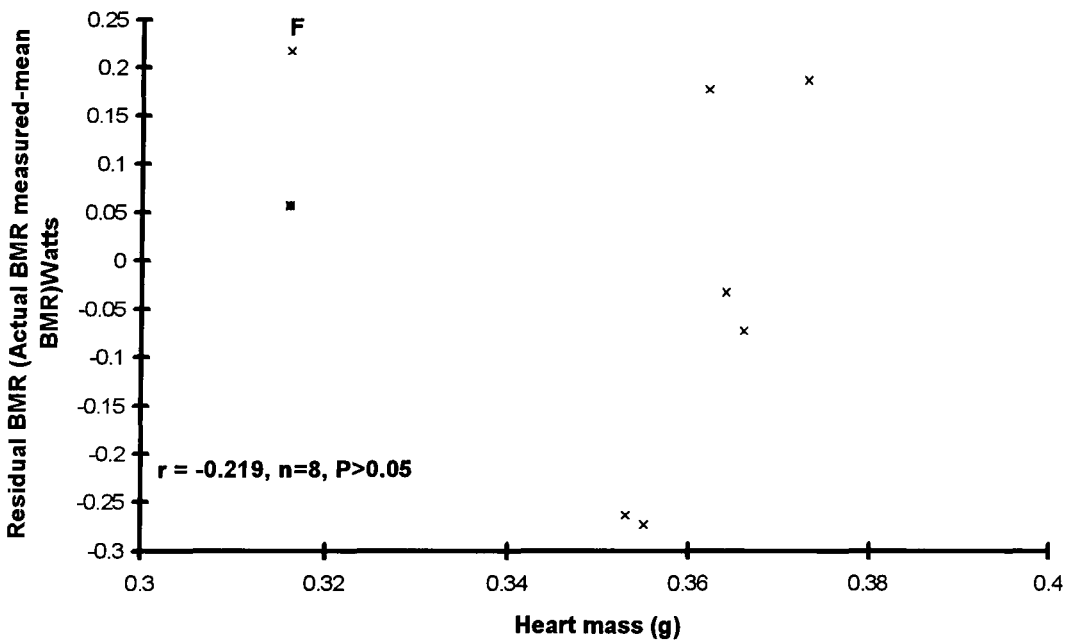


Figure 4.3.2.3. The relationship between residual BMR* and lean dry mass of kidney

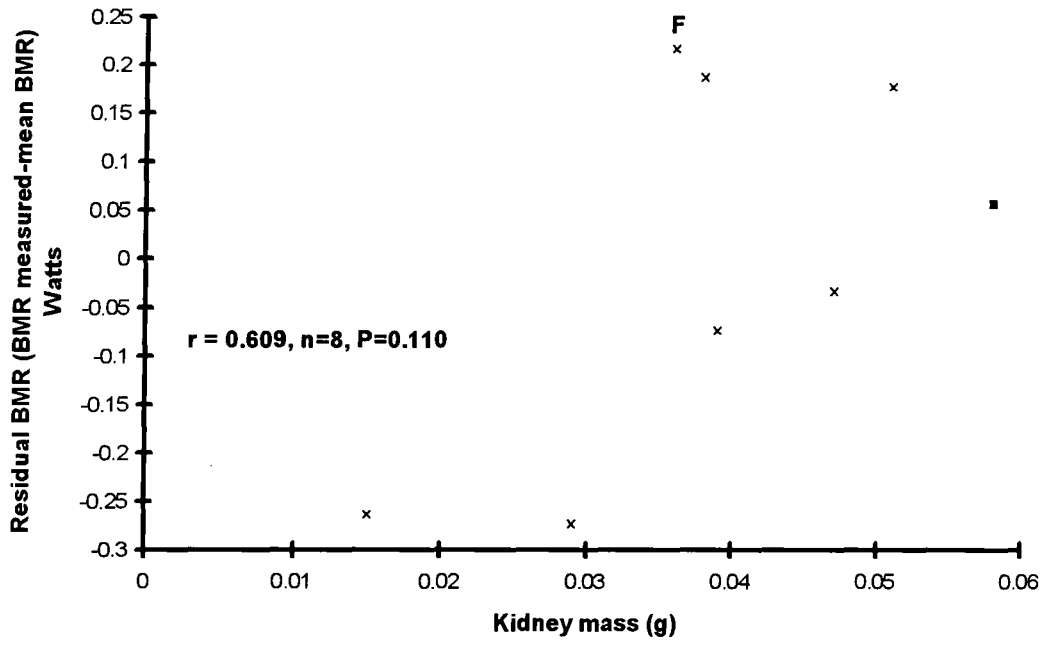


Figure 4.3.2.4. The relationship between residual BMR* and lean dry mass of small intestine

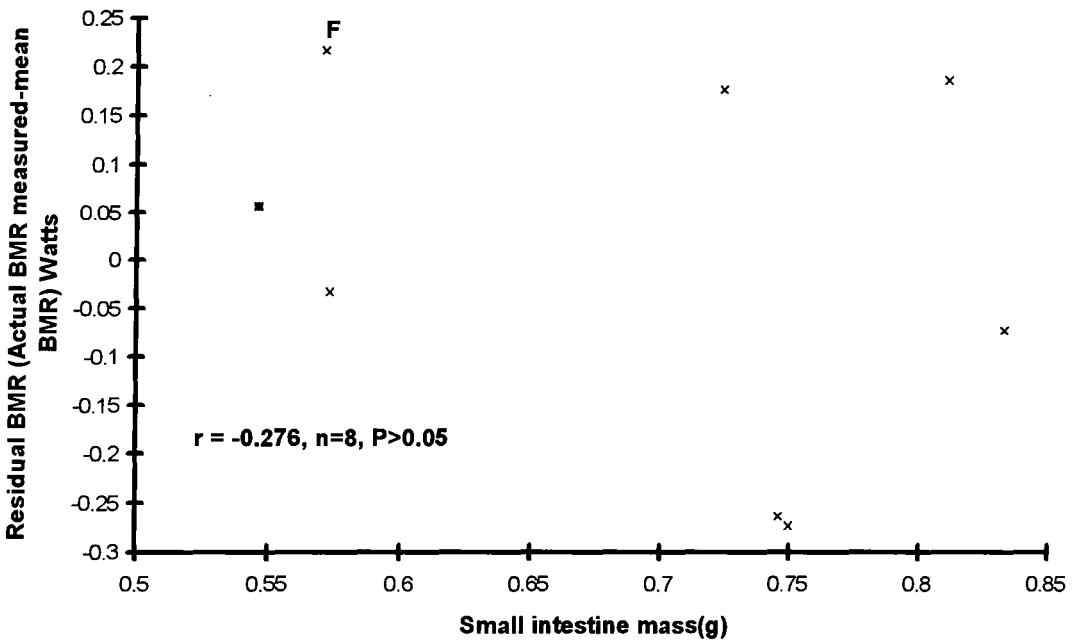


Figure 4.3.2.5. The relationship between residual BMR* and lean dry mass of stomach

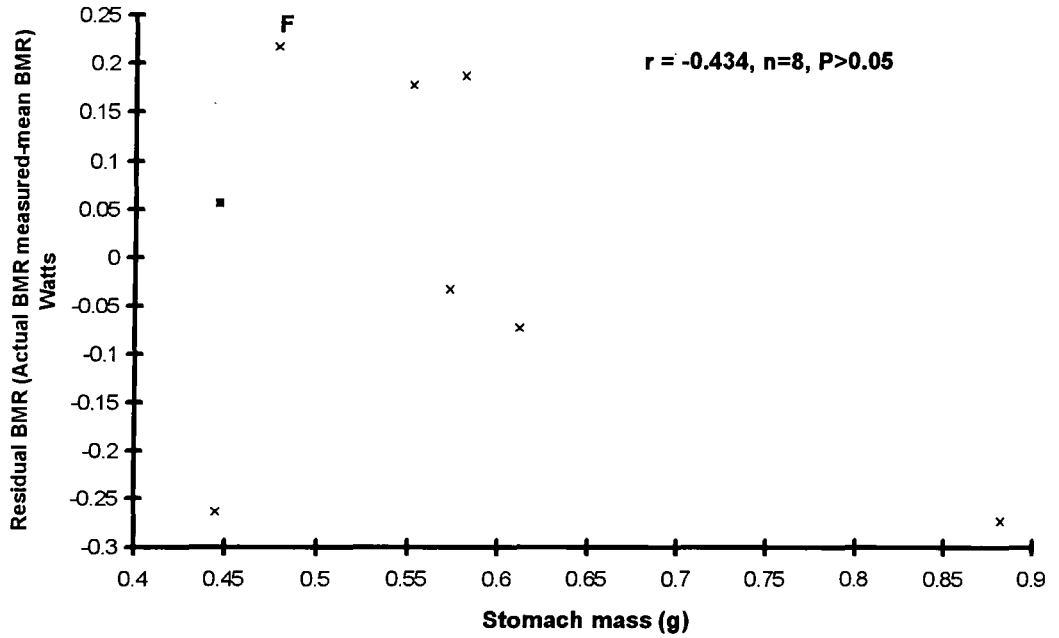


Figure 4.3.2.6. The relationship between residual BMR* and lean dry pectoral muscle mass

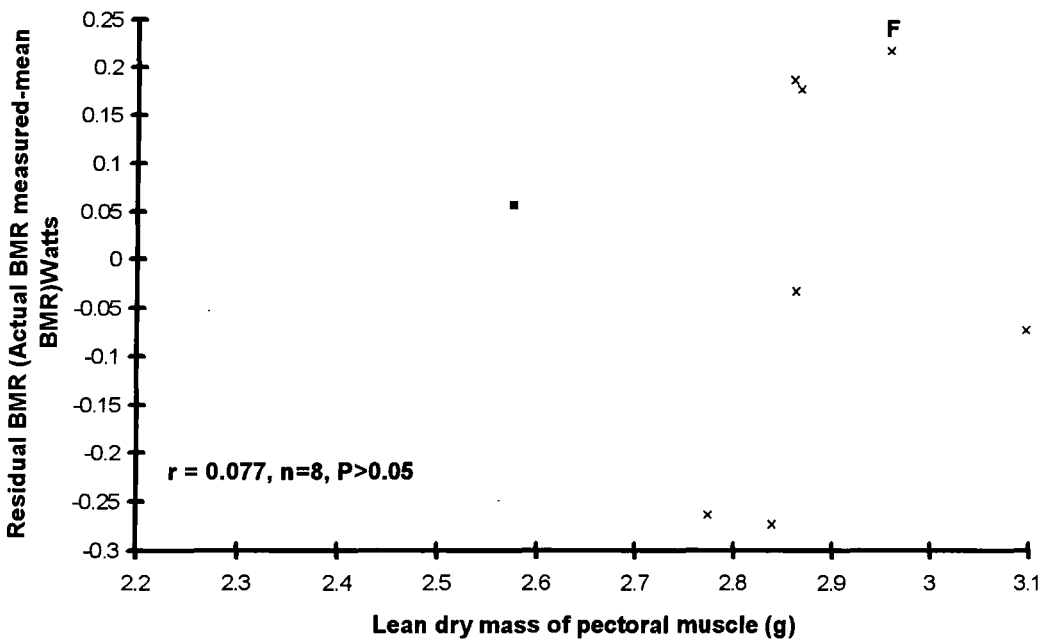


Figure 4.3.2.7. The relationship between residual BMR* and total fat mass

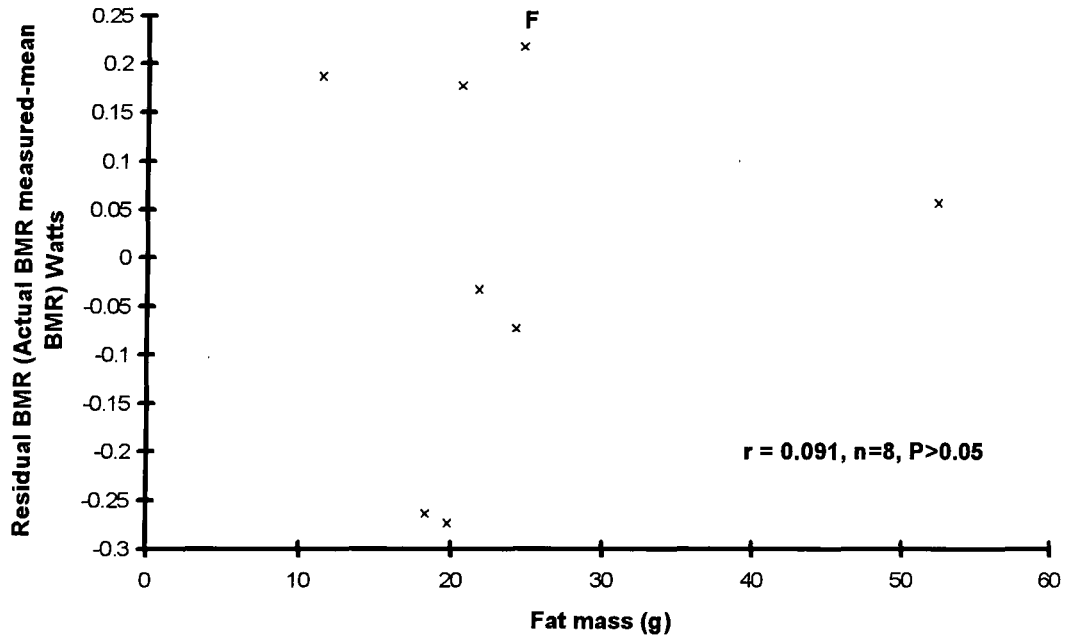
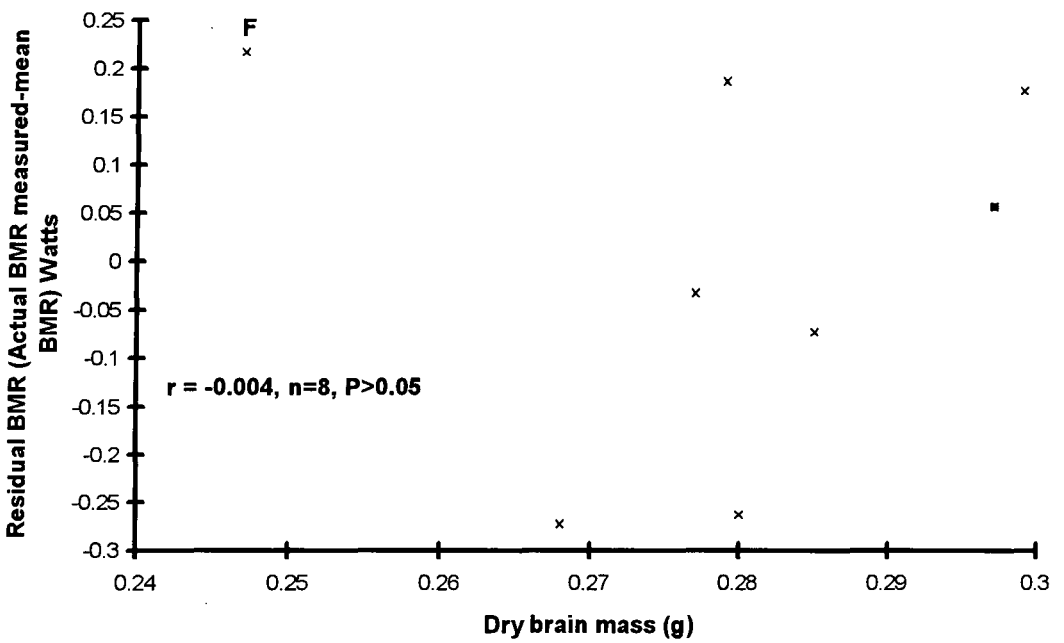


Figure 4.3.2.8. The relationship between residual BMR* and dry mass of brain



these negative correlations were significant the 10% level, possibly due (in part) to the small sample size. One way in which individuals with larger liver, heart, small intestine and stomach masses could have smaller BMR would be if birds with larger organs had lower metabolic activities per gram of tissues, as determined by aerobic enzymatic analysis or mitochondrial counts (This possibility will be examined later in this chapter). Those Knot that exhibited higher than average BMR* did tend to have higher lean dry masses of the highly metabolically active, (though relatively small), organ the kidney (r_8 = 0.609, P=0.109). Of the other organs and tissues correlated with residual BMR* no discernible trends were obvious i.e. pectoral muscle mass (r_8 = 0.083, P>0.05) and dry brain mass (r_8 = -0.004, P>0.05). The strength of the above correlations were not affected markedly when the significantly heavier bird (Knot RGG) was excluded from the correlation analyses.

Those individuals with larger lean dry liver masses tended also to have larger heart (r_8 = 0.697, P=0.06, *Bonferroni* correction in all cases), small intestine (r_8 = 0.795, P<0.05) and stomachs (r_8 = 0.827, P<0.05) lean dry masses. Individuals with larger lean dry heart masses tended to have larger small intestine masses (r_8 = 0.743, P<0.05) and, but not significantly so, stomach masses (r_8 = 0.422, P>0.05). No other pairwise combinations of organ masses were significantly correlated. When the different organs were separately correlated against the lean dry kidney mass, the trends produced were exclusively, but not significantly, negative. Since individuals that had larger lean dry liver masses also tended to have larger heart, small intestine and stomach masses, but similar total lean dry masses, these individuals must have possessed other tissues that contributed less to overall lean mass than the average bird. I correlated lean dry liver mass with the mass of the remainder of the carcass (minus all organ masses) to determine if this remainder was indeed less. The correlation produced was negative (r_8 = -0.301) but not significant.

Data on BMR*, lean dry liver and pectoral muscle mass were also available for Knot sacrificed in physiological period 1 (BM increasing in spring) and 2 (BM decreasing after spring BM peak). The total lean dry masses (see Table 4.3.1.1) in each group varied. To allow for this, BMR* residuals were calculated from the regression of Log BMR* against Log TLDM. These residuals in turn were correlated against lean dry liver and lean dry pectoral muscle mass. For those individuals sacrificed as they increased mass in spring, the residuals of BMR* showed a positive trend with liver mass ($r_7 = 0.480$, $P>0.05$) but a negative trend with PM mass ($r_8 = -0.373$, $P>0.05$), although neither correlation was significant at the 10% level. In those individuals that were losing mass in spring the correlation between residual BMR* and lean dry liver mass was both positive and highly significant (liver $r_9 = 0.899$, $P<0.01$), that is residual BMR* was significantly higher in birds with larger liver masses. The correlation between residual BMR and PM mass was also positive but not significant at the 10% level ($r_9 = 0.255$).

Therefore, although evidence is weak that differences in organ masses can explain differences in BMR* seen amongst individual Knot sacrificed outside the spring migratory period, during the period of body mass loss in spring some of the differences seen amongst individual Knot in BMR* are associated with differences in the mass of a metabolically active tissue, the liver. Nevertheless, it would appear that variation amongst birds in the metabolic intensity of these lean tissues must also be involved to explain the differences seen amongst individual Knot in BMR*.

4.3.3 Can intraspecific variation in BMR be explained by differences amongst individuals in aerobic enzyme activity and mitochondrial volume composition in various lean tissues?

In the previous section, it was shown that outside the spring migratory period individuals that had larger than average liver, heart, small intestine and stomach lean dry masses, for a given TLDM, also tended to have below average BMR. Thus, individuals with larger organs may exhibit lower mass-specific metabolic outputs of these organs. I therefore investigated whether differences amongst individuals in the metabolic intensity of various lean tissues could explain any of the differences in BMR amongst individuals in the same physiological state, i.e. states 1, 2 and 3, by correlating mitochondrial enzyme activities (succinate dehydrogenase and citrate synthase) and mitochondrial volume composition counts with residual BMR*. The residuals used in this analysis for individuals in physiological states 1 and 2 were calculated from the regression equations of LBMR on LTLDM and are shown in Table 4.3.1.2. For those individuals sacrificed outside the spring migratory period the residuals of BMR* were calculated from the regression line below, as the range of TLDM could not be assumed to be the same in these individuals, unlike the 8 birds used in the analysis of BMR* and organ mass:

LBMR= 1.442(1.10) - 0.081(0.71)LTLDM **Equation 4.3.3.1**

r2=0.176 n=8 P>0.05,

where figures in parentheses are standard errors

In birds sacrificed outside the spring migratory period, those individuals with higher than average BMR* for a given body mass did not show elevated levels of succinate dehydrogenase (SDH) in the liver (r_8 = -0.003), pectoralis major muscle (r_8 = -0.064),

small intestine (r_8 = 0.096) or heart (r_4 = -0.023), where P>0.05 in all cases. However, those birds with high SDH activity in their PM had significantly lower activities of SDH in their small intestines (r_8 = -0.780, P<0.05). Trends found when the residuals of BMR* were correlated with citrate synthase activity in the same four lean tissues (Liver r_8 = -0.529, PM r_8 = 0.162, small intestine r_8 = 0.377 and heart r_4 = 0.211), were not significant at the P=0.05 level. In individuals that were sacrificed during the period of fat deposition in spring, those individuals with higher than average BMR for a given lean dry mass tended to have lower SDH activities in the 4 lean tissues but these correlations were not significant even at the 10% level (liver r_7 = -0.421, PM r_7 = -0.310, heart r_7 = -0.468 and small intestine r_7 = -0.655). Similar negative (but non-significant) trends were seen when the activity of CS in individuals sacrificed during fat deposition were correlated with the residuals of BMR in both the gut (r_7 = -0.655) and heart (r_7 = -0.468) but not in the liver (r_7 = 0.011) or PM (r_7 = 0.198). Those individuals with a higher SDH activity in their liver tended to have a higher SDH activity in their small intestine, although the correlation was not significant (r_7 = 0.666, P>0.05).

During the period of BM loss in spring, those individuals with a higher than average BMR for a given lean mass had significantly higher levels of SDH in their PM mass (r_7 = 0.832, P<0.05) Similar trends in the other 3 tissues were also all positive but not significant at the 5% level (liver r_7 = 0.247, heart r_7 = 0.138 and small intestine r_7 = 0.168). Those individuals with high SDH activities in their PM also tended to have high SDH activities in their small intestines (r_7 = 0.833, P<0.05) and to a lesser extent in their livers (r_7 = 0.418, P>0.05). Correlations between residual BMR* and CS activity in liver (r_7 = -0.417), PM (r_7 = 0.271), heart (r_7 = 0.584) or small intestine (r_7 = -0.005) were all non-significant at the 5% level. The lack of significance in the above analyses may be partly due to the small sample size employed. Clearly, some of the variation in BMR seen amongst individual Knot during the period of BM decrease in spring could be explained partly by variation in the metabolic output per gram of

the PM muscle mass.

The second aim of section 4.3.3 was to determine whether individual birds in a particular physiological state with higher organ lean dry masses, for a given TLDM, tended to have lower enzyme activities per gram in these tissues. Unfortunately in those birds sacrificed outside the migratory period, data were available only for both enzyme activity and pectoral muscle lean dry mass. In the other two physiological groups data were available only for both enzyme activity and the lean dry mass of the liver and PM.

Birds sacrificed outside the spring migratory period, individuals with larger pectoral muscle lean dry masses tended to have higher SDH activity in their pectoral muscle ($r_8 = 0.587$) but lower CS activity ($r_8 = -0.322$), but neither of these relationships was significant at the 10% level. During physiological state 1, SDH activity in the liver tended to be lower, but not significantly so in those individuals with larger liver masses ($r_7 = -0.418$, $P>0.05$). No correlation was seen between CS activity and liver mass in those same birds ($r_7 = 0.080$). In birds losing mass after the spring BM peak, aerobic enzyme activity did not correlate with liver mass at all (SDH $r_7 = 0.154$ and CS $r_7 = -0.298$). Knot depositing fat in spring that had larger lean dry pectoral muscle masses did not exhibit significantly higher or lower levels of SDH ($r_7 = -0.116$) or CS ($r_7 = 0.017$) in the pectoral muscle, nor was any significant correlation seen between pectoral muscle mass and enzyme activity in birds sacrificed as they lost mass in spring (SDH $r_7 = 0.231$, CS $r_7 = 0.354$, $P<0.05$).

The second technique employed in this study attempted to estimate the mass-specific metabolic activity of various tissues indirectly, by measuring the volume composition of mitochondria contained in those tissues. Mitochondrial volume counts were then

correlated with the residuals of BMR* produced when Log BMR* was regressed against Log TLDM. Those birds sacrificed outside the migratory period with a higher than average BMR for a given lean mass did not exhibit significantly higher mitochondrial volume counts in either their livers (Pearson rank correlation, r_4 = 0.022), or in the superficial (r_4 = -0.098) or deep aspects of pectoralis major (r_4 = 0.152). In those birds sacrificed while increasing in mass in spring no significant relationship (P>0.05) was found to exist between residual BMR and mitochondrial volume composition in the liver (r_4 = -0.017) or in the superficial (r_4 = 0.189) or deep aspect (r_4 = -0.414) of pectoralis major. This was also the case in those birds sacrificed during the period of BM loss in spring (Liver r_4 = 0.163, PM superficial r_4 = 0.399 and PM deep r_4 = 0.485), although a significant trend was present between residual BMR and mitochondrial volume composition in the PM (The SDH activity in the PM during this period was also higher, indeed significantly so, in those individuals with higher BMR for a given lean mass). The lack of any significance in these correlations and the fact that volume composition of mitochondria did not correlate significantly even at the 10% level with enzyme activity may simply reflect that the sample sizes of 4 used in this study were too low. However, another possible reason for the lack of a relationship between mitochondrial volume composition and enzyme activity may be that, amongst individuals of the same species, mitochondrial volume within the lean tissues does not affect the metabolic output per gram of those lean tissues as much as the enzymes situated within the inner-mitochondrial membrane (cristae). From Chapter 3, it is known that the levels of SDH in the PM of captive Knot varied on a seasonal basis but the mitochondrial volume remained fairly constant. Therefore, it is perhaps not surprising that mitochondrial volume composition of the lean tissues did not explain any of the variation seen in BMR amongst individuals.

4.4 Discussion

4.4.1 Intraspecific variation in BMR and its relationship with body mass and body composition.

The main aim of the work carried out in this chapter was to identify the factors that lead to differences in BMR amongst individual Knot, that are in the same physiological state. The results presented show clearly that in groups of captive Knot, irrespective of physiological state, only small proportions of the differences amongst individuals in BMR can be explained simply by differences in body mass (BM), or in any major component of body mass, i.e. total lean mass (TLM), total lean dry mass (TLDM) or total fat mass (TFAT).

No significant relationship existed amongst individuals when Log BMR (LBMR) was regressed against Log BM (LBM), in any of the 3 distinct physiological states. Indeed, the coefficients of determination (r^2) were particularly low in those birds sacrificed outside the spring migratory period ($r^2 = 0.052$, n=24), and in birds ($r^2 = 0.091$, n =7) sacrificed during the period of BM increase in spring (period 1). Around 22% of the variation in BMR amongst individuals sacrificed during period 2 (BM falling from spring peak) was attributable to differences in BM amongst them, though even this relationship was non-significant at the 10% level. However, there is evidence from the literature that differences in BMR amongst individual birds of the same species can be partly explained by variations in BM. Scott *et al* (1996) found that differences in BM explained some 54% of the variation seen in BMR amongst 21 captive Redshank *Tringa totanus*, sampled outside the 'breeding period'. Weber & Piersma (1996) also calculated that differences in BM seen amongst a group of captive Knot (*islandica* subspecies, n=14), losing BM after the spring migratory peak, explained some 44% of the

differences seen in BMR amongst these birds. Scott (1991) suggests that when employing least-squares linear regression analysis, high levels of individual variation in BMR and BM will lead to a high degree of scatter and hence low coefficients of determination. These reasons possibly allied to the small sample sizes (periods groups 1 and 2 particularly), may partly explain the low r^2 values obtained in my study when LBMR was regressed against LBM. But the amount of individual variation in my study, e.g. in birds losing mass after peak BM in spring was actually less that that calculated by Weber & Piersma's (1996) for captive *islandica* Knot measured during period 2 (see Table 4.4.1 below).

Table 4.4.1: Differences in body composition in two groups of captive Knot sacrificed as BM was decreasing in spring from peak BM

(Values in parentheses are standard errors)

BM- Body mass **TLM- Total lean mass**

TFM- Total fat mass **TLDM- Total lean dry mass**

	Weber & Piersma (1996) n=14 sub-species islandica	**This study n=9 sub-species islandica**
BM	143(8.4)	150(3.5)
TLM	104(2.1)	102(1.7)
TFM	39(6.3)	48(3.6)
TLDM	38(1.0)	35(1.2)

In my study the means and coefficients of variation in parentheses for BM and BMR were 150g (12%) and 1.84 Watts (15%) respectively, whereas in Weber & Piersma's (1996) study the mean values were 143g (21%) and 0.92 Watts (24%). So although the mean values of BM and BMR in my study were higher than those measured by Weber & Piersma (1996), the individual variability in BM and BMR amongst the Knot in my study was considerably less. Therefore, the suggestions

made by Scott (1991) to explain low r^2 values probably do not apply to my study. Piersma *et al* (1996) investigated whether differences in BMR seen between four different categories of Knot (wild and captive, of the races *islandica* and *canutus*) sampled in mid-winter, could be "explained by" any of the variation seen between the 4 categories in BM and body composition. In their analyses they admit that pooling of data into the 4 distinct categories removed much of the variability seen amongst individual birds within a group, thereby reducing the inherent scatter in their regression analyses. Scott *et al* (1996) also used mean values (measured over several days from each individual Redshank) of BMR, BM and body composition in their study, thereby decreasing the between individual variation in these parameters and possibly leading to the higher r^2 values seen in their (and Weber & Piersma's) studies.

It might be thought that the higher levels of BMR, for a given body mass or total lean mass, measured in this study when compared to Weber & Piersma's (1996, see Table 4.4.1) might have been in part due to BMR measurements being taken during the day and not at night as in Weber & Piersma's (1996) study. However, no significant differences occurred in BMR of individual Knot measured during the day and during the night (see Section 3.3.2). The mean RQ value in this study was 0.754 (0.01SE), where n=514, which would be expected if the Knot in this study were post-absorptive during BMR measurements.

No significant linear regression equations were obtained in my study between Log BMR and Log TLM or between Log BMR and Log TLDM amongst individual Knot, in any one of the three distinct physiological states examined. Indeed, variation in TLDM amongst individual Knot, irrespective of physiological state, explained less than 5% of the differences seen amongst these individuals in BMR. These findings are in direct contrast to those found by previous workers, e.g. Daan *et al* (1989) in Kestrels; Piersma *et al* (1996) in Knot and Scott *et al* (1996) in Redshank, who all reported that differences in BMR seen amongst individuals

of the same species were explained primarily by variations in TLM or more particularly TLDM, rather than in BM or TFAT. Piersma *et al* (1996) suggest that fat-free dry mass (TLDM) alone determines the BMR of an individual. In their study, differences in total lean mass (not TLDM) did explain some 99% of the variability seen in BMR amongst 4 distinct groups of Knot, where the regression was based on 4 points (mean BMR and mean TLM of each group). However, in Chapter 3 of this thesis, it was shown clearly that the activity of the aerobic enzyme succinate dehydrogenase in several lean tissues is also considerably lower in captive Knot, when compared to wild conspecifics. Therefore, some of the differences in BMR between wild and captive Knot in Piersma *et al's* (1996) study may have been due to differences in the metabolic activity per gram of the lean tissues. Scott *et al* (1996) suggest that part of the reason why BMR correlates with TLM rather than with TFAT in many intraspecific studies in which LBMR correlates with LBM, is because most of the variation in total body mass between individuals of the same species is due to variations in body (skeletal) size and therefore in lean mass. This will certainly be the case in birds not carrying considerable fat stores, e.g. in the Redshank outside the breeding season studied by Scott *et al* (1996) and in wild Knot studied during the winter by Piersma *et al* (1996) but this was not the case in the captive Knot used in my study, where most of the variation seen amongst individual Knot in BM was due to TFAT and not TLM (see Table 4.4.1).

While it is certainly true that almost all of the metabolic heat produced in a bird is generated by the lean tissues (Piersma, 1994), variations in fat mass amongst individuals, (due to direct and indirect costs on BMR) may explain some of the differences seen amongst those individual's in BMR. I have shown earlier when calculating BMR* that the estimated O_2 consumption of adipose tissue in Knot is negligible, even in those birds carrying considerable fat stores. Therefore, it is likely that, if variation in fat load does have an effect on variation in BMR amongst individuals, the effect is indirect due to the support and carrying of the fat mass (Witter & Cuthill, 1993). My results show clearly that that differences in

TFAT that exist amongst individual Knot explain little of the variation seen amongst these individuals in BMR, although the highest r^2 (0.335) value obtained in the least-squares analyses in this chapter was obtained when LBMR was regressed against LTFAT, in birds increasing in BM in spring. My results corroborate well with those of Scott *et al* (1996) in (albeit leaner) Redshank, that differences in fat mass amongst individual birds of the same species explain little of the variation seen in BMR amongst individuals. But there is evidence that the indirect costs of supporting and carrying this fat mass may affect an individual's BMR (see Chapter 3). Weber & Piersma (1996) actually found that it was variations in TFAT and not in TLM that best explained the differences in BMR seen amongst a group of captive Knot that were losing BM in spring. They suggested that their findings showed that there were either indirect costs to carrying fat or that the fat mass itself is not metabolically inert, or both.

It can clearly be seen from my data that differences in BMR amongst individual captive Knot in this study, irrespective of physiological state, were not explained simply by variations that existed amongst these individual birds in either BM or in BM composition. Therefore, the differences must be associated with variations in the average metabolic output per gram of the lean tissues. Such differences must be due to either:

1. Differences amongst individuals in the contributions of various metabolically active lean tissues and organs to an individual's total lean mass and /or
2. The metabolic activity of these lean tissues differing amongst individual Knot in a particular physiological state.

These possible explanations for intraspecific variation in BMR will now be discussed further.

4.4.2 Intraspecific variation in BMR and its relationship with organ mass.

It is well known that the various lean tissues and organs that make up the total lean mass of an individual bird or mammal vary considerably in their metabolic activities per gram (Schmidt-Nielsen, 1984; see Table 4.4.3). Therefore, differences in BMR* amongst individuals with the same total lean mass could be due to differences in the masses of various metabolically active tissues that make up an individual's total lean mass.

My results showed that amongst 8 individual Knot, sacrificed outside the migratory period and with very similar total lean dry masses (range 32-33g), the differences in BMR* cannot be explained simply by differences amongst them in the lean dry masses of various metabolically active organs and tissues that make up TLDM. Although the BMR* amongst these 8 individuals did tend to increase with an increase in the LDM of the kidney, the correlation was not significant ($P=0.109$). The kidney is known to have a extremely high metabolic activity per gram in rats (see table 4.4.3), but due to its small mass it accounts only for around 5% of total organismic O_2 consumption. There is some published evidence that the relative contributions of various organs that make up TLDM can explain some of the differences seen in BMR, amongst individual birds of the same species. Daan *et al* (1989) reported that the lower than expected BMR seen in Kestrels fed on low maintenance diets were strongly correlated with a reduction in the lean dry masses of the heart and kidney and to a lesser extent the liver and lungs. Of course correlation does not imply causation and Daan *et al* (1989) did not measure whether the metabolic activity per gram of the various lean tissues also differed between kestrels kept on low and high maintenance diets.

Table 4.4.3 Oxygen consumption (measured using an O_2 electrode) of various tissues and organs in a 150g rat. The O_2 consumption per gram, the total O_2 consumption of the tissue (or organ) and total O_2 consumption of this tissue as a percentage of total body O_2 consumption (100%) are shown. (Adapted from Field *et al*, 1939)

Organs denoted by asterisk were correlated against residual BMR* in 8 Knot sacrificed outside premigratory period

Organ	Organ weight (g)	ml^{-1} O_2 g^{-1} hr^{-1}	Whole organ ml^{-1} O_2 g^{-1} hr^{-1}	% of total
Skeletal muscle*	61.4	0.875	53.72	48.8
Diaphragm	1.0	1.800	1.8	1.6
Skin	27.8	0.416	11.55	10.5
Skeleton	10.0	0.153	1.53	1.4
Blood	9.7	0.025	0.24	0.2
Liver*	9.2	2.010	16.48	15.0
Alimentary canal*	8.0	1.010	8.08	7.3
Ligaments	7.4	0.070	0.52	0.5
Brain*	2.3	1.840	4.23	3.8
Kidneys*	1.4	4.120	5.76	5.2
Testes	1.2	1.030	1.24	1.1
Lungs	0.9	1.250	1.13	1.0
Heart*	0.7	1.930	1.35	1.2
Spleen	0.4	1.330	0.53	0.5
Remainder	9.6	0.200	1.92	1.7
Total	150.0	-	110.08	100

Of the 8 individuals used to investigate the relationships between BMR and organ mass, those individuals with larger lean dry liver, heart, small intestinal and

stomach masses tended to have lower that average BMR*. This may indicate that in birds with these larger organs may have had lower metabolic activities per gram, and hence lower overall BMR*. This is seen between families of mammals (Schmidt-Nielsen, 1984). It is possible that the individuals in this sample with higher than average BMR*, but lower organ masses, had a larger mass of some other highly metabolically active organ/tissue not measured in my study. Table 4.4.3 confirms that the organs sampled in my study are known to be among the most metabolically active organs (Field *et al,* 1939). The skin of the rat, although having a fairly moderate O_2 consumption per gram, accounted for over 10% of total body O_2, due to its large mass. The mean (±SE) lean dry mass of the skin of wild Knot, of the *islandica* subspecies, was calculated at 4.64±1.37g or around 12% of total fat-free dry body mass (Piersma, 1994). Therefore, it is feasible, but unlikely, that differences in tissues such as the skin, either in mass or metabolic activity per gram, could lead to significant differences in BMR amongst individual Knot. The mean (±SE) skeletal mass, which is known to have low metabolic activity per gram, varied little amongst these 8 individuals (3.725±0.08g). Those individuals with lower BMR* ,for a given TLDM, did not have considerably greater skeletal masses.

During the period of BM increase in spring, differences in BMR* seen amongst individual Knot were not explained by differences in the LDM of the liver or in pectoral muscle mass. However, in those birds sacrificed during period 2, around 90% of the variation that existed amongst these individuals in BMR*, for a given TLDM, was explained by differences seen amongst those birds in TLDM of the liver, i.e. those individuals with larger lean dry liver masses, for a given TLDM, tended to have higher than average BMR*. This strong correlation was not seen between the residuals of BMR and TLDM of the pectoral muscle mass. Table 4.4.3 confirms that the liver is a highly metabolically active lean tissue. Therefore, in Knot in physiological state 2, i.e. rapidly losing BM, there is good evidence that differences amongst individual Knot in the contribution of the liver to overall lean dry mass may explain some of the differences seen in BMR amongst these

individuals. Weber & Piersma (1996) suggested that differences seen amongst captive Knot in BMR during period 2, were explained by differences in the lean dry mass of the PM and heart (but not liver) and not by differences in the metabolic activity per gram of the tissues. From these results there appears to be clear evidence that the differences seen in BMR amongst individual Knot, particularly outside the spring migratory period and during the period of BM increase in spring, are not wholly explicable in terms of tissue/organ masses but must also reflect differences in the metabolic activity per gram of these lean tissues. However, during the period of rapid decrease in BM in spring there is some evidence that differences in the lean dry mass of liver, for a given TLDM, may explain some of the differences seen amongst these individuals in BMR.

4.4.3 Intraspecific variation in BMR and its relationship with mitochondrial volume and aerobic enzyme activity.

To determine whether the differences in BMR* amongst individual Knot that appeared to be due to differences in the metabolic activity per gram of the lean tissues, residual BMR* produced solely by the lean tissues was correlated with aerobic enzyme activity (succinate dehydrogenase and citrate synthase) in 4 metabolically active lean tissues: the liver, pectoralis major muscle, heart and small intestine. The mitochondrial volume compositions of liver and PM (superficial and deep aspects) were also calculated and correlated with residual BMR*.

In 8 birds (n=8) sacrificed outside the spring migratory period (but not in the 8 individuals used for organ/BMR analysis), those individuals with higher enzyme activities per gram in the liver, pectoralis major muscle, heart and small intestine did not tend to have higher BMR* for a given TLDM. A similar result was obtained when the mitochondrial volume composition in the liver and PM was

correlated with TLDM. This suggests that, in those individuals sampled, the differences seen in BMR* amongst these individuals were not explained by variations in the metabolic activity per gram in the lean tissues sampled. It is unfortunate that in the 8 individual Knot used to investigate whether differences in BMR* could be explained by differences amongst individuals in organ mass (see section 4.4.2), SDH and CS activity were not measured, as some of the differences in BMR* amongst those individuals appeared to be due to differences in the metabolic activity per gram of certain lean tissues.

During the period of BM increase in spring, those individuals with higher than average BMR*, for a given TLDM, tended to have lower levels of SDH in their liver, pectoralis major muscle, heart and small intestine and lower levels of CS in their heart and small intestine, although the correlations produced were not significant at the 10% level. If these findings are biologically, although not statistically, significant, they may indicate that in those individuals with higher BMR*, for a given mass, although tending to have lower SDH activities in the four tissues tested, actually had increased masses and/or aerobic enzyme levels in other tissues (e.g. kidneys) that were not tested. Differences amongst individuals in BMR* could not be explained in these individuals by differences in mitochondrial volume composition of the liver and PM. However, in individuals with higher than average BMR*, for a given TLDM, lower mitochondrial volume counts (as with SDH activity) were measured in the deep aspect of the pectoralis major muscle.

In those individuals losing BM after the spring peak, individuals with higher than average BMR for a given TLDM also had significantly higher SDH activities in their pectoralis major muscle and also higher levels of SDH in the other three lean tissues tested. There was also a significant positive correlation between SDH activity in the pectoralis major muscle and small intestine and a positive correlation between SDH activity in the pectoralis major muscle and liver. That is,

those individuals with high activities of SDH in their pectoralis major muscle also tended to have high activities of SDH in their livers and guts. Those individuals with higher than average BMR* also tended to have a higher mitochondrial volume composition in their pectoralis major muscle but not in the liver, but these correlations were not significant possibly due to the very small sample size (n=4). Variations in the metabolic activity of skeletal muscle may have a considerable effect on an individual's BMR*, due to the high contribution that skeletal muscle makes to total lean mass. Field *et al* (1939) reported that although the metabolic activity of muscle was only moderate, due to its mass the skeletal muscle accounted for approx. 50% of total body O_2 consumption. Therefore, even small variations in the metabolic activity of the skeletal muscle of Knot may have considerable effects on that individual's BMR*. Elevated levels of SDH activity in the pectoralis major muscle leading to a higher than average BMR may be a mechanism 'employed' by captive Knot to enable the catabolism and loss of the fat stores, which obviously cannot be burnt off by long-distance flight in captive birds. It is also possible that the elevated levels of SDH in the pectoralis major muscle leading to a high BMR*, increase the metabolic scope of the individual and enable it to undertake long-distance migration, during which energy consumption may reach 10 x BMR (Jenni-Eiermann & Jenni, 1992). This would be a mechanism to increase power output of the flight muscles prior to flight, with or without an increase in the pectoralis major muscle mass (Marsh, 1984).

In summary, it can be seen that the differences in BMR that are so clearly seen amongst individual captive Knot that are in the same physiological state, are not explained simply by variations amongst them in total body mass, total lean mass, total lean dry mass or in total fat mass. The low coefficients of determination seen in this study may also be partly explained by the high degree of mass-independent variation in BMR seen within captive Knot (see Chapter 3). Variability does exist amongst individuals in their average metabolic output per gram of the lean tissues and organs that make up total lean mass. Differences amongst individual Knot in BMR can partly be explained both by differences amongst individuals in the mass

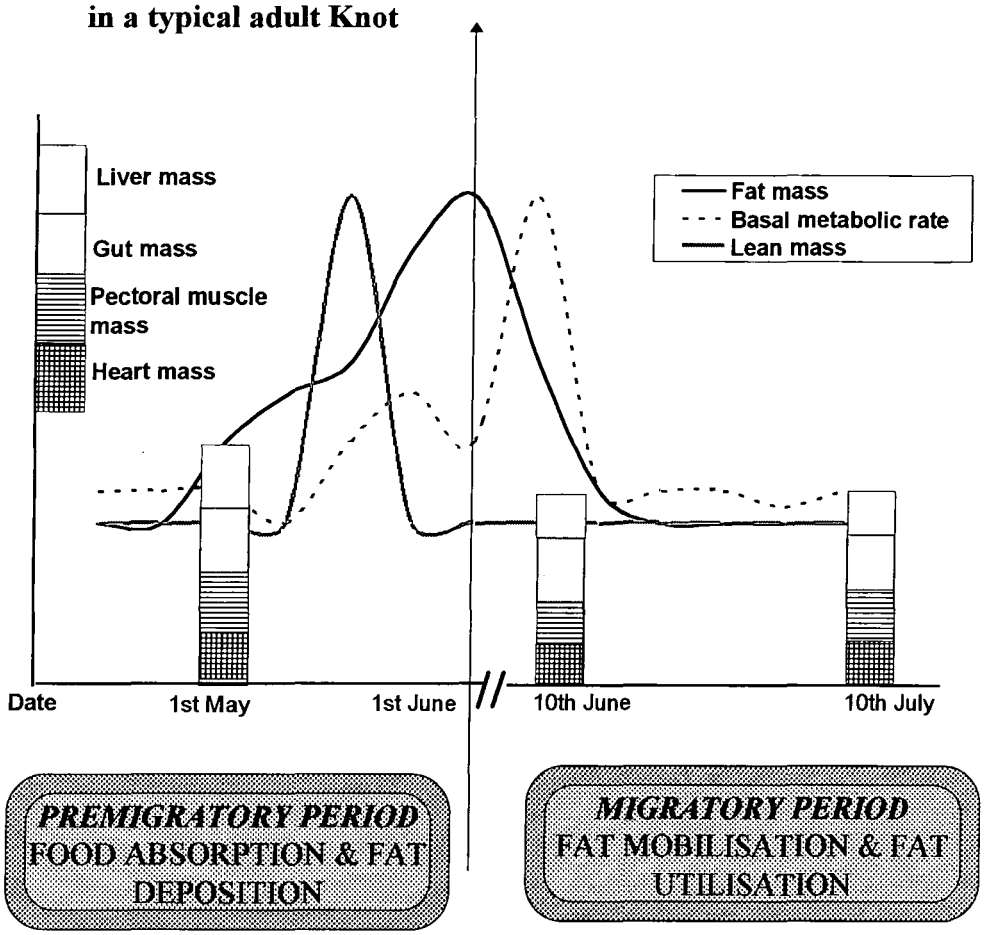
of various metabolically active organs that contribute to total lean mass but also due to differences amongst them in the metabolic activity per gram of various lean tissues. In much of the work described in this chapter, clear trends which could make biological sense can be seen in many cases but these trends lack statistical significance. Much of this lack of significance is due to the small sample sizes used in many of the analyses. These constraints on sample size were due to the logistic difficulties of keeping large numbers of Knot in captivity; and because many of the procedures, particularly mitochondrial volume composition analysis, were extremely time consuming. The finding that the mean BMR measured in this study are higher than those measured in Knot by Piersma (1994) and Weber & Piersma (1996) is unexplained but does not affect the validity of the findings because the factors causing variation in BMR, both intra-individually and intraspecifically, in both studies are likely to be the same, irrespective of the mean BMR encountered.

5.0 General Discussion

5.1. Seasonal variation in Basal Metabolic Rate (BMR) and its relationship to long-distance migration.

The relationships, detailed in Chapter 3, between BMR and body composition in a captive adult Knot during the spring and early summer are summarised schematically in Figure 5.1.

Figure 5.1 Seasonal variation in BMR, lean mass, fat mass and organ mass in a typical adult Knot



In my study, a total of 13 captive Knot (12 adult and 1 'atypical' juvenile) out of 16 (81%) displayed seasonal peaks in BMR, often to levels of double the seasonal minimum recorded. These occurred as body mass was decreasing rapidly, a few days after its spring peak. Cadee (1992) and Piersma *et al* (1995) also found increases in body mass to levels similar to those seen in my study during the spring in captive Knot, although they concluded that peak BMR occurred *during* (my italics) the early summer peak in body mass. However, as they measured BMR in individual Knot only about every 6 weeks they must usually have missed the peak of BMR. Indeed, they recorded a maximum increase in BMR during spring of only 40% (in one individual) with a peak level of only 1.5 Watts, considerably lower than the peak level of 2.0 Watts consistently achieved by Knot in my study (Chapter 3, see Graphs 3.3.3A-F). I measured BMR far more frequently, often every 48 hours during spring. The peak BMRs seen in my study, occurred during May and early June, coinciding approximately with the period in the wild when Knot undertake northward migration to the breeding grounds (Evans, 1992; Piersma & Davidson, 1992; Davidson & Wilson, 1992).

Wild Knot typically display two distinct and recurring physiological states during migration to and from the breeding grounds (Piersma & Davidson, 1992), both of which were mirrored in captivity:

(i) **Premigratory period**, a period of fat deposition and rapid increase in body mass, before migration and at refuelling sites.

(ii) **Migratory period**, a period of fat mobilisation and catabolism leading to body mass decrease, chiefly during flight.

The findings of my study will now be discussed in the context of long-distance migration and the physiological adaptations necessary for Knot to achieve this.

5.1.1 Premigratory period

Preparation for migration by birds, insects and certain species of bat typically involves a period of rapid body mass increase (up to 8g/day in captive birds in this

study). Evans (1992) reported that the average overall rate of body mass increase of Knot at a refuelling site was around 3.8g/day, although the maximum recorded value for an individual was 8g/day. This premigratory body mass increase in birds primarily results from rapid fat deposition to fuel long-distance flight (Blem, 1980). Captive Knot, on average, increased their fat mass in spring by nearly 30% over prefattening levels. Some bird species also deposit lean tissue before migration, particularly in the flight muscles (Evans, 1969; Marsh, 1984; Klaassen *et al*, 1997). An average increase in dry pectoral muscle mass of 6%, equivalent to an increase in (wet) muscle mass of some 2-3g (depending on body size), was measured in wild Knot *en route* from the southern North Sea coasts to the Neararctic breeding grounds by Evans (1992). Captive Knot in my study exhibited only small increases in total lean mass (approx. 5% or 3-4g) during the spring.

Three main mechanisms have been identified through which this rapid premigratory fat deposition may be achieved (see Blem, 1980; Ramenofsky, 1990):
(i) Premigratory hyperphagia- an increase in appetite (Ramenofsky, 1990); (ii) Increased efficiency of assimilation of food; (iii) Temporary decrease in overall basal energy requirements.

I will now discuss whether there is evidence from my study that any of these three mechanisms were involved in premigratory fattening in captive Knot.

(i) Premigratory hyperphagia, leading to hyperlipogenesis, rapid fat deposition and elevation of body mass has been studied primarily in songbirds (Biebach, 1996). For example, captive Garden warblers *Sylvia borin* increased their daily gross food intake by almost 50% (Bairlein, 1985) during the premigratory period. Hyperphagia has also been reported in various wader species feeding in the wild during spring (Metcalfe & Furness, 1984; Ens *et al*, 1990). Captive Knot in my study displayed hyperphagia during the premigratory period, with the daily gross food intake consumed by groups of individual increasing considerably during this time (*pers. obs*). Unfortunately, as Knot were kept in groups of 8-10 and as

different individuals did not commence fattening on exactly the same day I cannot quantify the increase in daily intake rate per individual. In the wild, Knot appear to reach a threshold body mass before migrating (Evans, 1992), with the subsequent fall in body mass between refuelling site and breeding area being due chiefly to the catabolism of fat during flight, but also due to the cessation of feeding and catabolism of protein (including pectoral muscle) in flight (see Evans *et al*, 1992).

(ii) While it would appear that there is an upper limit to the rate that birds and mammals can digest and assimilate food (Kirkwood, 1983), there is good empirical evidence that the highest levels measured can also alter on a seasonal basis. Bairlein (1985) reported that hyperphagia in captive Garden warblers *Sylvia borin* during premigratory fattening was associated with both an increased efficiency of digestion and an increased assimilation of dietary fat, protein and carbohydrate. The possible mechanisms behind this reported increase in digestive efficiency are unknown (Bairlein, 1985). However, such an increase has not been seen in House wrens *Troglodytes aedon* that were acclimated to cold (Dykstra & Karasov, 1992), or in mice acclimated to cold (Konarzewski & Diamond, 1994), or indeed in another study of captive Garden warblers experimentally undergoing migration related body mass changes (Klaassen & Biebach, 1994).

It is generally thought that when the daily demand for food energy exceeds the gut's capacity to supply that energy, the energy assimilation rate is increased primarily through temporary growth of the alimentary tract and associated organs (for review see Piersma & Lindstrom, 1997). Such hypertrophy has been shown experimentally to occur both in Mice *Mus musculus* (Hammond *et al*, 1994; Speakman & McQueenie, 1996) and in Wrens acclimated to cold (Dykstra & Karasov, 1992), although no direct evidence for gut hypertrophy has been obtained for premigratory birds (Karasov, 1996). However, Piersma *et al* (1993) did report that the stomach masses of Knot sampled during early spring on the Wadden Sea were significantly greater than those of Knot sacrificed in Iceland later in spring, just before departure to the arctic breeding grounds. Piersma & Lindstrom (1997) also report that Bar-tailed Godwits *Limosa lapponica* showed a

30% increase in stomach mass in the 'first half' of a 3 week refuelling period, but a 20% decrease in the second half. From my study there is some evidence that gut hypertrophy may have occurred in captive Knot during the premigratory period. The mass of the small intestine was higher (but not significantly so) for a given body mass during this time, and the spring peak in total lean mass (as predicted using TOBEC) generally occurred during this period of fat deposition, on average 6 days before the peak body, and therefore fat, mass.

Captive Knot in my study probably increased their digestive efficiency during the premigratory period, as a result of the increase in the metabolic activity per gram of the small intestine. The mean succinate dehydrogenase (SDH) activity in the small intestine during this time rose by nearly 50% compared to that measured outside the spring migratory period (Chapter 3). This increase in digestive efficiency, allied to premigratory hypertrophy, will enable captive Knot to increase their daily energy assimilation rate during the period of premigratory fattening. However, this increase in gut metabolic activity, coupled to the significant increase in liver mass seen in captive Knot during the premigratory period, will aid fat deposition only if the net energetic benefits of these changes exceed the net energetic costs. Speakman & McQueenie (1996) suggest that the 'penalty' incurred through hypertrophy of the alimentary tract and associated organs is an 'inevitable increase in overall BMR'. The small intestine is known to be relatively metabolically active (Krebs, 1950), therefore any increase in its metabolic activity, allied to the increase in liver mass, is likely to increase total BMR as a consequence. One mechanism which could be employed to aid fat deposition further at this time would be through decreasing the energetic costs derived from other contributors to total BMR. This could counter the 'inevitable' increase seen in BMR, thereby decreasing the net energetic costs of that individual overall.

(iii) A decrease in BMR and hence total daily energy expenditure will aid fat deposition even if the food intake rate remains constant. Evidence to support this has been reported in Svalbard ptarmigan *Lagopus mutus hyperboreus* (Stokkan, 1992; Lindgard *et al*, 1992) through a reduction in locomotory activity, and in

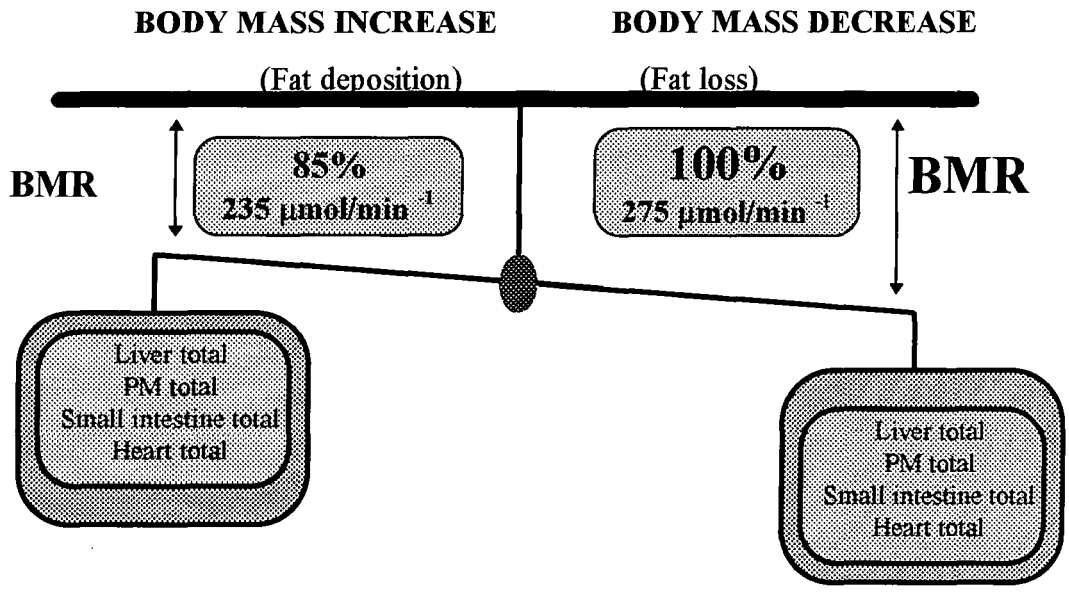
Rufous Hummingbirds *Selasphorus rufus* (Hiebert, 1993) through employing torpor. Indeed, Stokkan (1992) reported that the reduction in total daily energy expenditure was sufficiently great to enable fat deposition despite a simultaneous reduction in overall food intake rate during autumn. However, he did not take into account the changes in diet that occur in Svalbard ptarmigan during autumn. A change in diet from insectivory (chiefly) to frugivory (chiefly) has been suggested as another mechanism that may increase the rate of fat deposition in small passerines before autumn migration (Bairlein, 1990).

There is direct evidence from my study (Chapter 3, section 3.3.3) that a temporary plateau in BMR occurred during the premigratory period. The seasonal peak in BMR typically did not occur until some 5 days after peak body mass, with BMR tending to increase rapidly immediately after body mass started to decrease in early summer (see Figure 5.1). The apparent suppression of BMR during the period of fat deposition did not result from either a decrease in the total mass of the metabolically active lean tissues or a decrease in the mass of the metabolically active liver, heart, small intestine or pectoral muscle. The mean masses of these four organs during the premigratory period (see Figure 5.1) were actually higher, for a given body mass, than during the other two physiologically distinct periods, i.e. the period as body mass decreased after the spring peak and the period before/after the spring migratory period. Therefore, the plateau in BMR measured during the premigratory period must have been caused by some other mechanism.

If mean organ mass (liver, pectoral muscle, heart, gut) is multiplied by mean SDH activity, then the combined total activity for these 4 metabolically active tissues (see Table 4.4.3) is 15% less during the period of fat deposition than during the period of fat loss (see Figure 5.2). Thus any 'inevitable' increase in BMR occurring during the premigratory period, as a result of digestive adaptations that lead to rapid and efficient food processing (i.e. increased small intestine metabolic activity and increased liver mass), was countered by a marked decrease (60%) in the metabolic activity per gram of the pectoral muscle at this time. This led to a decrease in overall BMR because the mass of pectoral muscle (approx. 20-25% of

total lean mass) is much greater than the mass of the digestive organs (approx. 10% of total lean mass). Dreidzic *et al* (1993) reported a similar reduction in the activity of a mitochondrial enzyme, citrate synthase, in pectoral muscle of wild Semipalmated sandpipers *Calidris pusilla* during a period of intense premigratory fattening. Recently, Bishop *et al* (1998) also reported that citrate synthase activity in the pectoral muscle of premigratory Barnacle geese *Branta leucopsis* actually declined with increasing body mass.

Figure 5.2. Total combined succinate dehydrogenase activity (μmol min^{-1}) in the 4 lean tissues measured during the period of body mass increase and then body mass decrease in spring. (Total activity as body mass decreased in spring is set at 100%. Data obtained from Chapters 3 and 4)



My captive Knot were sacrificed soon after fattening commenced in spring. Therefore I do not know what changes actually occurred in organ mass or enzyme activity at peak body mass or peak BMR. There is evidence from studies of wild Knot, however, that the liver, small intestine and stomach all decrease in mass immediately before migration (Piersma *et al*, 1993; Piersma & Lindstrom, 1997). Therefore, it is feasible that a reduction in the masses of the digestive organs also

occurs in captive birds just before they reach peak body mass and this, in part, may explain the plateau in BMR measured at this time (Chapter 3).

5.1.2 Migratory period

The spring peak in BMR generally occurred as body mass and fat mass were decreasing and was not caused by a peak in total lean mass, which tended to precede the peak in body mass by about 6 days and in BMR by around 11 days. If BMR was related only to total lean mass as suggested by Piersma *et al* (1995), the 5% increase in lean mass from 100g to 105g would have resulted in an individual's BMR increasing from 2.0 Watts to 2.1 Watts. The mean BMR increase seen in captive Knot in my study was far greater than this however, with BMR actually increasing on average from 2.00 to 2.88 Watts (44%).

Perhaps peak BMR during this time could be explained by increases in the mass of the most metabolically active lean tissues/organs that make up total lean mass, as suggested by Piersma *et al*, (1996); Weber & Piersma (1996); and Lindstrom, (1997). Variations in organ masses have been shown to explain some of the interspecific and intraspecific differences that exist in BMR, both within mammals (Konarzewski & Diamond, 1995; Speakman & McQueenie, 1996) and birds (Daan *et al*, 1991). However, as body mass decreased rapidly after the spring peak, the mean liver, small intestine, pectoral muscle and heart masses in captive Knot were actually lower (for a given body mass), than those measured during the period of body mass increase or outside the spring migratory period. Due to the obvious difficulty in obtaining carcasses of birds sacrificed as they actually migrate to the breeding grounds, there is very little direct evidence that the mass of any of the flight 'support' organs (Kersten & Piersma, 1987), e.g. kidney, heart, liver, are actually elevated during migratory flight in wild shorebirds. Captive Knot were sacrificed, on average, 15(±4.5SE) days after the peak in BMR. It is therefore feasible that significant increases in various components of total lean mass had occurred at peak BMR but had decreased by the time of death. Piersma & Lindstrom (1997) suggest that seasonal flexibility in organ mass in various

species of birds and mammals may actually take place over a 'matter of days', although a longer time-scale for change (2-3 months) has been suggested to occur in birds (Redig 1989; McWilliams & Karasov, 1998). It is also possible that seasonal changes had occurred in the mass of metabolically active organs and tissues that I did not measure, e.g. kidney. However, I believe that the tissues measured in this study were the most metabolically important, either because they had very high mass-specific metabolic activities or moderate metabolic activities but a large mass.

The maximum rate of decrease in body mass in a captive Knot after the spring peak was 8g/day, the same as the maximum rate of body mass increase. However, the time-scale of body mass loss was far more rapid. For example, Knot "OO" took 33 days from commencement of fattening to peak body mass but only 10 days to return to the pre-fattening level. The only measured parameter that increased by a similar amount to the 44% increase in BMR was SDH activity in the pectoral muscle, which almost trebled between the premigratory period and the migratory period, although due to sampling difficulties already outlined the time-scale of these changes could not be determined. It is also known, however, that citrate synthase activity (another estimator of metabolic activity) is significantly elevated in the pectoral muscle of various migratory passerines when compared to non-migratory conspecifics (Lundgren, 1988).

Little of the variation in BMR amongst individual Knot at a given time of year, i.e. birds in the same physiological state, was caused by differences in body mass, total lean mass or fat mass. This is clearly shown by the example given below of captive Knot sacrificed outside the spring migratory period (see Chapter 4). One individual (WYY) had a BMR over 40% higher than another (BW), although its body mass, total lean mass and fat mass were only 5%, 1% and 11% respectively higher (see Table 4.3.1.1.). Both individuals had very similar liver, pectoral muscle and heart masses with "BW" actually having a 20% greater mass of the small intestine. However, the combined SDH activity for the four tissues was almost 20% higher for individual "WYY" than "BW".

Similarly, for two individuals sacrificed as they decreased in body mass during spring, although Knot "OO" had a BMR some 13% lower than Knot "GG", it had a body mass, total lean mass and fat mass some 30%, 27% and 31% respectively higher. Both individuals had similar liver and heart masses to one another, but "OO" had a 10% smaller pectoral muscle mass than "GG" but a 25% larger small intestine mass. The total combined SDH activity for the four tissues was however almost 20% higher for individual "GG" (which had the higher BMR) than for individual "OO", although much of this difference could be explained by "GG" having a 5-fold greater SDH activity in the heart than that measured in "OO".

5.1.3 What is the function of the spring peak in BMR?

The peak in BMR seen in captive Knot, as mentioned previously, occurs at approximately the time wild birds undergo migratory flights to the breeding grounds. In section 5.1.2 the possible mechanisms causing this spring peak in BMR were discussed. I will now examine the possible adaptive value of this peak in BMR.

Metabolic scope has been described as the amplitude between which the metabolic rate of an individual can vary (Fry, 1947). Drent and Daan (1980) calculated that the 'optimal working capacity' (later termed the maximum sustainable metabolic rate, MMR) of breeding passerines was around four times BMR. They suggested that a daily energy expenditure greater than 4 times BMR could not be maintained for a period greater than 1-2 days, without inflicting some subsequent fitness cost on that individual. More recently, Bryant and Tatner (1991) calculated that the ratio between BMR and MMR, in a sample of 28 passerine species, actually varied between +1 to +7 (mode of 3). There is some evidence that an elevated daily energy expenditure during chick rearing does indeed increase the risk of subsequent mortality in both Kestrels *Falco tinnunculus* (Daan *et al*, 1996) and in Northern house-martins *Delichon urbica* (Bryant, 1991).

There has been much recent debate as to whether a relationship between BMR and MMR actually exists in birds and mammals, although theories assuming such a relationship are widespread in the literature, e.g. Bennett and Ruben's (1979) theory on the evolution of endothermy, and Kersten & Piersma's (1987) 'Energetic Margin Hypothesis' (1987) which suggested that the higher BMRs than expected for a given body mass in certain shorebird species were simply a metabolic consequence of an energetically expensive way of life. Koteja (1987), however, suggested that the assumption of a relationship existing between BMR and field metabolic rate did not have sound experimental support in animals, while Meerlo *et al* (1997) found no evidence of an intraspecific or intra-individual relationship between BMR and daily energy expenditure (DEE) in the Field vole *Microtus agrestis*. Recently, Ricklefs *et al* (1996) concluded that a relationship between BMR and MMR exists in mammals but not in birds. However, Dutenhoffer & Swanson (1996) found that an interspecific relationship existed ($r = 0.861$) between BMR and maximal cold-induced 'summit' metabolism in 10 species of passerines, even when the effects of body mass and phylogeny were removed. They did admit, however, that as birds do not use the full aerobic potential of their muscles during thermogenesis, unlike during exercise-induced MMR (Gessaman & Nagy, 1988), the actual summit metabolic rate measured in their study will not be the actual MMR.

If a fixed relationship does exist between BMR and MMR, any increase in BMR seen in captive Knot during spring indicates an increase also in MMR, although the direction of causality remains unclear (Ricklefs, 1996) i.e. it is not known whether a high MMR 'pulls up' BMR, or whether a high BMR 'pushes up' MMR. Either way, an increase in BMR during this time will indicate an elevation of MMR and therefore an increase in the metabolic scope of that individual. This will thus enable Knot to cope energetically with the demands of long-distance migration, with less chance of deleterious effects on fitness.

The work reported in this thesis confirms that the BMR of captive Knot varies considerably on a seasonal basis, (as suggested in outline by Cadee, 1994; Piersma

et al, 1995) and enables me to reject the hypotheses that seasonal and intraspecific variation in BMR are simply a consequence of variation in total lean mass (as suggested by Piersma *et al*, 1995), in total body mass or in total fat mass. My work indicates that although some of the variation in BMR both within and amongst individual captive Knot may be caused by variation in the mass of various metabolically active lean tissues, (claimed to be the sole source of variation in BMR, intraspecifically, in captive Knot losing mass during early summer, (Weber & Piersma, 1996)), variation in BMR is also caused by differences in the *metabolic intensity per gram* of organs with a considerable mass, e.g. pectoral mass.

Since variation in the metabolic activity of various tissues and organs leads to variation in BMR and so perhaps also in MMR, the spring increase in BMR from this increase in metabolic activity may also be advantageous to Knot during migratory flight. Premigratory fattening, leading to an increase in body mass, is known to lead to a rapid increase in the flight costs per unit distance (Pennycuick, 1978). In order to meet these increased power requirements, an individual bird can increase the total aerobic capacity of its flight muscles by:
(i) An increase solely in pectoral muscle mass, as reported to take place in premigratory Grey catbirds *Dumetella carolinensis* by Marsh (1981), or
(ii) An increase in the metabolic activity per gram of that flight muscle (Lundgren, 1988), or (iii) a combination of both methods.

Method (i) will increase total lean mass and hence body mass significantly, but method (ii) will not. Since migratory flight is energetically very expensive, there should be a strong selective pressure to minimise the mechanical power required to fly (Norberg, 1996). I suggest that if the organs and tissues involved in migration increase chiefly in their metabolic activity (rather than in mass as suggested by Kersten & Piersma, 1987; Piersma *et al*, 1995; Weber & Piersma, 1996), this will minimise the mechanical power needed for flight because total lean mass and total body mass increases will be minimised.

The enzyme activity of various tissues cannot vary unchecked, however. Hochachka *et al* (1988) suggest that 3 main intrinsic building blocks are required for elevating aerobic output in skeletal muscle: (i) Myofilaments; (ii) Mitochondria and aerobic enzymes (iii) Sarcoplasmic reticulum. They argue that a point is reached when any further increase in any one of these blocks will impair the performance of the other two, and hence overall aerobic output. Therefore, there may come a point when some increase in cell number or cell size and hence mass of various organs is necessary. This may be why limited pectoral muscle hypertrophy is evident in several premigratory birds (Evans, 1969; Evans *et al*, 1992), and why premigratory Knot in my study exhibited an increase in liver mass. An increase preferentially in the metabolic activity of various organs and tissues may be an energetically 'prudent' way in which migratory birds can increase their metabolic ceiling and metabolic scope, without increasing the energetic costs of flight. A reduction in flight costs will in turn allow an individual Knot to fly further for a given mass of fuel.

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Appendix I.

Table I.1- I.5: Date of capture, experimental procedures carried out and the fate of all Knot referred to throughout this thesis.

Table I.1: Date of capture

Batch	Date of capture	Number of Knot taken from wild
1	2/12/94	11
2	1/3/95	5
3	6/11/95	22
4	4/3/96	5
5	7/1/97	10
6*	*Feb 1994*	7
TOTAL number of Knot used during this thesis		**60**

** Individual Knot captured by I. Scott, prior to the start of this thesis.*

Table I.2: Fate of individuals caught on 2/12/94 (Batch 1).

	No. of birds used in each experiment	
Experiment 1.	*19*	Seasonal variation in BMR. (N = Total number of BMR measurements per individual)
Experiment 2.	*40*	Intraspecific variation in BMR
Experiment 3.	*20*	Production and testing of species-specific TOBEC calibration curve
Experiment 4.	*8*	Effect of weight manipulation on body mass, body composition and BMR.
Experiment 5.	*27*	Enzymatic assays
Experiment 6.	*12*	Mitochondrial volume composition
Experiment 7.	*8*	Effects of captivity on BMR
Experiment 8.	*49*	Effects of captivity on body composition and comparison of organ masses between wild and captive Knot.

Age at capture = 1 (Juvenile Knot) Age at capture = 2 (Adult bird)

Batch number	ID & (Age at capture)	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Date sacrificed
1	BW (2)		✓			✓			✓	1/5/96
1	OO (2)	✓ (33)	✓			✓		✓	✓	6/7/96
1	GG (2)	✓ (31)	✓			✓		✓	✓	4/7/96
1	YY (2)	✓ (18)	✓			✓		✓	✓	24/5/96
1	LL (2)	✓ (13)								Died Aug. 1995
1	WW (2)	✓ (30)						✓		20/06/97
1	GY (1)	✓ (21)	✓			✓			✓	28/6/96
1	GF (1)	✓ (32)	✓			✓		✓	✓	7/6/96
1	GO (1)	✓ (23)	✓			✓	✓	✓	✓	12/6/96
1	GL (1)	✓ (22)	✓			✓	✓	✓	✓	28/6/96
1	GW (1)	✓ (33)	✓			✓	✓	✓	✓	30/5/97

Table I.3: Fate of individuals captured in batch 2 (1/3/95) and batch 3 (6/11/95).

Batch number	ID & Age at capture	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp.8	Date sacrificed
2	16 (2)								✓	1/3/95
2	17 (2)								✓	1/3/95
2	18 (2)								✓	1/3/95
2	19 (2)								✓	1/3/95
2	20 (2)								✓	1/3/95
3	LW (2)		✓	✓		✓			✓	15/12/95
3	LG (2)		✓			✓			✓	1/5/96
3	LY (2)		✓			✓			✓	26/6/96
3	WO (2)		✓	✓		✓			✓	15/12/95
3	YG (2)		✓			✓			✓	24/5/96
3	YYY (2)		✓	✓		✓			✓	14/12/95
3	YW (2)		✓	✓		✓			✓	14/12/95
3	AA (2)		✓			✓				28/5/96
3	WGG (1)	✓ (16)	✓			✓	✓		✓	19/5/97
3	WGL (1)	✓ (20)	✓			✓			✓	25/6/96
3	WYG (1)	✓ (18)	✓	✓					✓	17/06/97
3	WWW (1)	✓ (11)	✓						✓	29/5/97
3	WYY (1)	✓ (13)	✓			✓			✓	3/7/96
3	WLG (1)	✓ (15)	✓			✓	✓		✓	28/5/97
3	WGY (1)	✓ (16)	✓	✓					✓	24/6/97
3	WG (1)	✓ (15)	✓	✓						24/6/97
3	WLL (1)	✓ (17)	✓			✓	✓		✓	28/5/97

Table I.4: Fate of individuals captured in batch 3 (6/11/95), batch 4 (4/3/96) and batch 4 (7/1/97).

Batch number	ID & (Age at capture)	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Date sacrificed
3	01 (2)								✓	6/11/95
3	02 (2)								✓	6/11/95
3	06 (2)								✓	6/11/95
3	07 (2)								✓	6/11/95
3	21 (2)								✓	6/11/95
4	A (2)					✓			✓	4/3/96
4	B (2)					✓			✓	4/3/96
4	C (2)					✓			✓	4/3/96
4	D (2)					✓			✓	4/3/96
4	E (2)					✓			✓	4/3/96
5	JLL (2)		✓	✓	✓				✓	27/6/96
5	JLW (2)		✓	✓	✓				✓	3/7/97
5	JWG (2)		✓		✓				✓	1/7/97
5	JWW (2)		✓		✓				✓	25/6/97
5	JYG (2)		✓	✓	✓				✓	27/6/97
5	JYY (2)		✓	✓	✓				✓	3/7/97
5	RGG (2)		✓	✓	✓				✓	3/7/97
5	BGG (2)		✓	✓	✓				✓	1/7/97
5	JWY (2)								✓	23/05/97
5	BYY (2)								✓	Died April 97

Table I.5: Fate of individuals (batch 6) that were caught in Feb 1994 by I. Scott.

Batch number	ID & (Age at capture)	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp.7	Exp. 8	Date sacrificed
6	YE (2)		✓	✓			✓			7/6/95
6	WH (2)		✓	✓			✓			7/6/95
6	WL (2)			✓			✓			5/12/94
6	GN (2)			✓			✓			6/12/94
6	OR (2)			✓			✓			7/12/94
6	YL (2)			✓			✓			8/12/94
6	OG (2)			✓						21/1/95

Appendix I

Figures I.1.-I.6: Seasonal variation in mean monthly body mass, predicted total lean mass and predicted total fat mass of adult and juvenile captive Knot. Error bars indicate 1 x SE.

Figure I.1: Adult mean monthly body mass (n=14 in all cases).

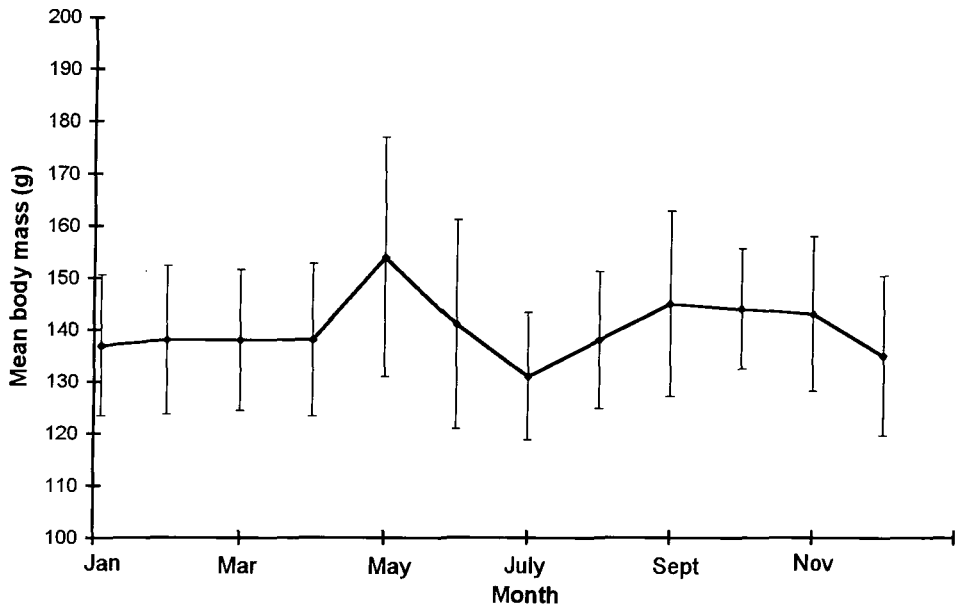


Figure I.2: Juvenile mean monthly body mass (n=10 in all cases).

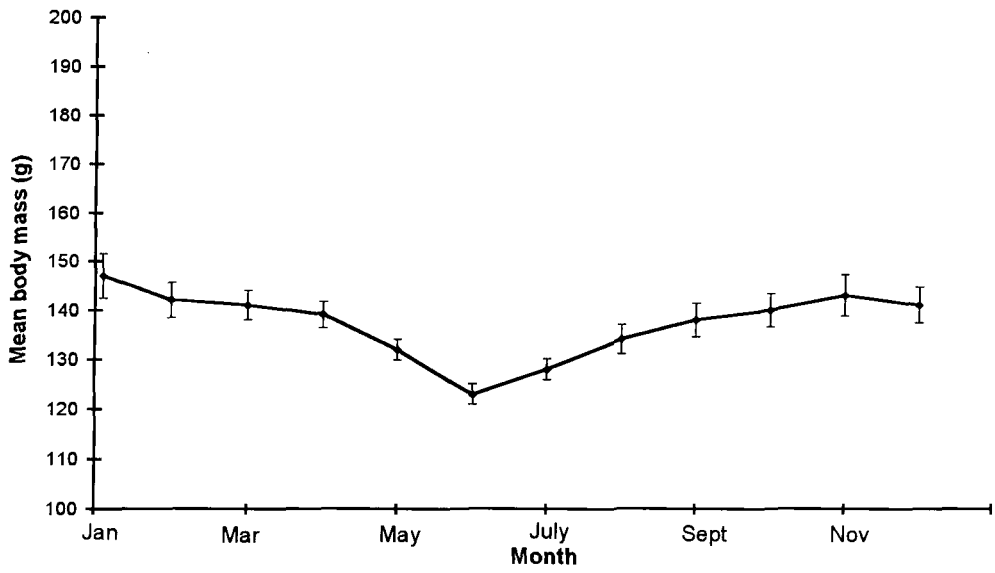


Figure I.3: Adult mean monthly predicted total lean mass (n=14).

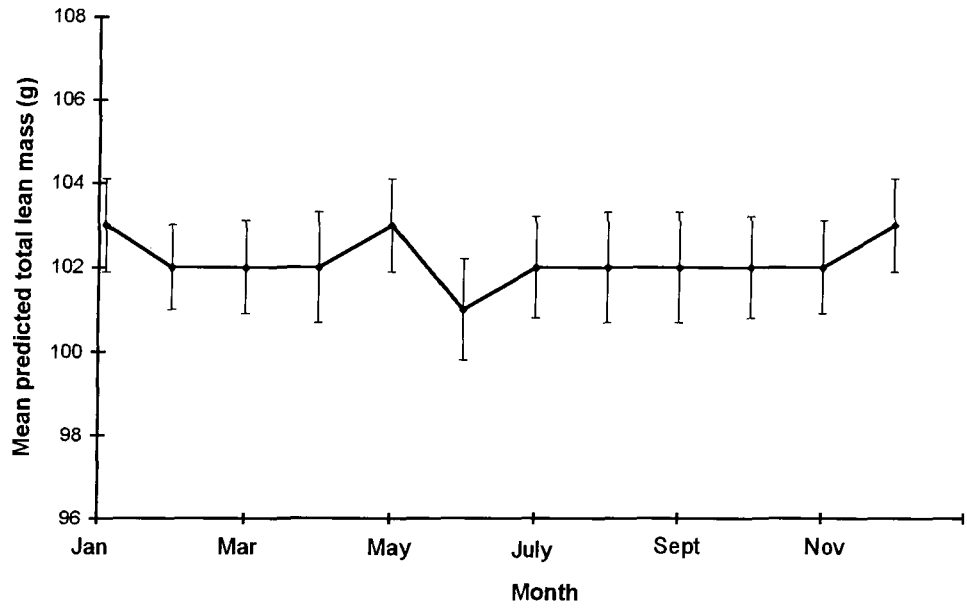


Figure I.4: Juvenile mean monthly predicted total lean mass (n=10).

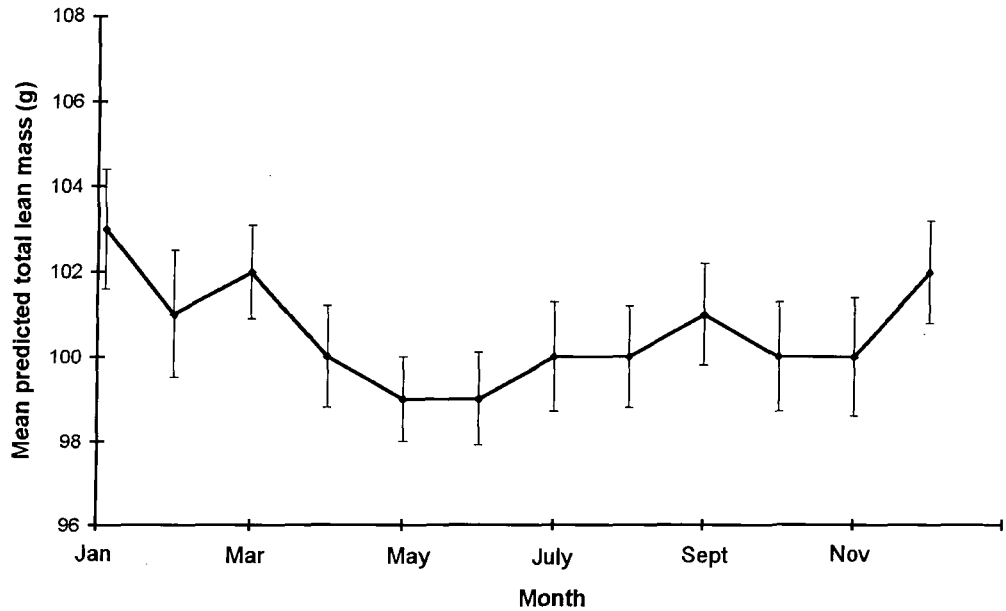


Figure I.5: Adult mean monthly predicted total fat mass (n=14).

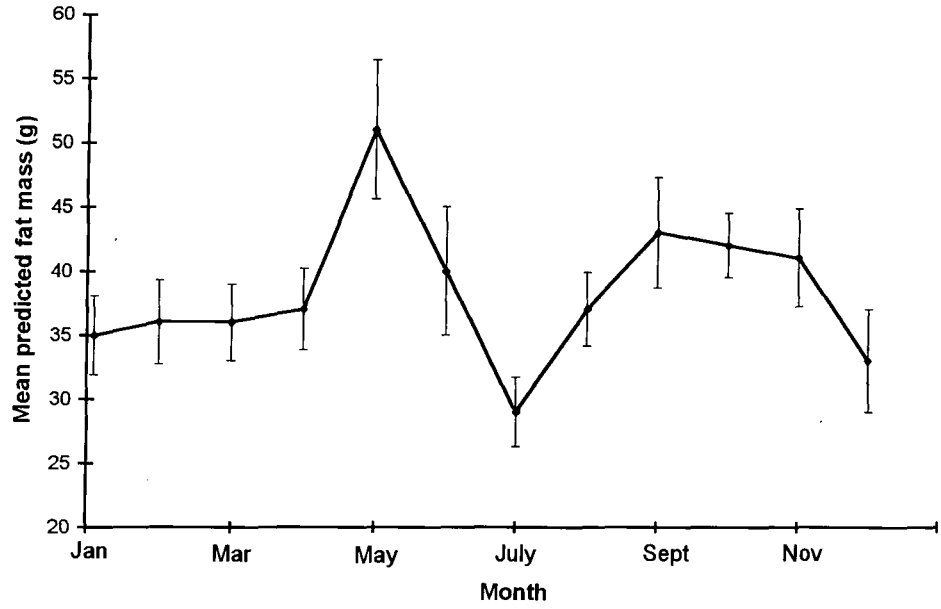
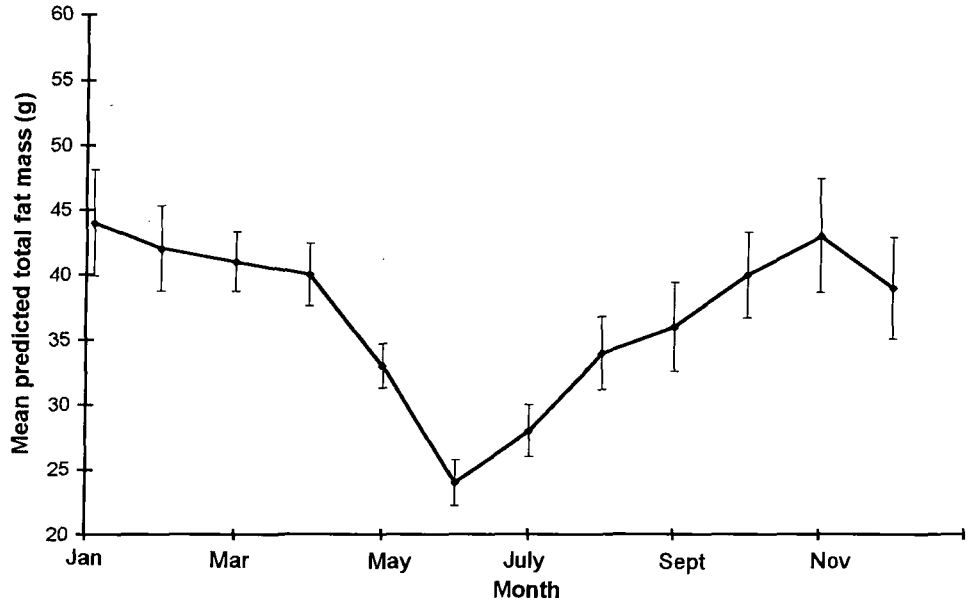


Figure I.6: Juvenile mean monthly predicted total fat mass (n=10).



Figures I.A-I.Y-: Seasonal changes in body mass and body mass components in adult and juvenile Knot. Upper line denotes overall body mass.

M= Prenuptial moult PM= Primary moult Day 0= January 1994

Adult- Entered captivity as adult
Juvenile-Entered captivity as first-year bird

Figure I.A: Adult Knot LL

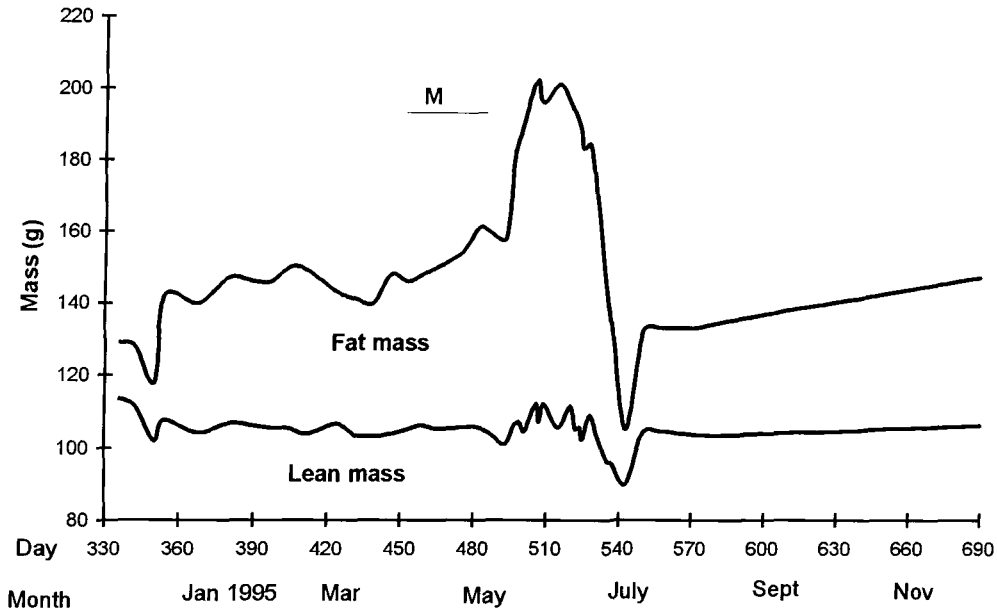


Figure I.B: Adult Knot WW

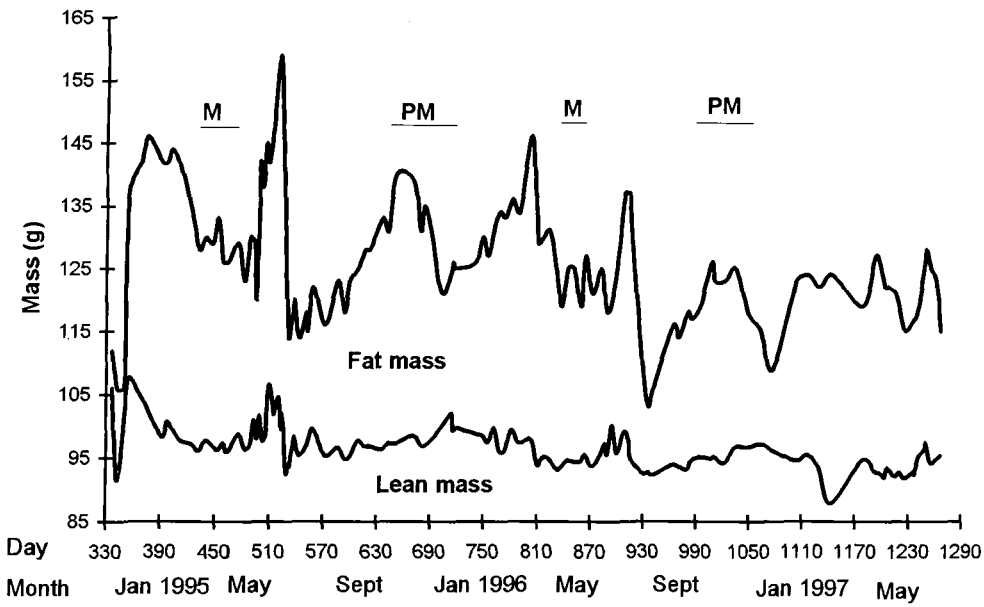


Figure I.C: Adult Knot YY

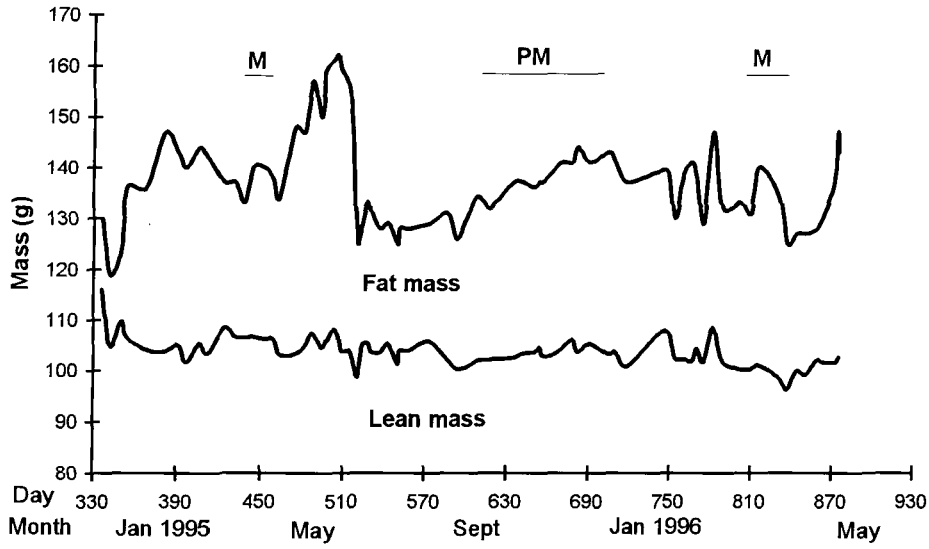


Figure I.D: Adult Knot LY

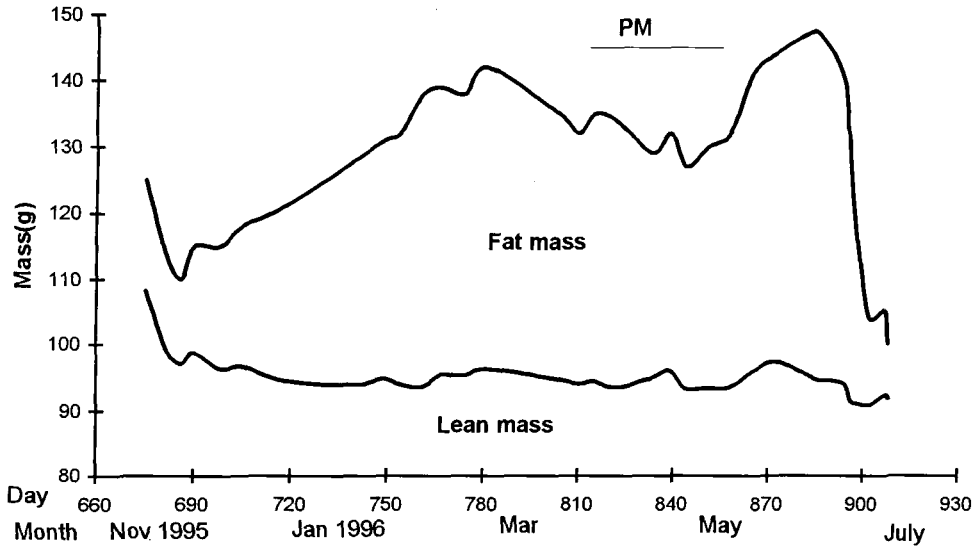


Figure I.E: Adult Knot LG

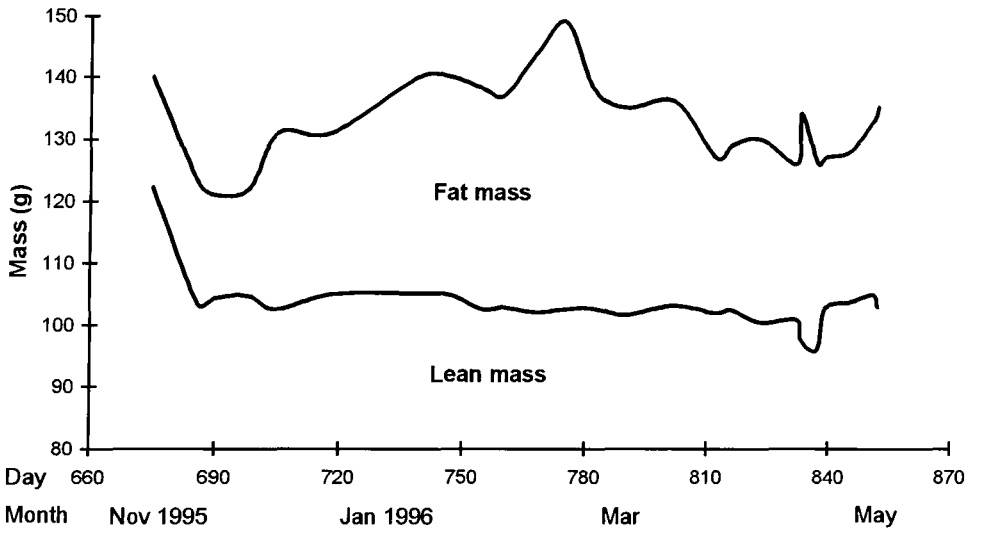


Figure I.F: Adult Knot YG

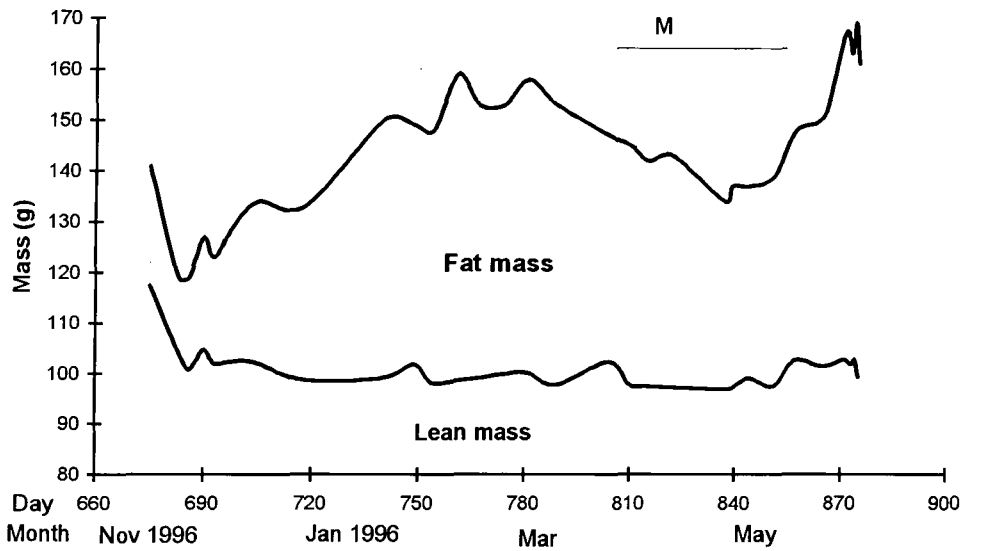


Figure I.G: Adult Knot BW

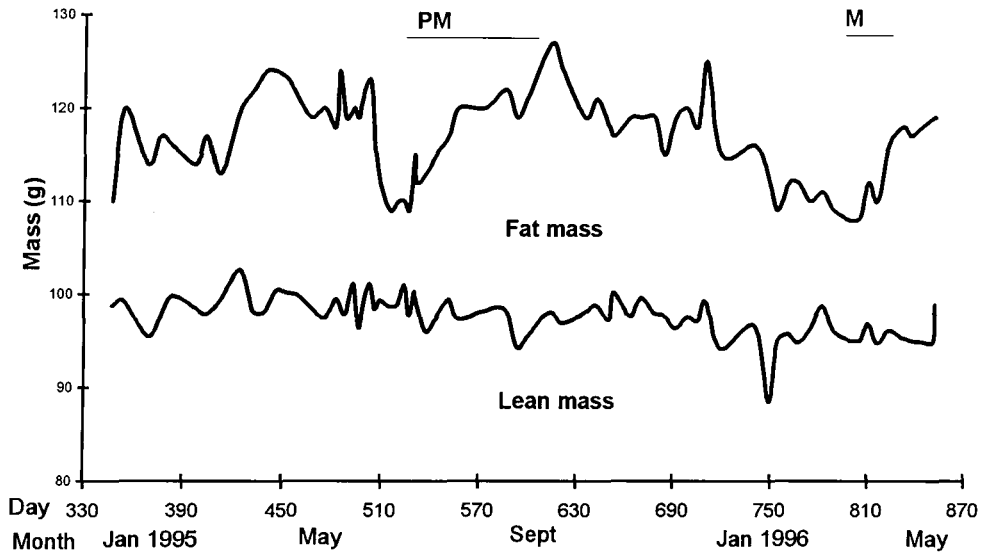


Figure I.H: Adult Knot WH

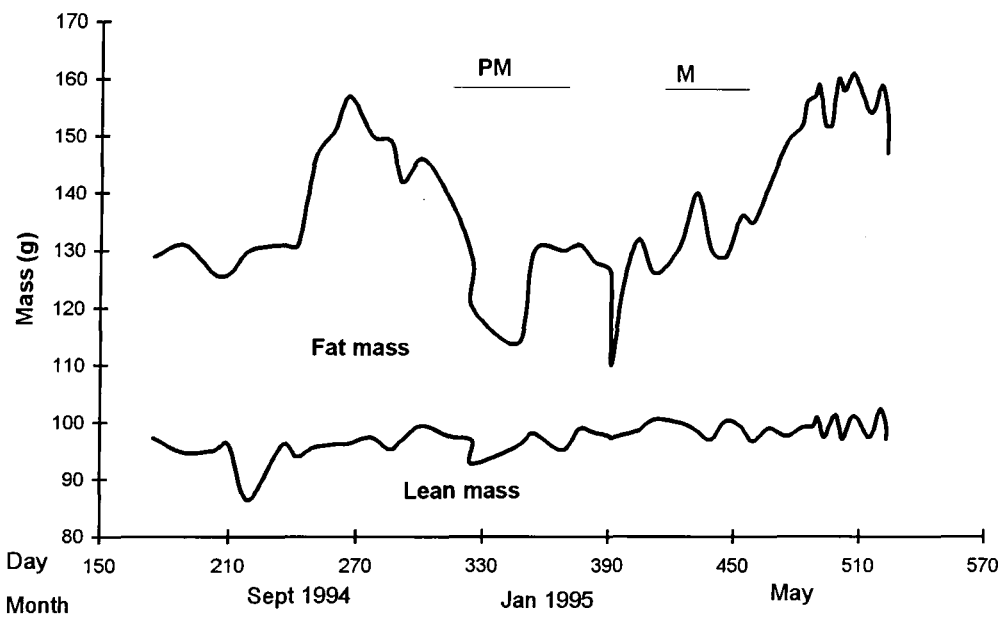


Figure I.I: Adult Knot YE

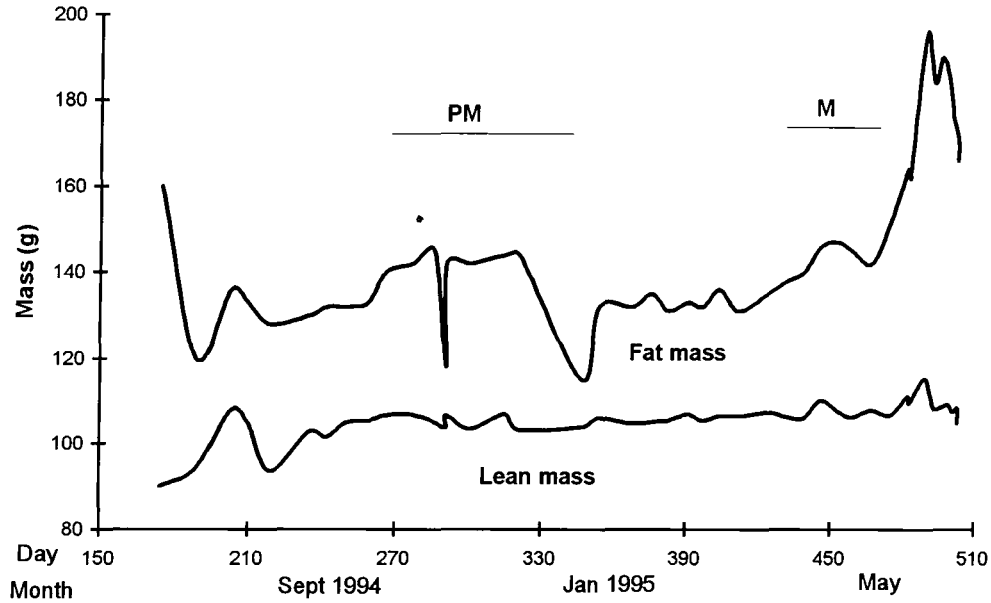


Figure I.J: Adult Knot YW

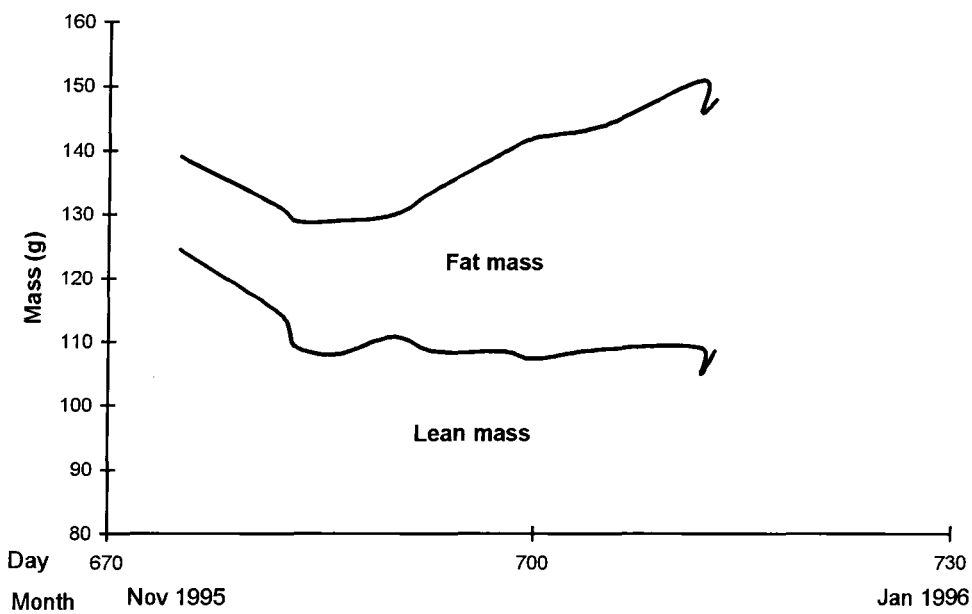


Figure I.K: Adult Knot LW

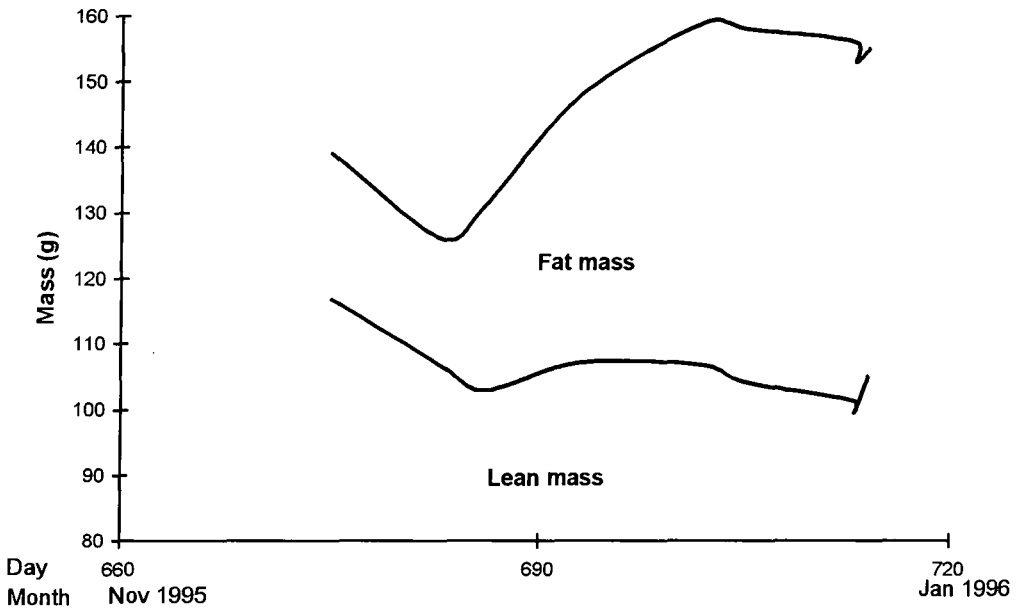


Figure I.L: Adult Knot WO

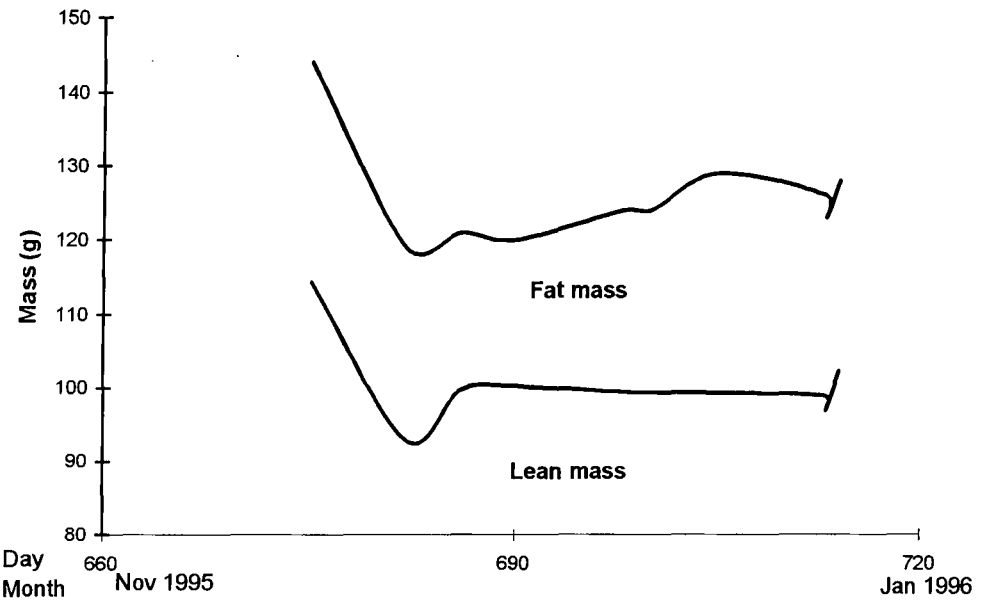


Figure I.M: Adult Knot YYY

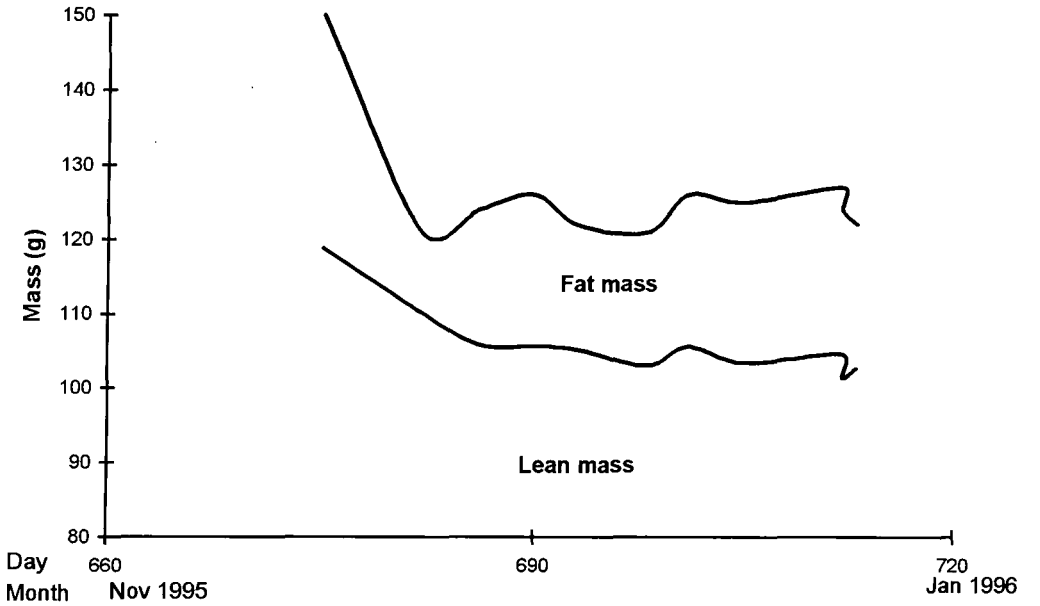


Figure I.O: Adult Knot AA

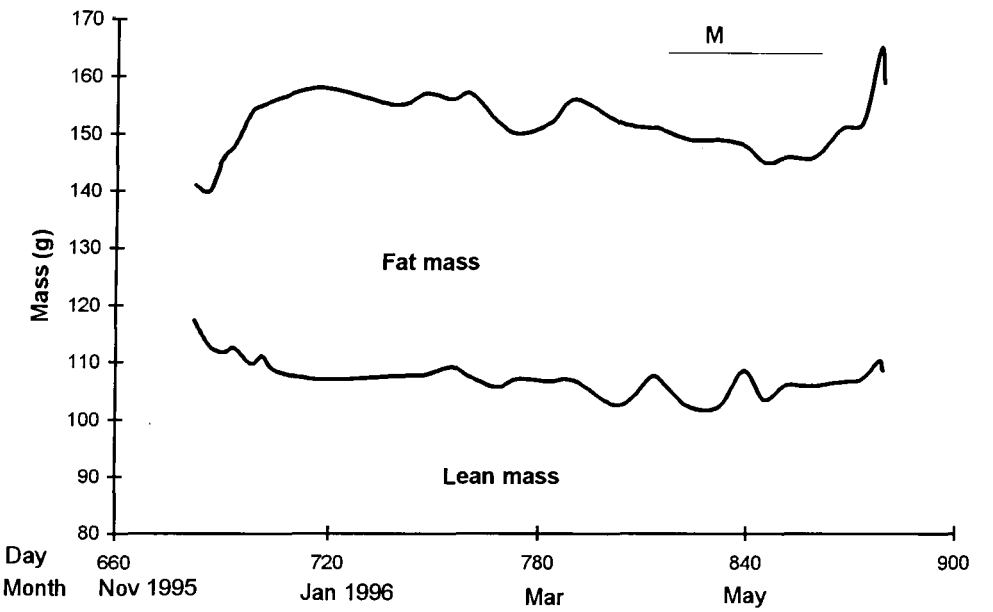


Figure I.P: Juvenile Knot GW

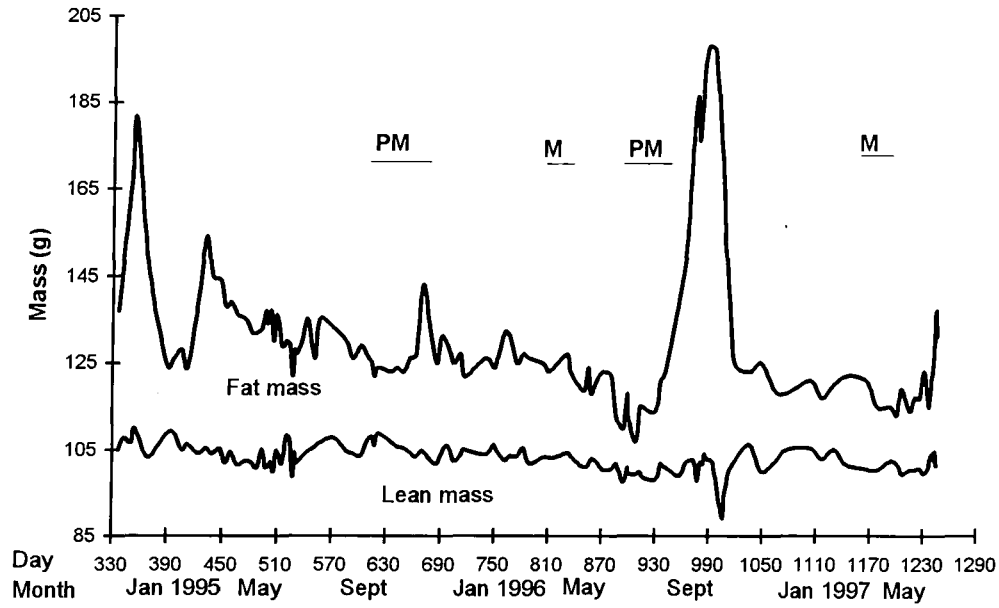


Figure I.Q: Juvenile Knot GY

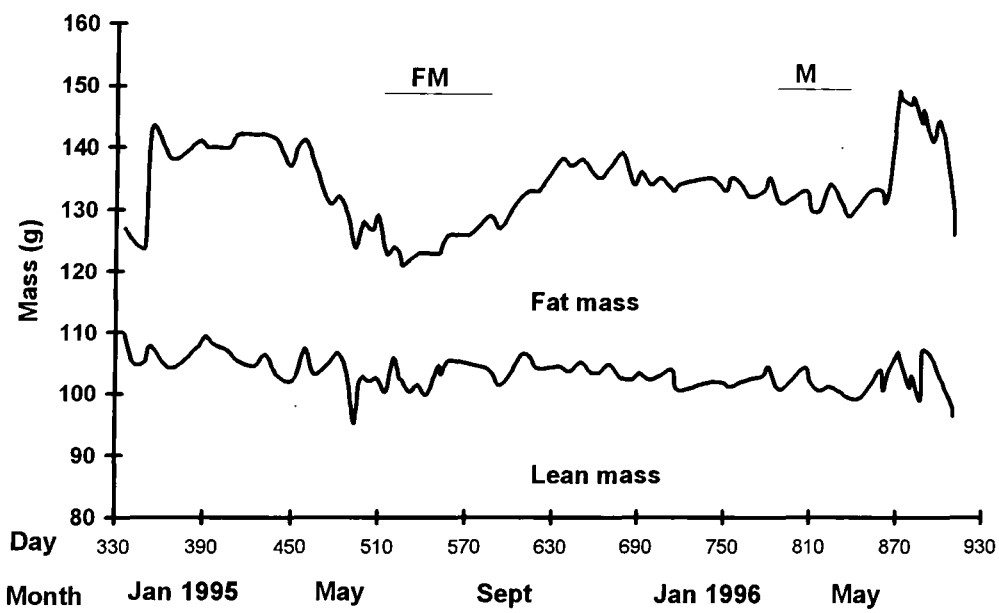


Figure I.R: Juvenile Knot WGG

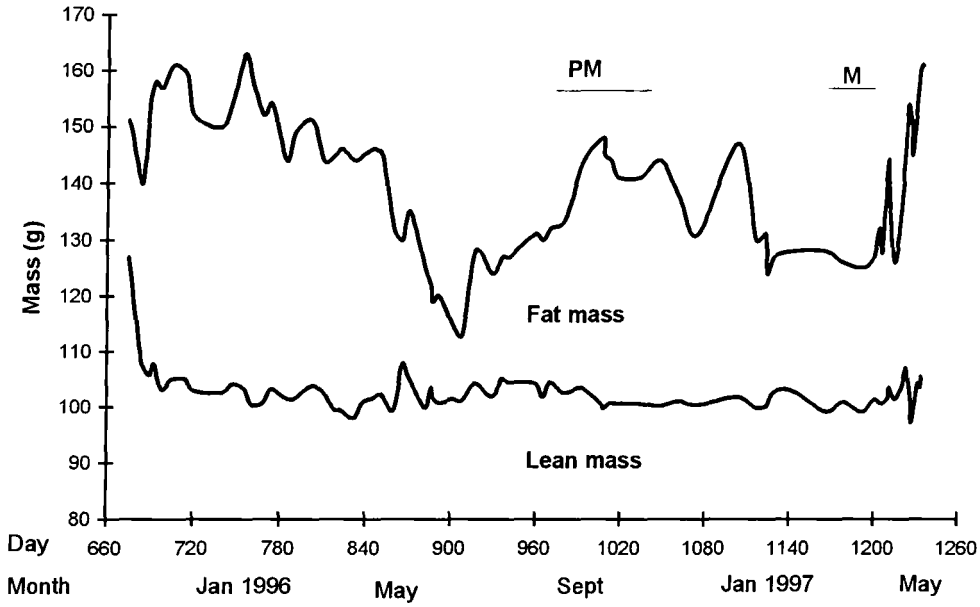


Figure I.S: Juvenile Knot WGY

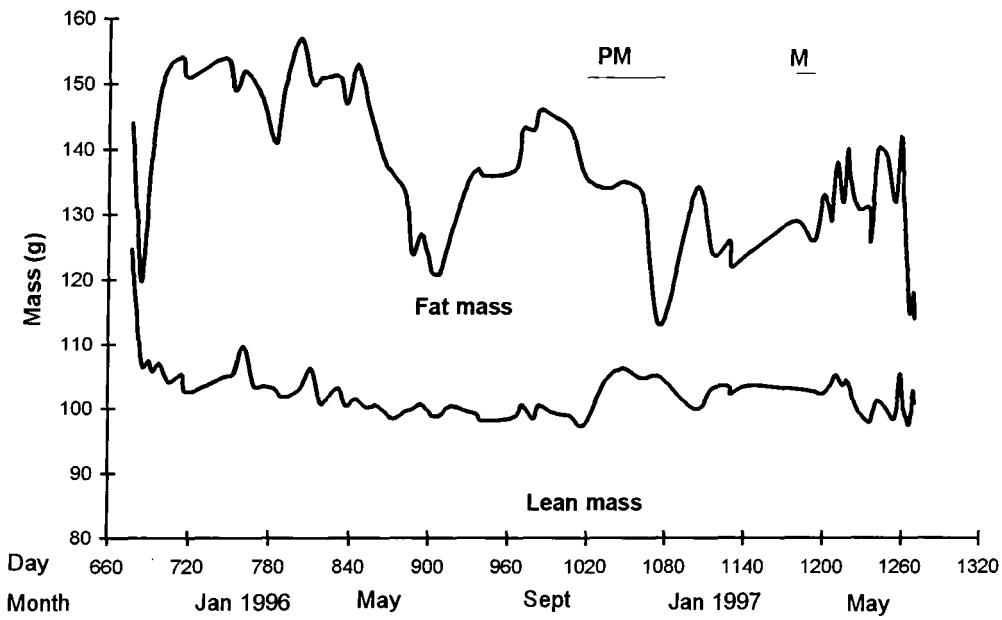


Figure I.T: Juvenile Knot WLL

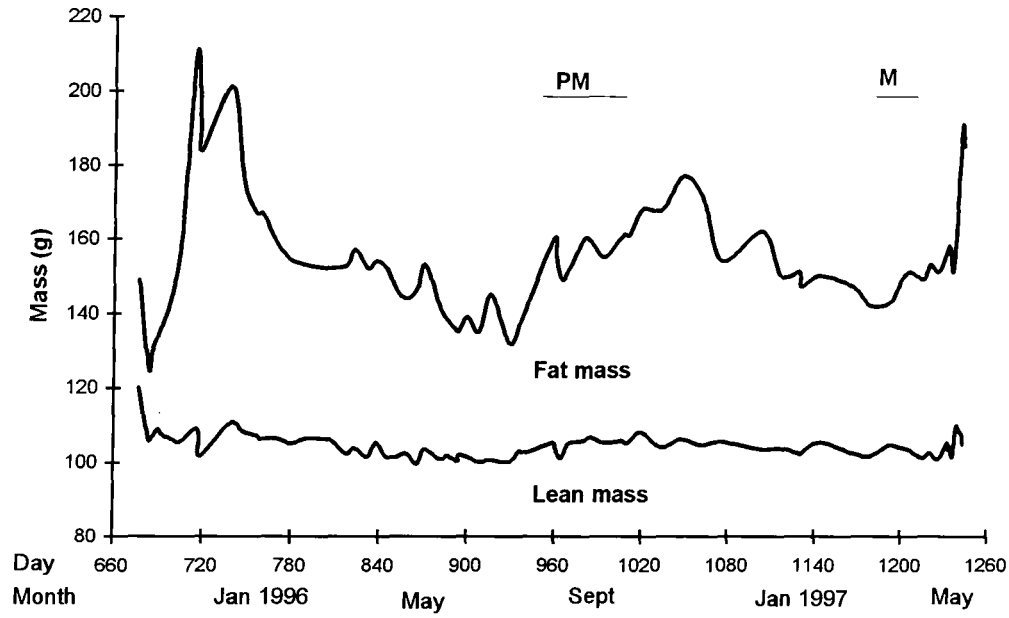


Figure I.U: Juvenile Knot WWW

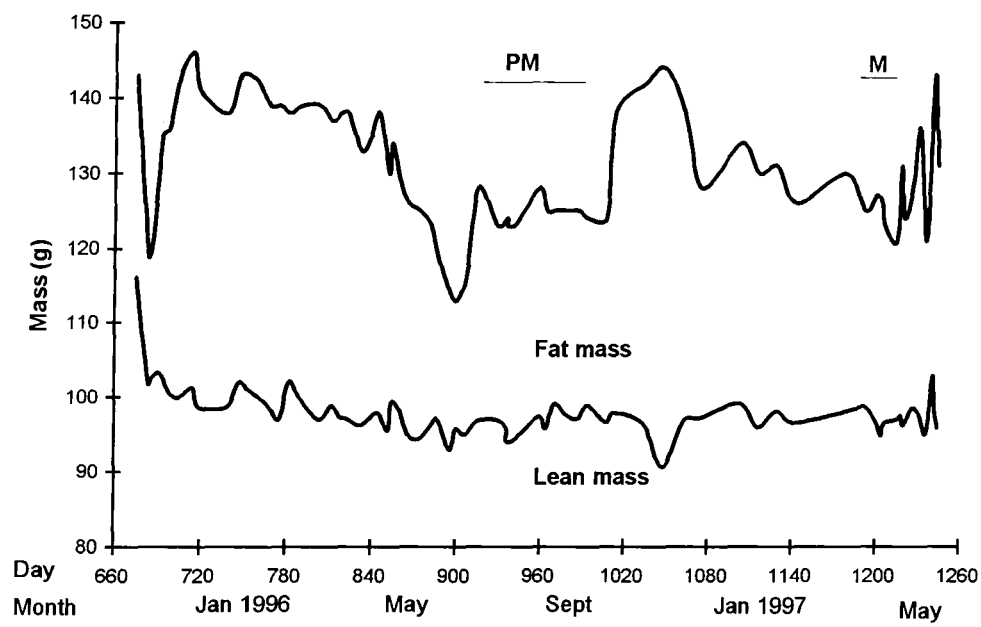


Figure I.V: Juvenile Knot WYG

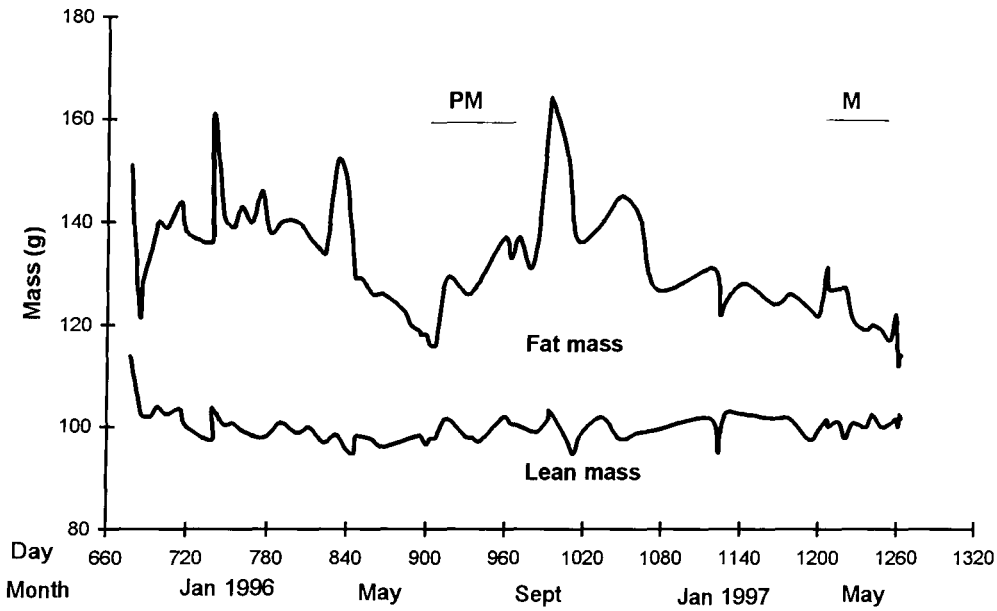


Figure I.W: Juvenile Knot WLG

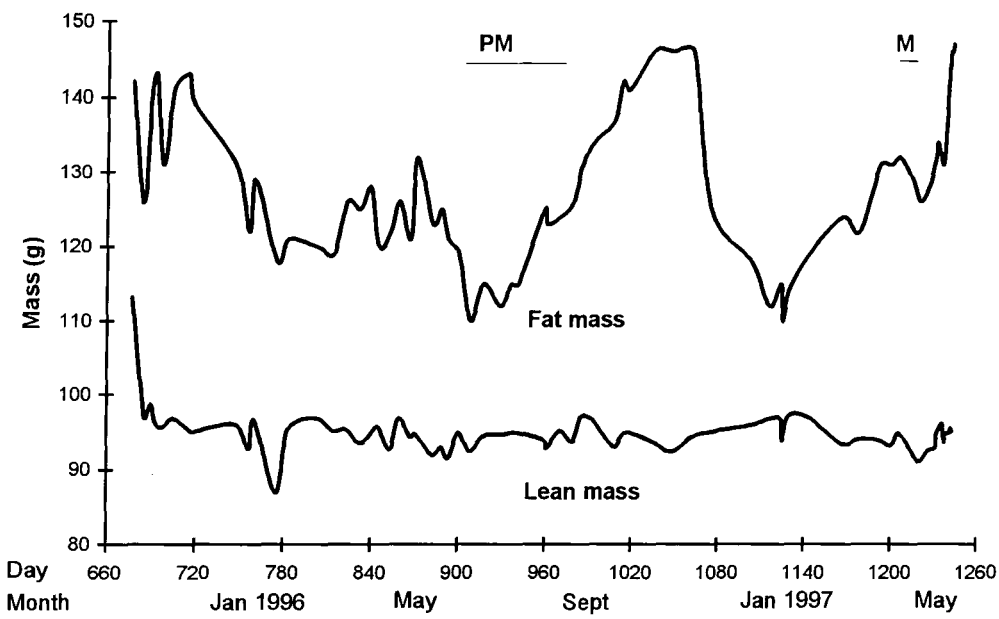


Figure I.X: Juvenile Knot WG

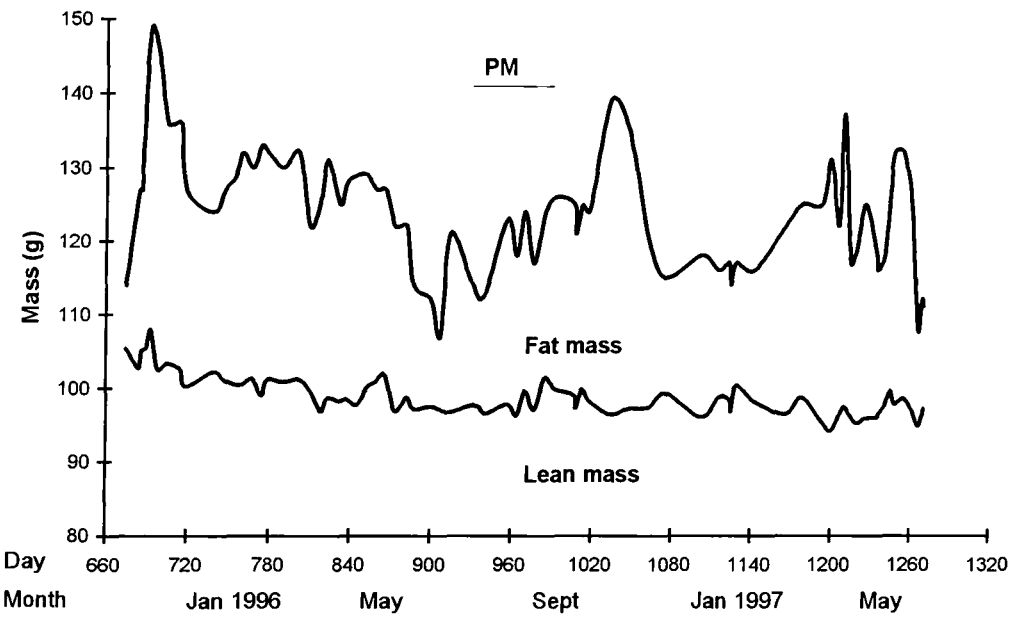
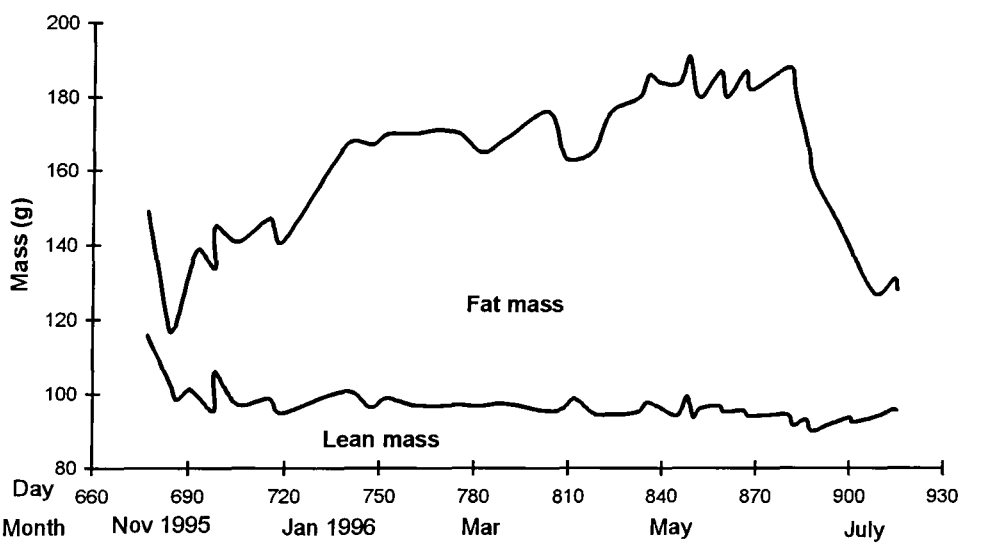


Figure I.Y: Juvenile Knot WYY



Figures I.1A-I.1T :Seasonal variation in total body mass and basal metabolic rate (BMR) in adult and juvenile Knot

Day 0= Jan 1994.

Figure I.1A: Adult Knot LL

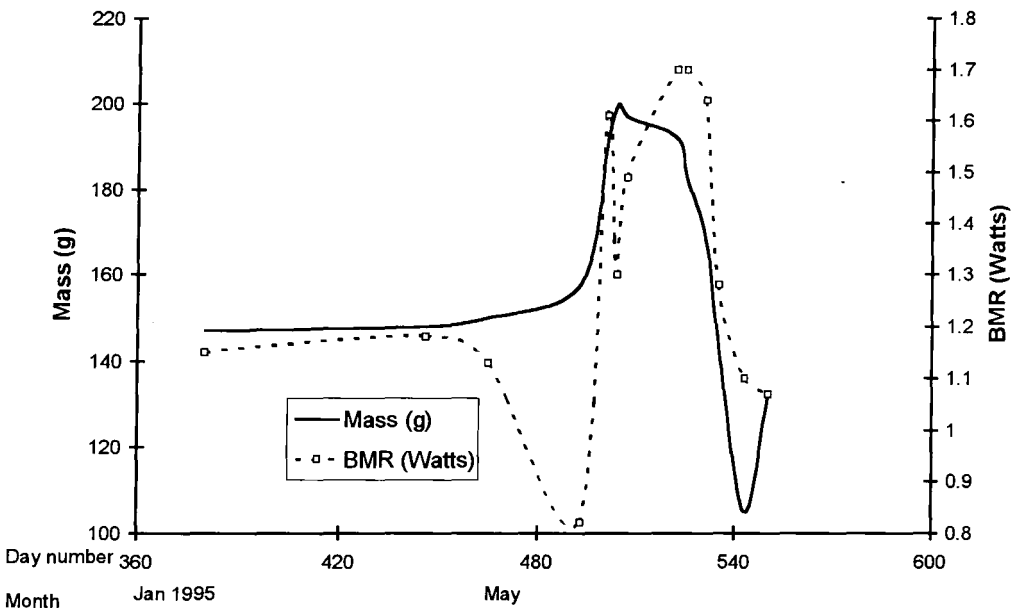


Figure I.1B: Adult Knot WW

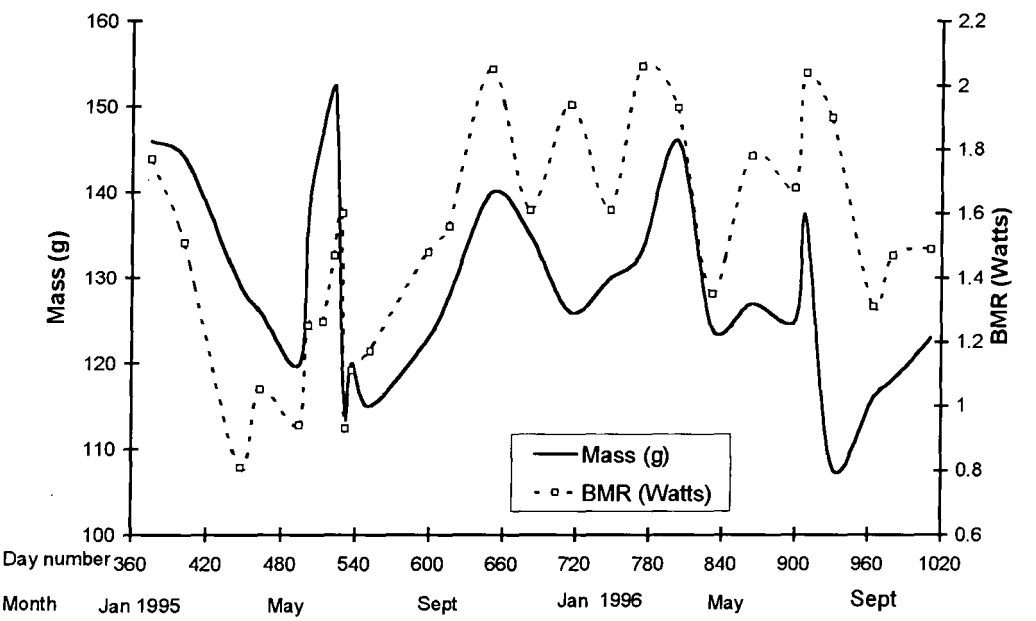


Figure I.1C: Adult Knot YY

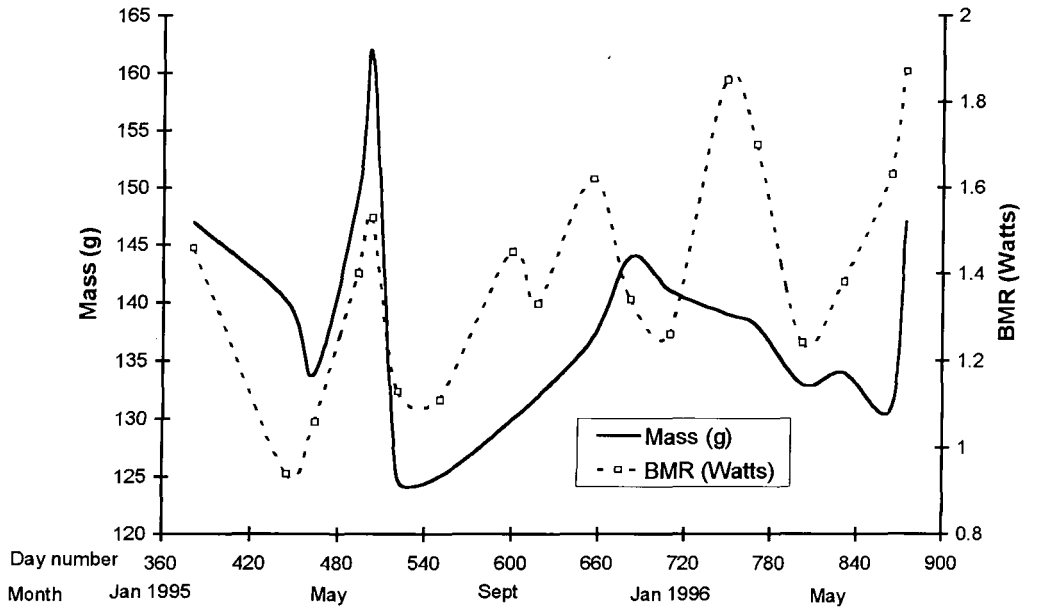


Figure I.1D: Adult Knot LY

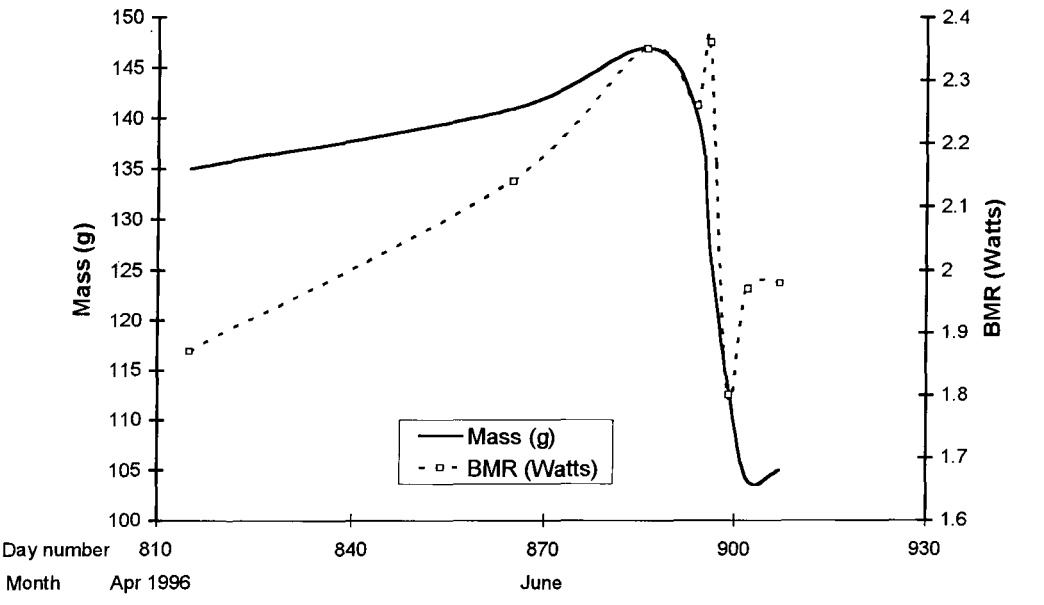


Figure I.1E: Adult Knot LG

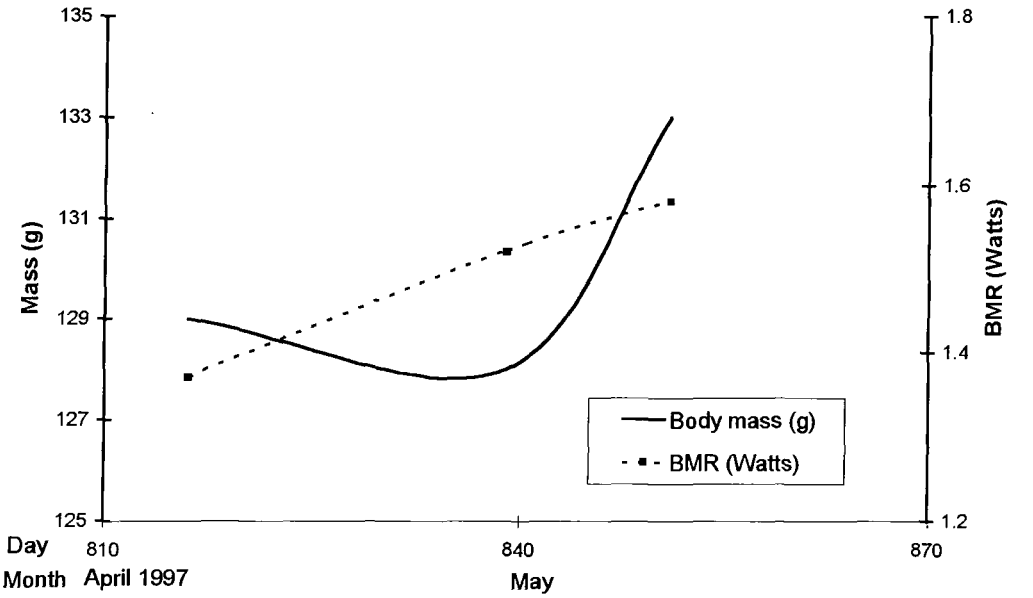


Figure I.1F: Adult Knot YG

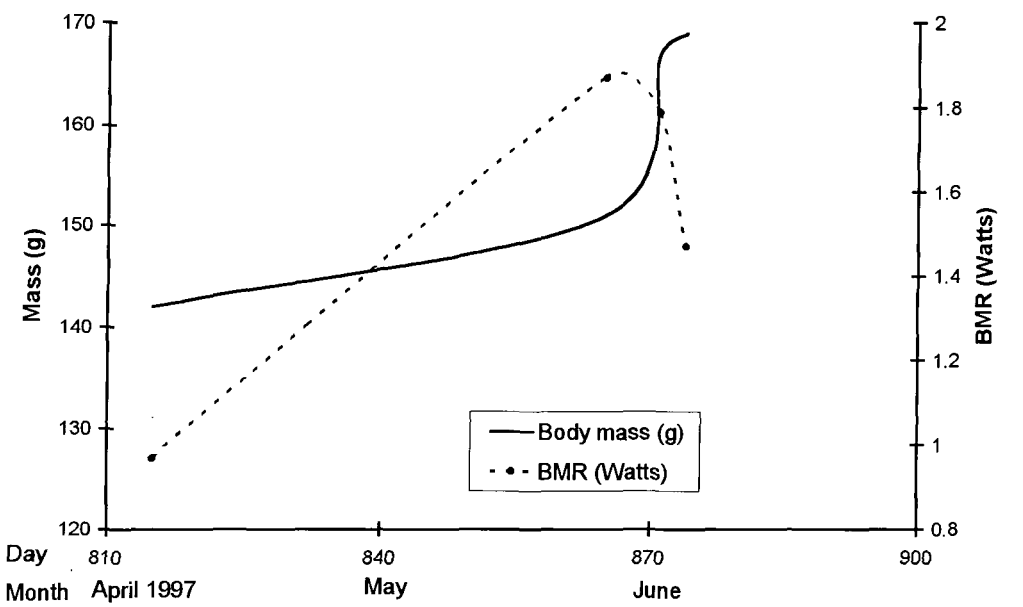


Figure I.1G: Adult Knot BW

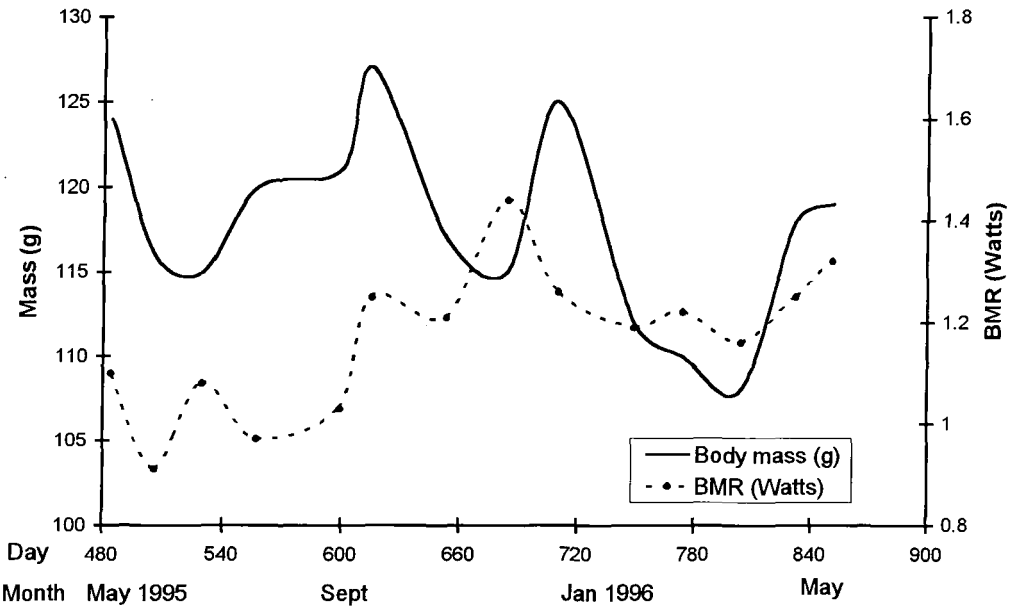


Figure I.1H: Adult Knot WH

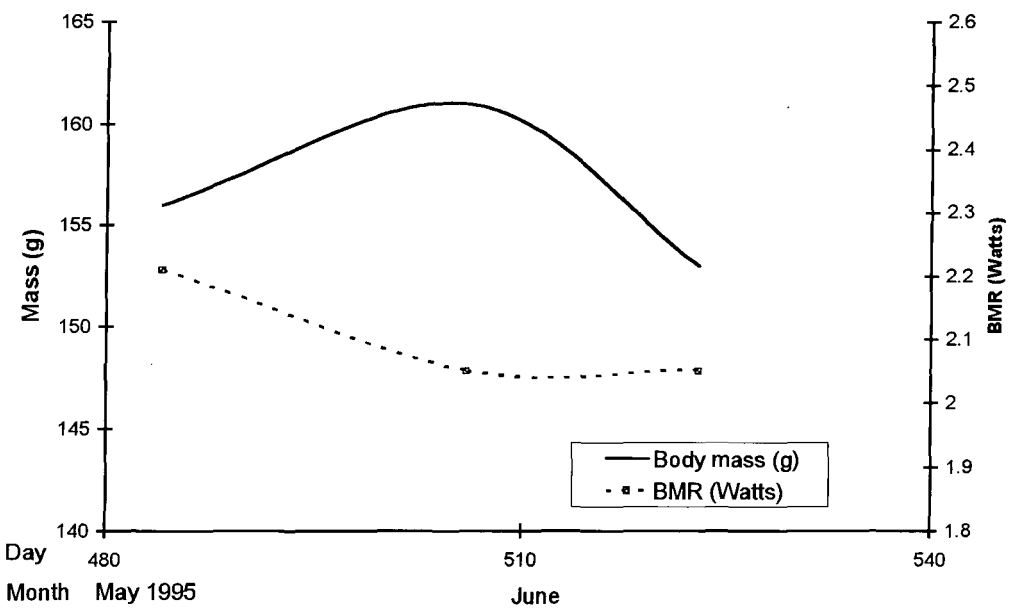


Figure I.1I: Adult Knot Yellow

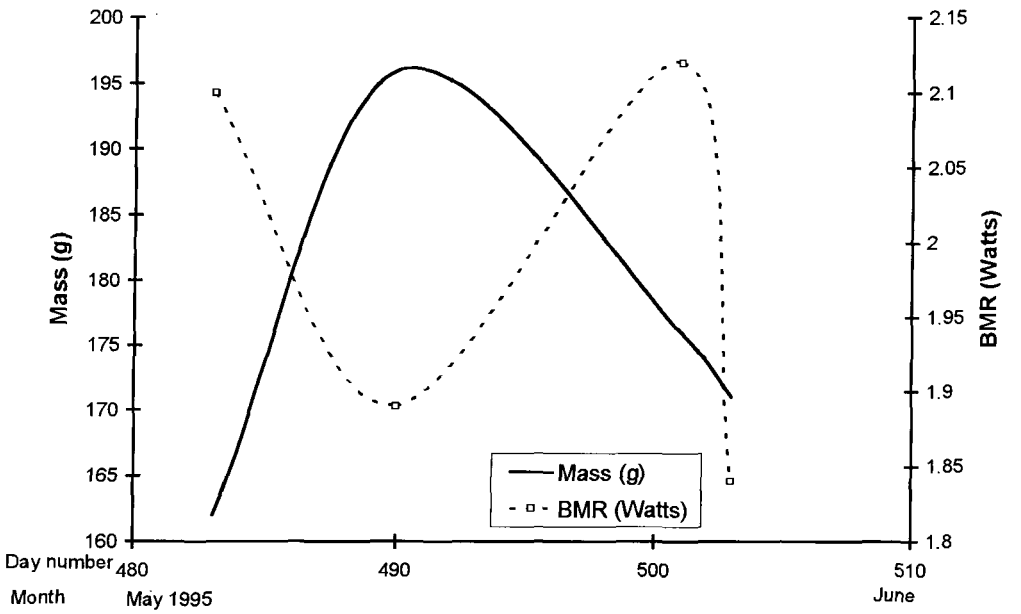


Figure I.1J: Adult Knot AA

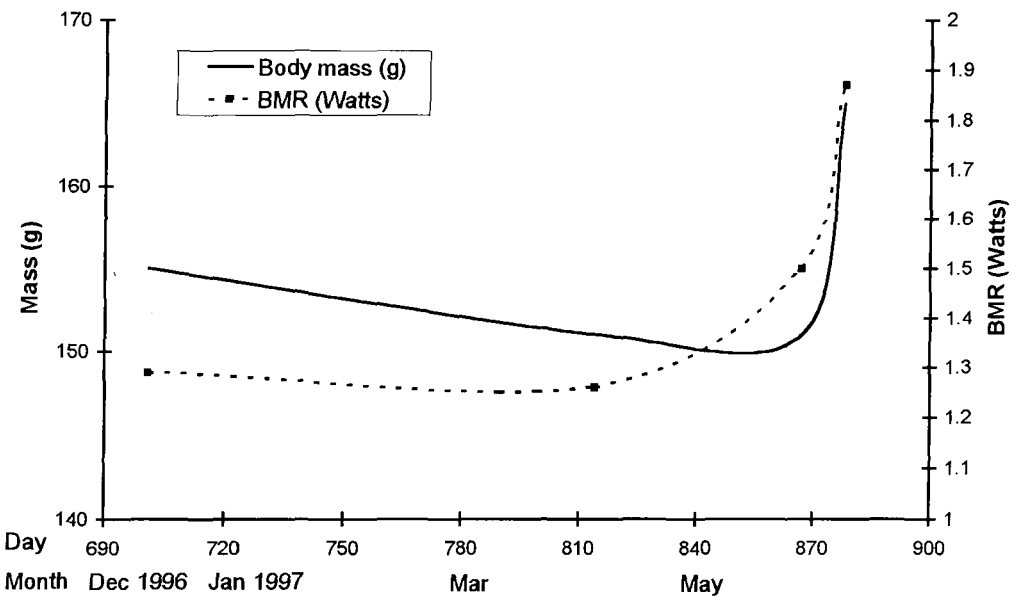


Figure I.1K: Juvenile Knot GW

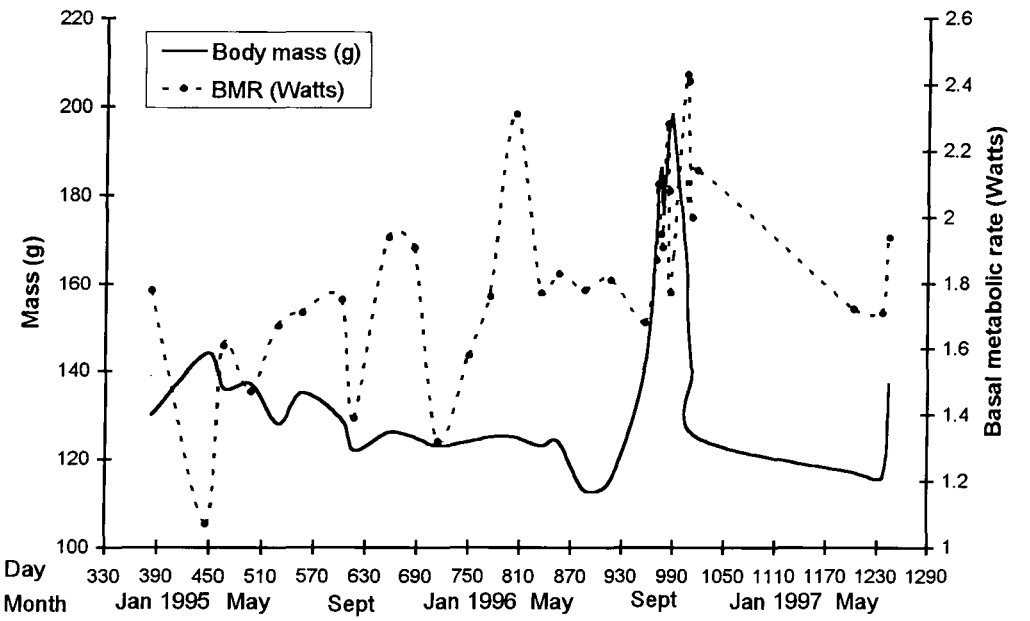


Figure I.1L: Juvenile Knot GY

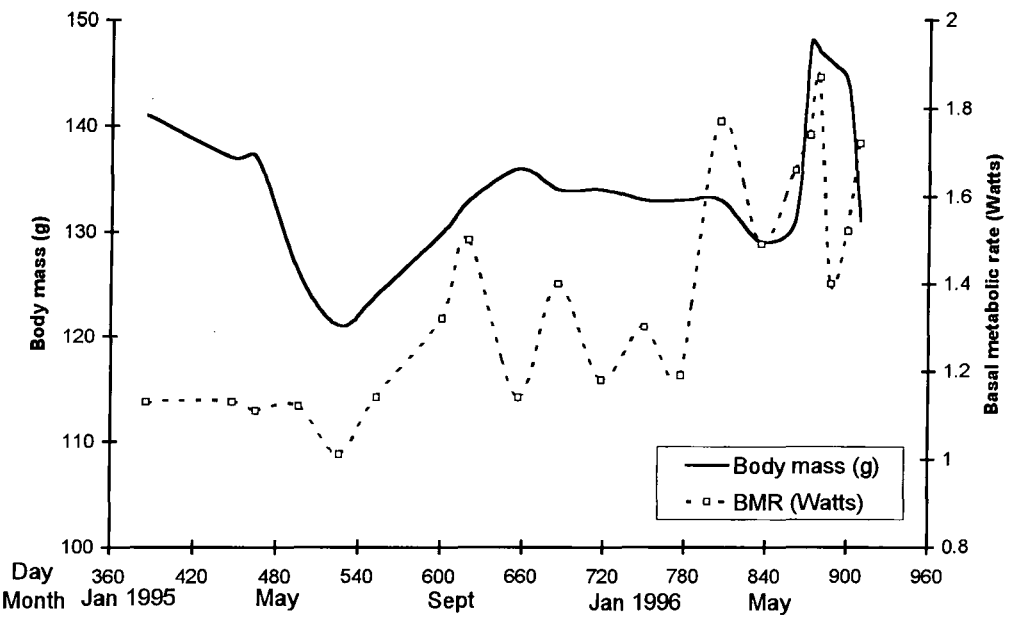


Figure I.1M: Juvenile Knot WGG

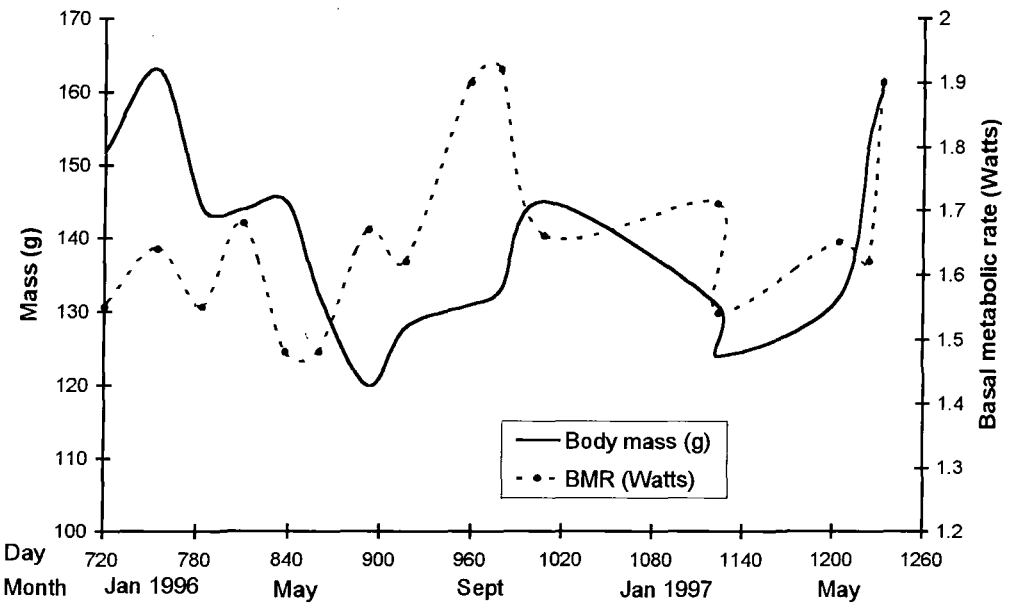


Figure I.1N: Juvenile Knot WGY

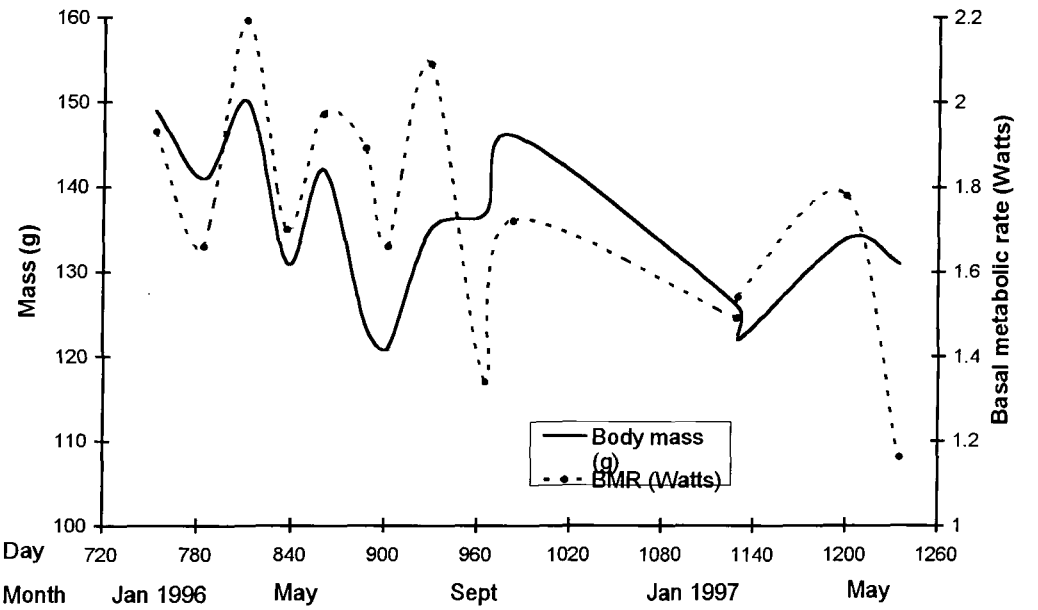


Figure I.1O: Juvenile Knot WLL

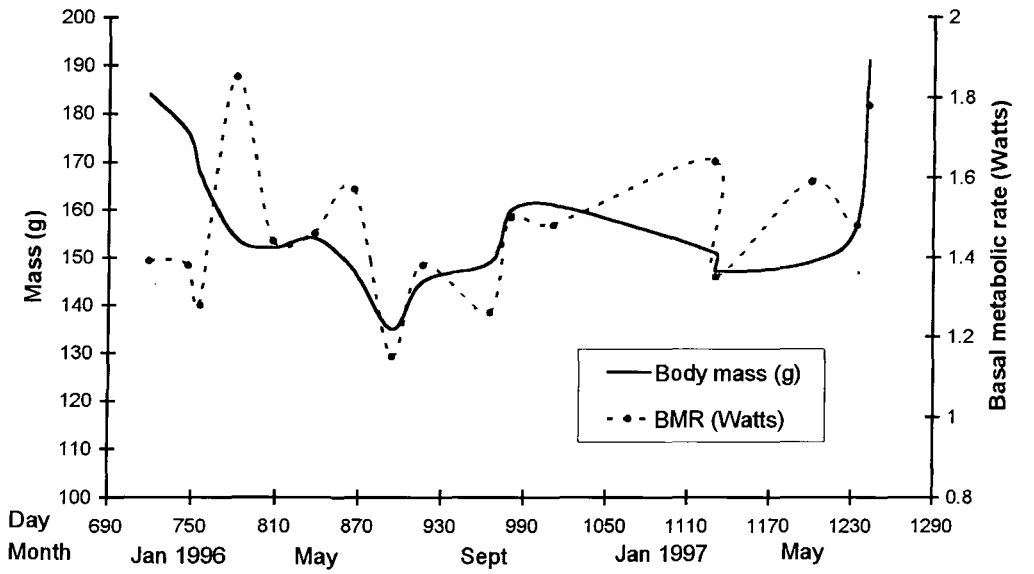


Figure I.1P: Juvenile Knot WWW

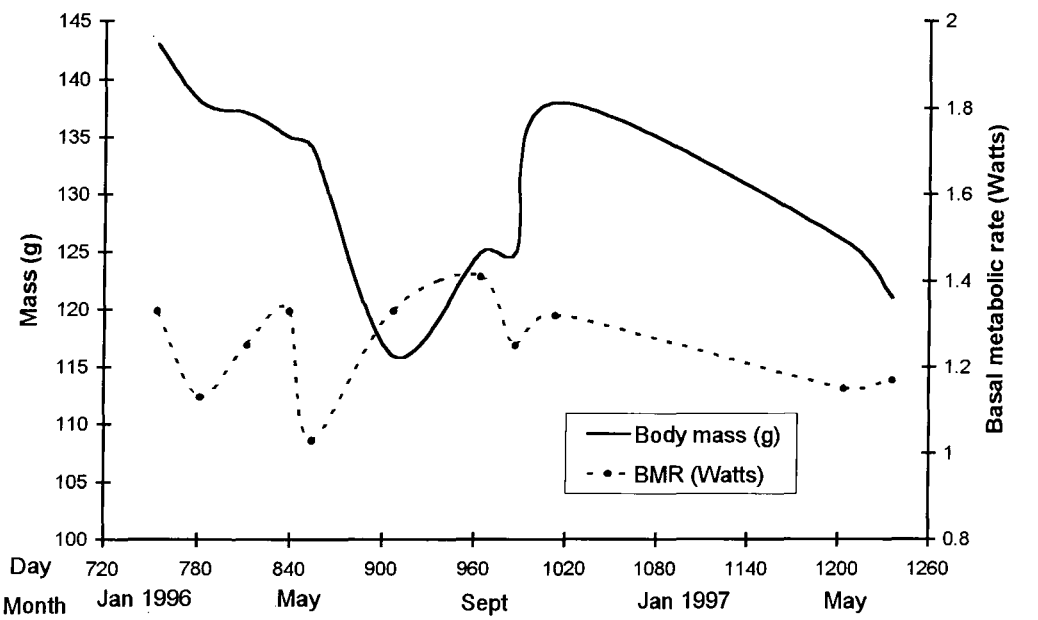


Figure I.1Q: Juvenile Knot WYG

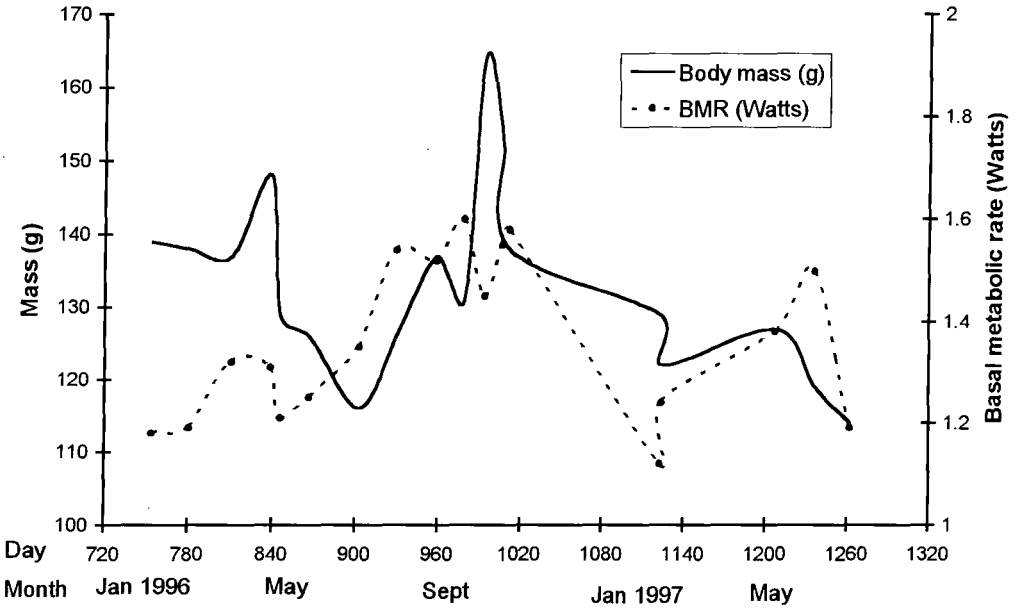


Figure I.1R: Juvenile Knot WLG

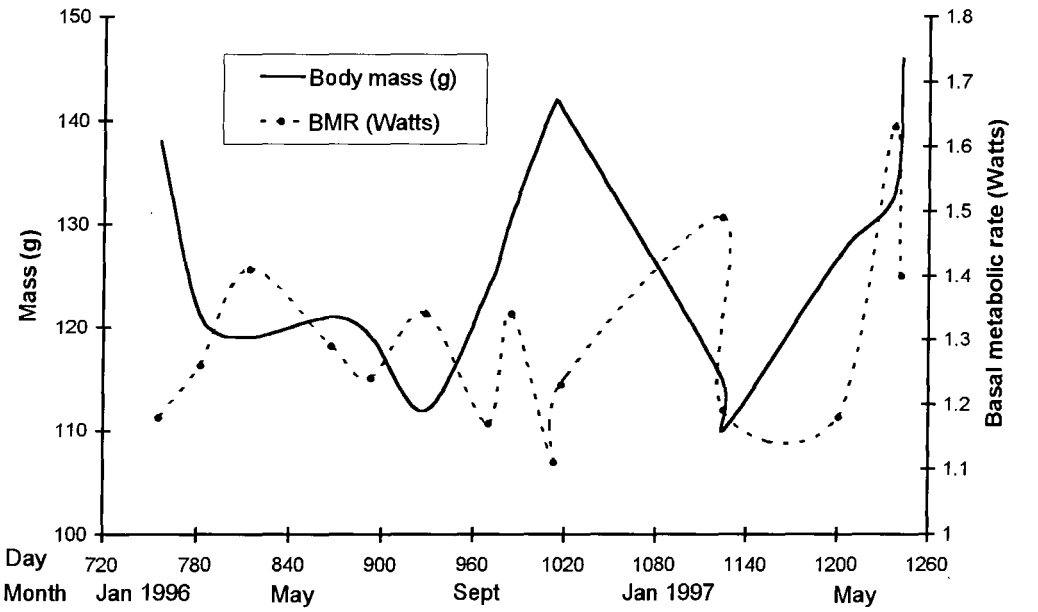


Figure I.1S: Juvenile Knot WG

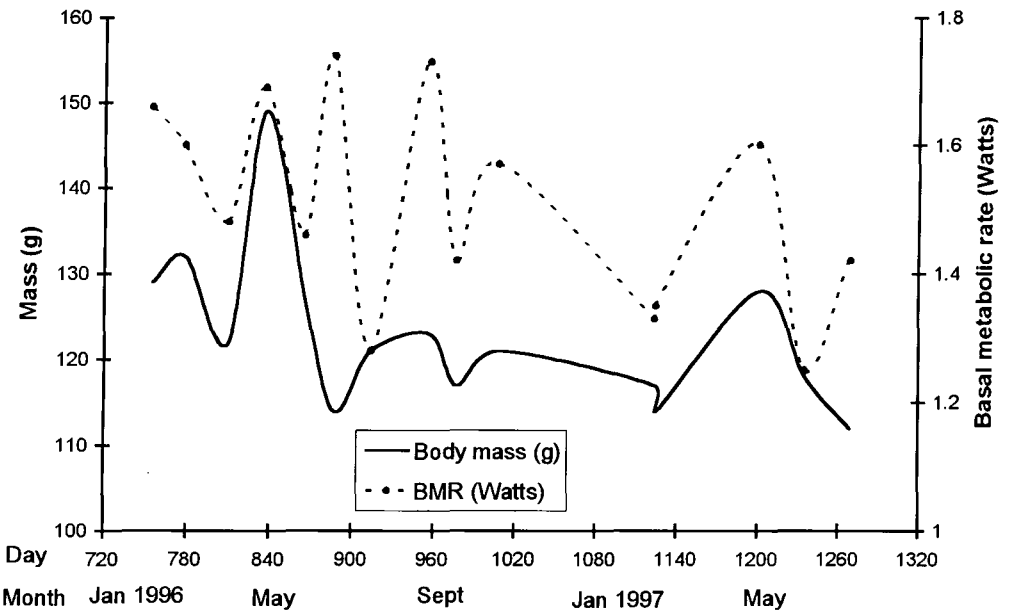
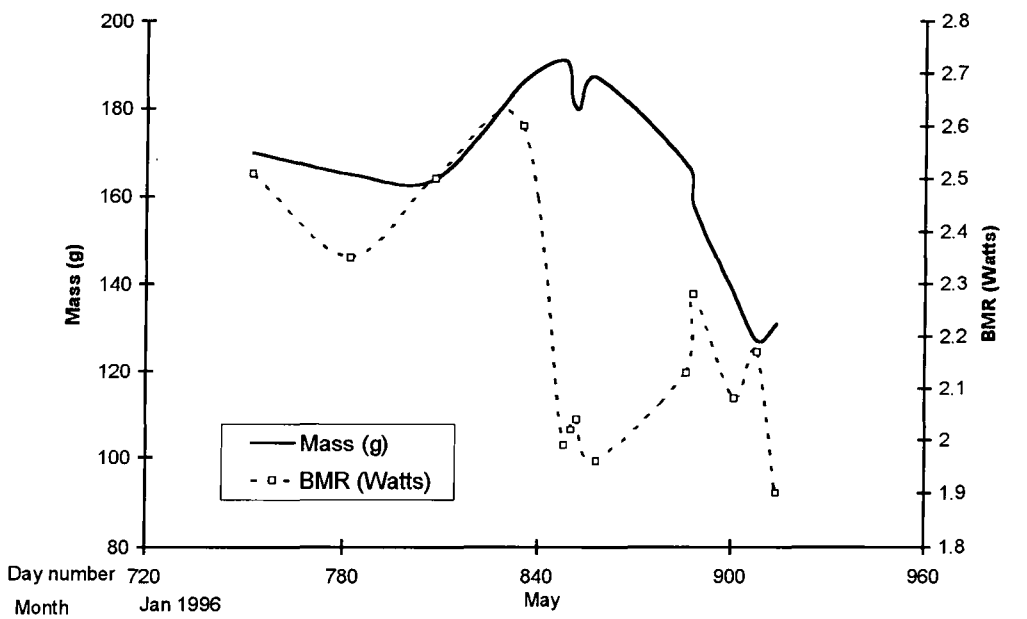


Figure I.1T: Juvenile Knot WYY



Figures I.1A-I.1T :Seasonal variation in total body mass and basal metabolic rate (BMR) in adult and juvenile Knot

Day 0= Jan 1994.

Figure I.1A: Adult Knot LL

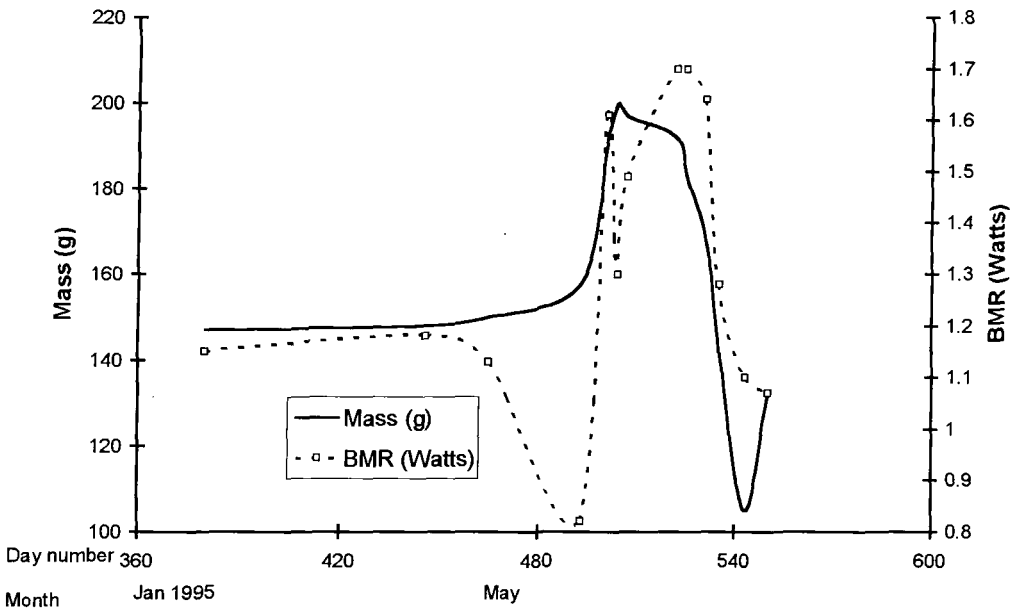


Figure I.1B: Adult Knot WW

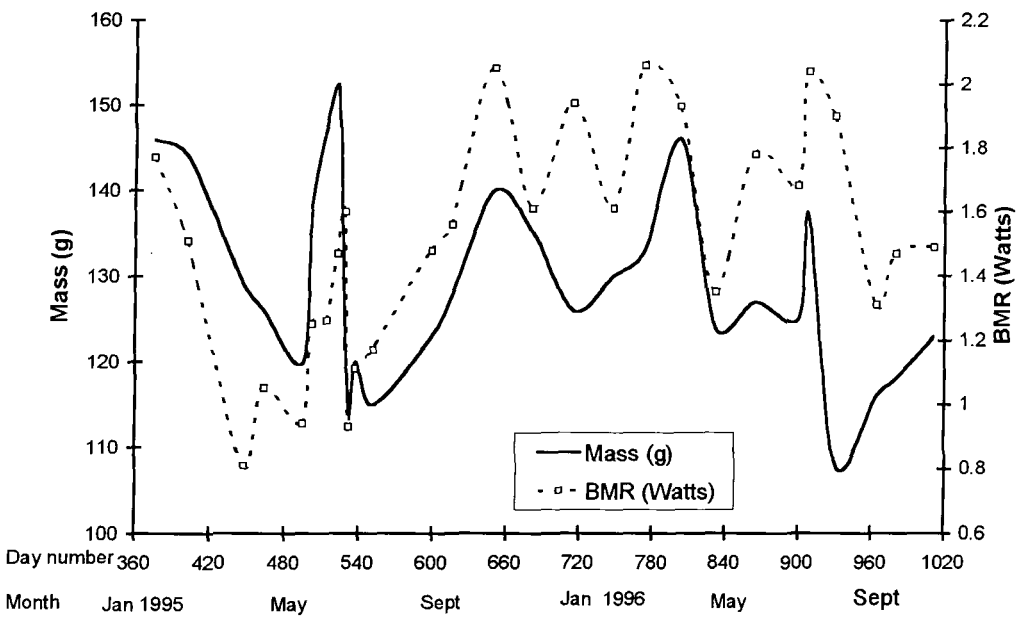


Figure I.1C: Adult Knot YY

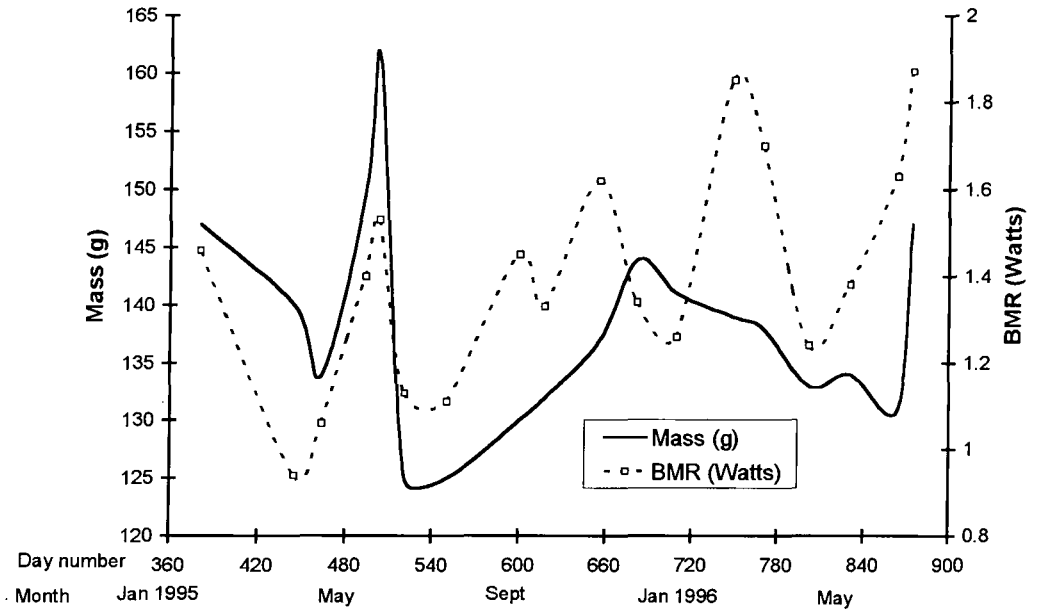


Figure I.1D: Adult Knot LY

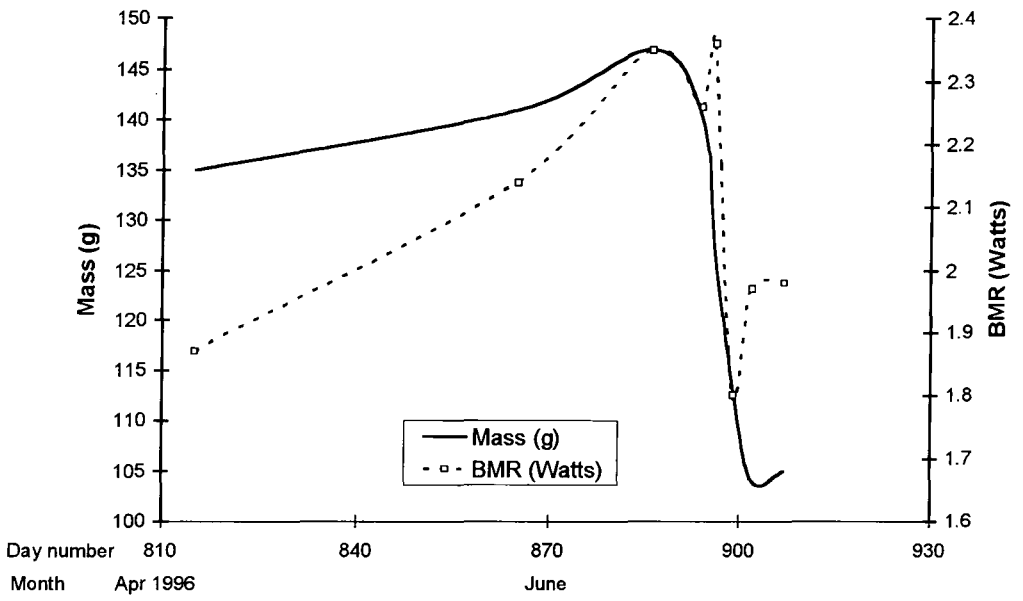


Figure I.1E: Adult Knot LG

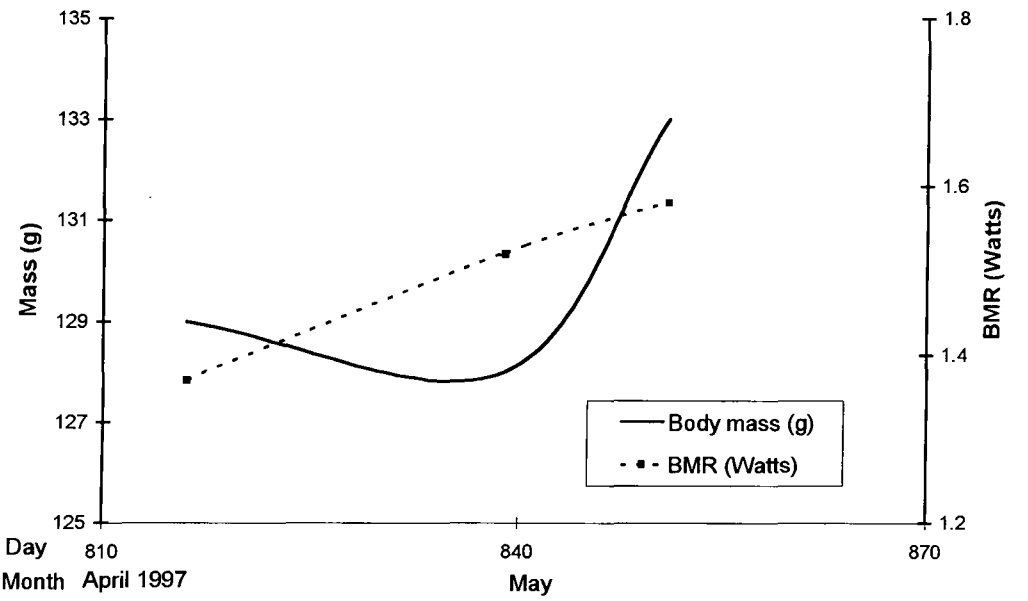


Figure I.1F: Adult Knot YG

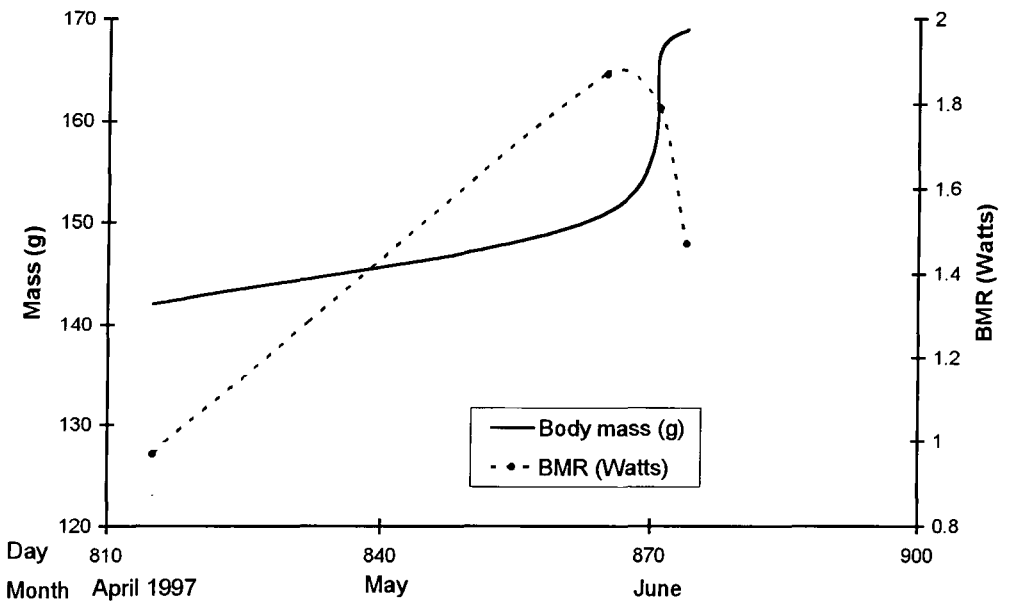


Figure I.1G: Adult Knot BW

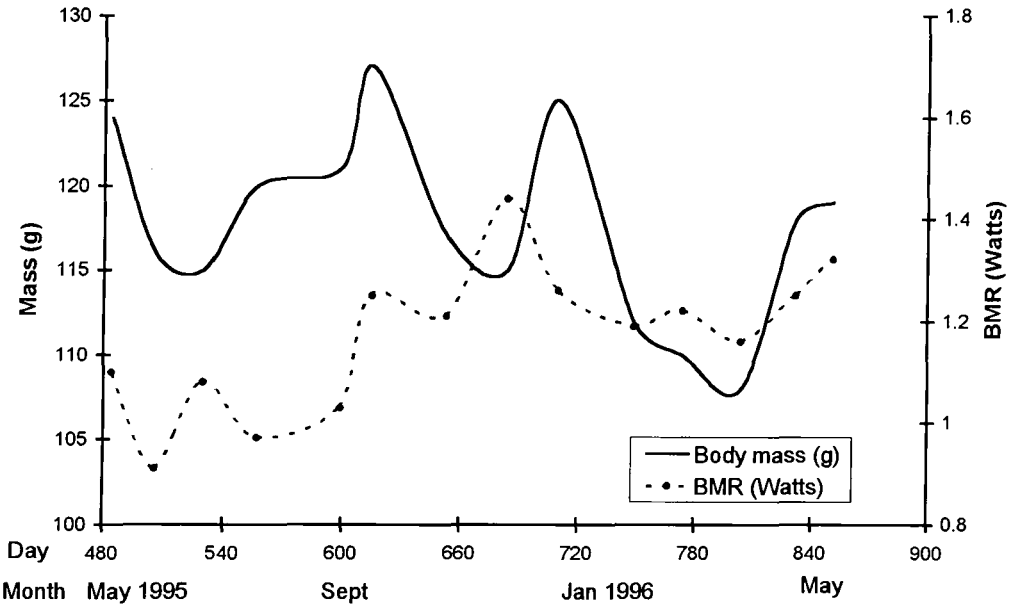


Figure I.1H: Adult Knot WH

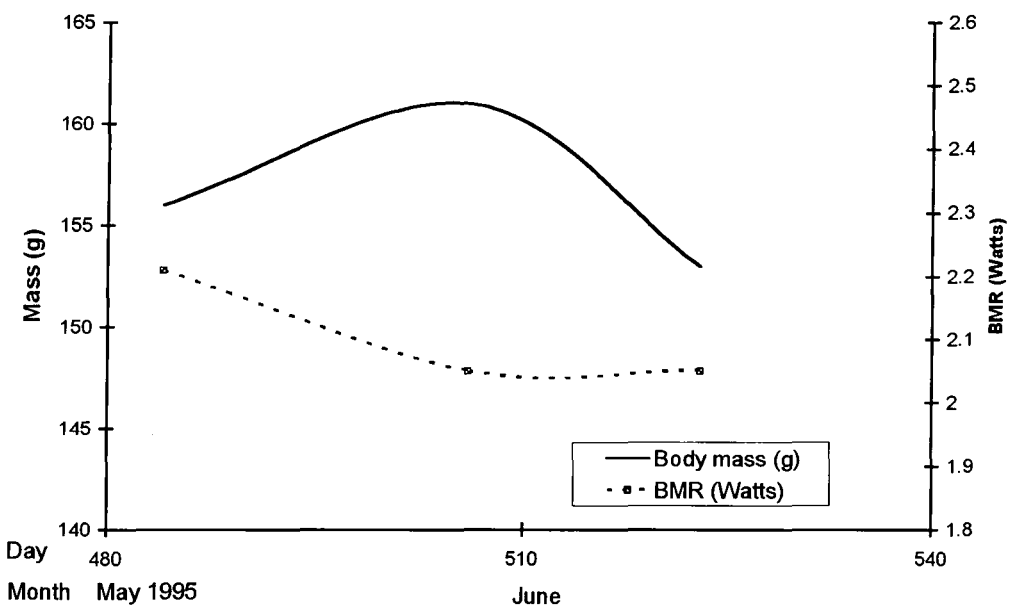


Figure I.1I: Adult Knot Yellow

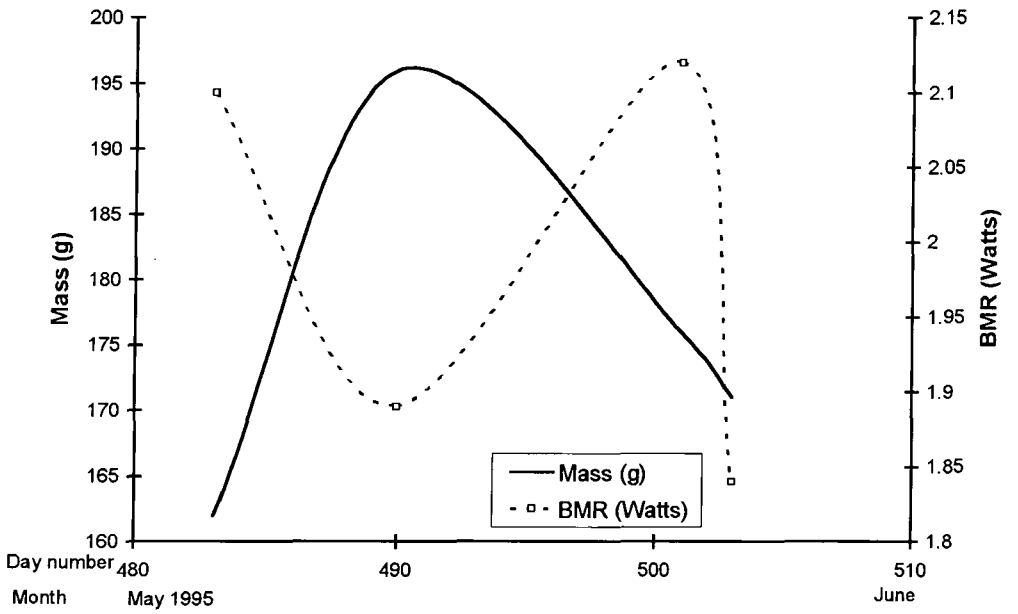


Figure I.1J: Adult Knot AA

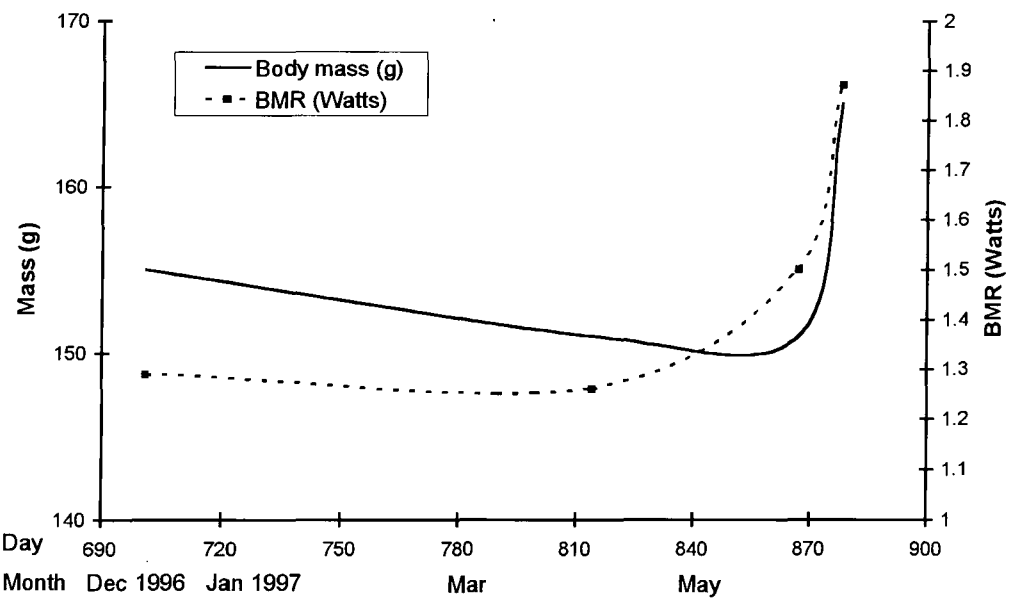


Figure I.1K: Juvenile Knot GW

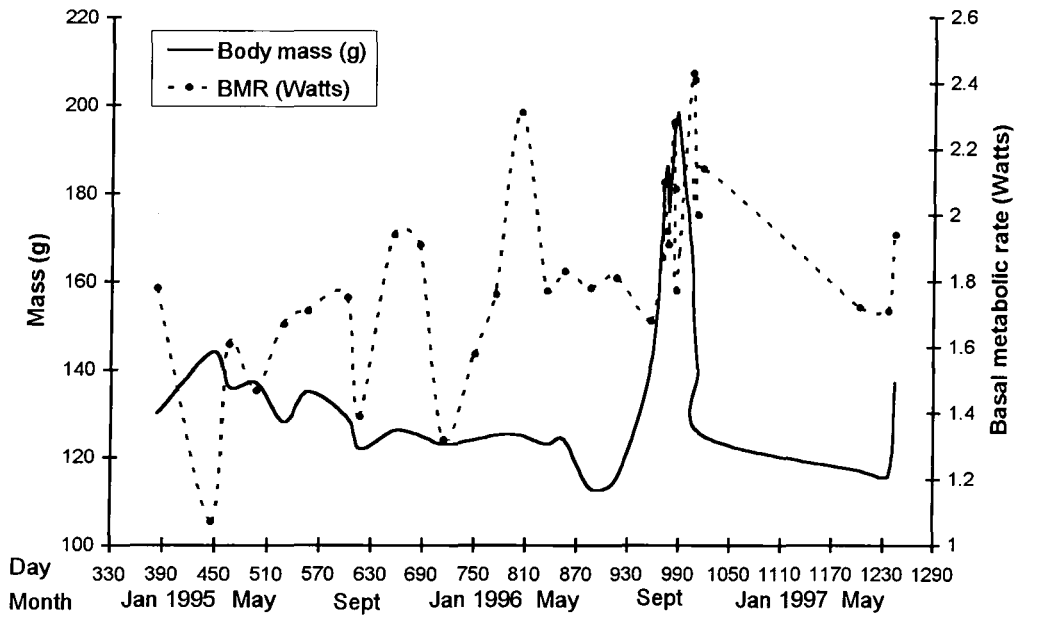


Figure I.1L: Juvenile Knot GY

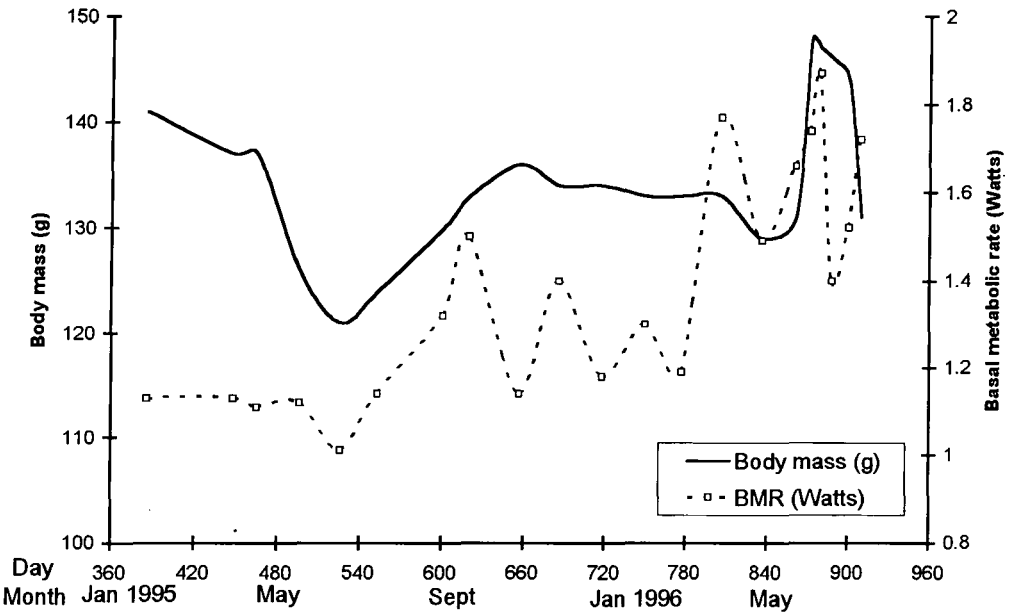


Figure I.1M: Juvenile Knot WGG

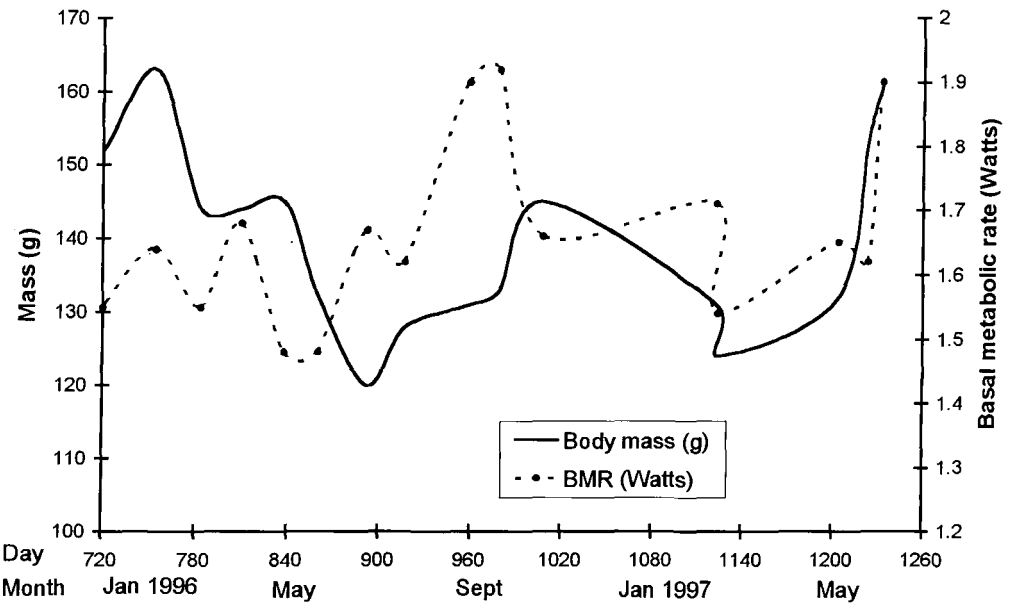


Figure I.1N: Juvenile Knot WGY

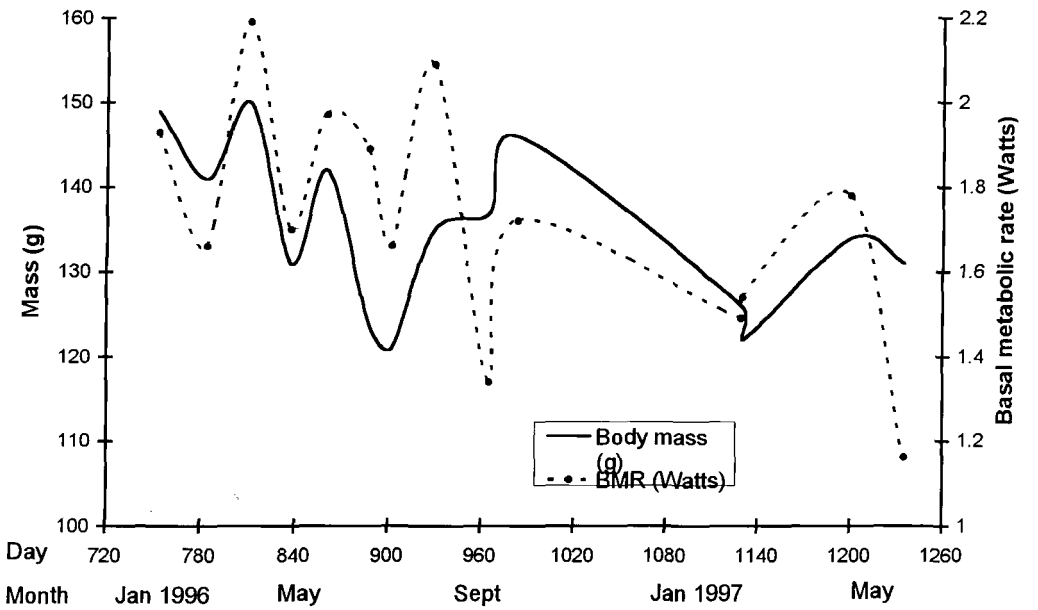


Figure I.1O: Juvenile Knot WLL

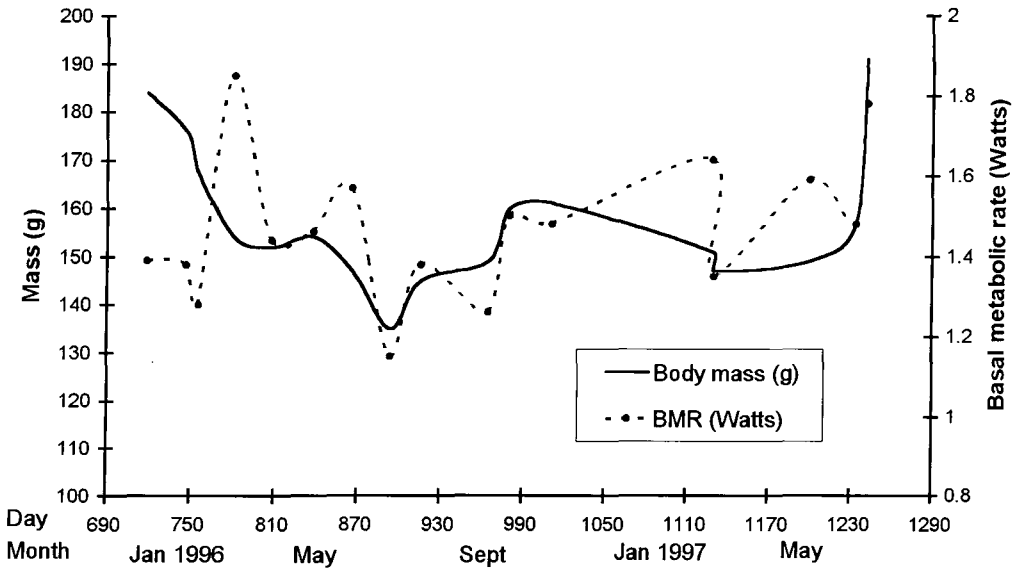


Figure I.1P: Juvenile Knot WWW

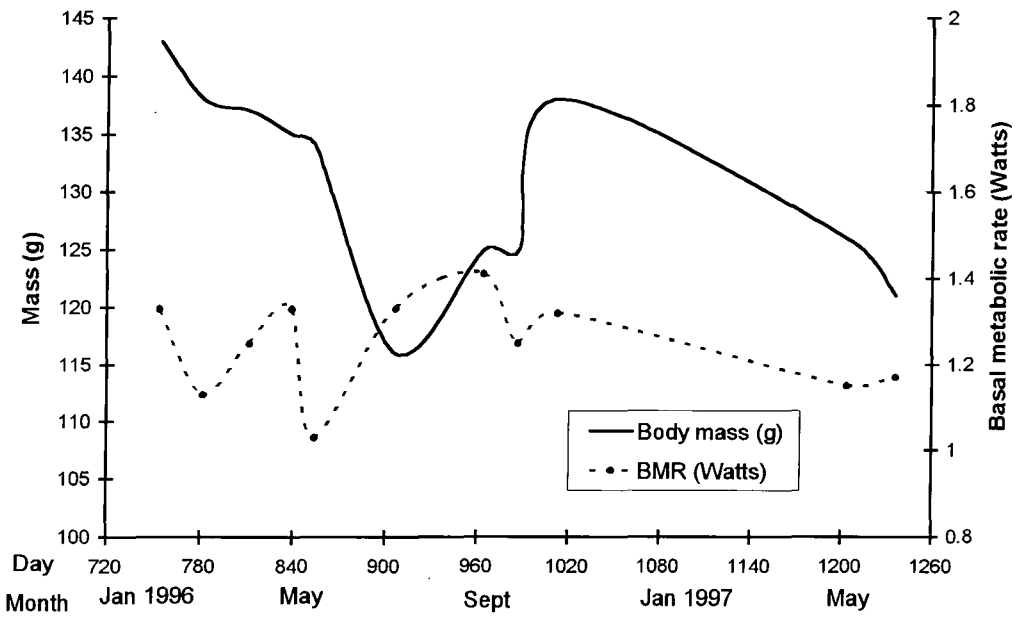


Figure I.1Q: Juvenile Knot WYG

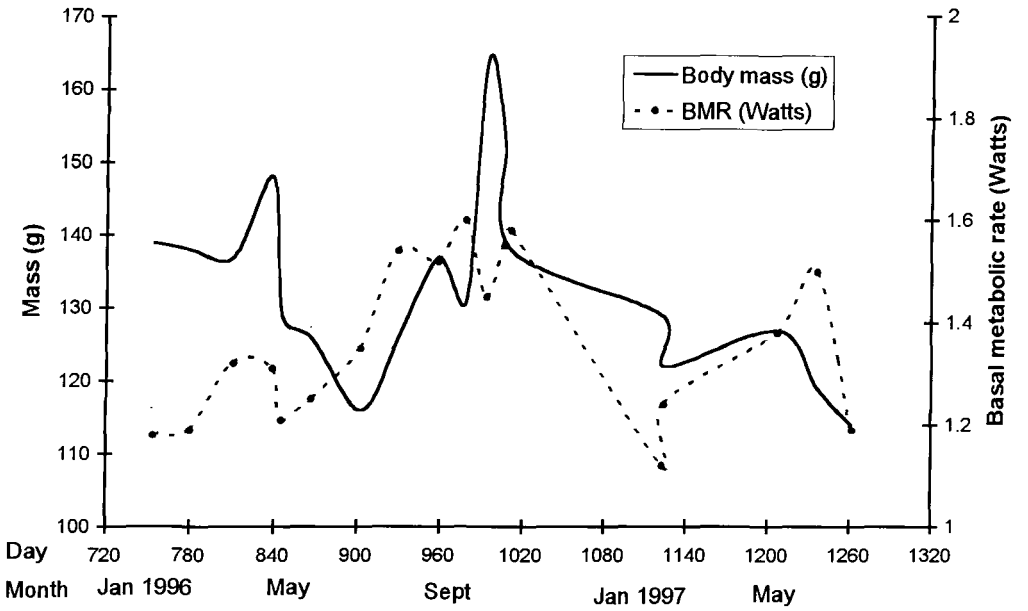


Figure I.1R: Juvenile Knot WLG

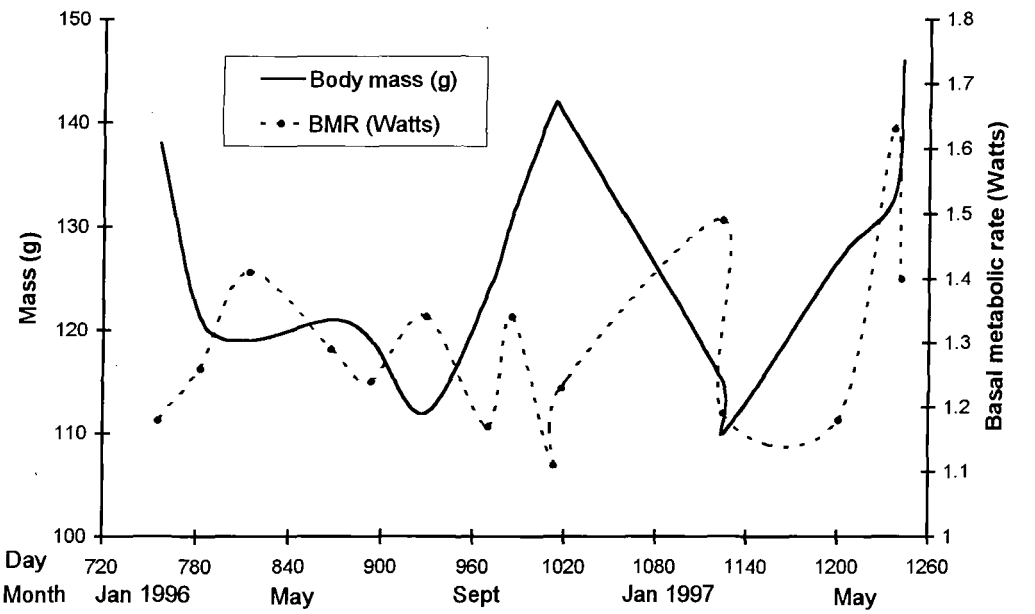


Figure I.1S: Juvenile Knot WG

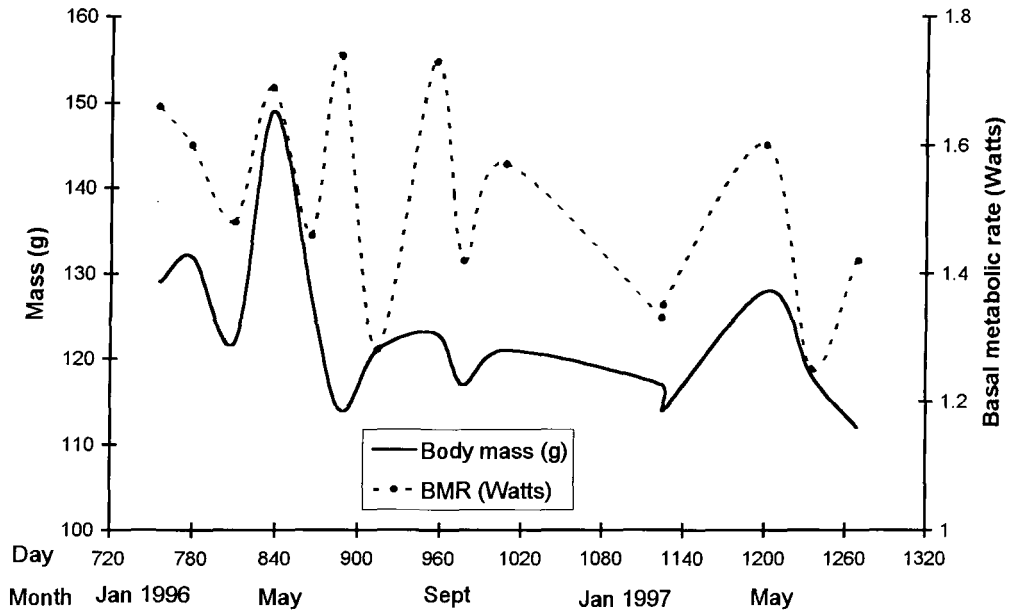
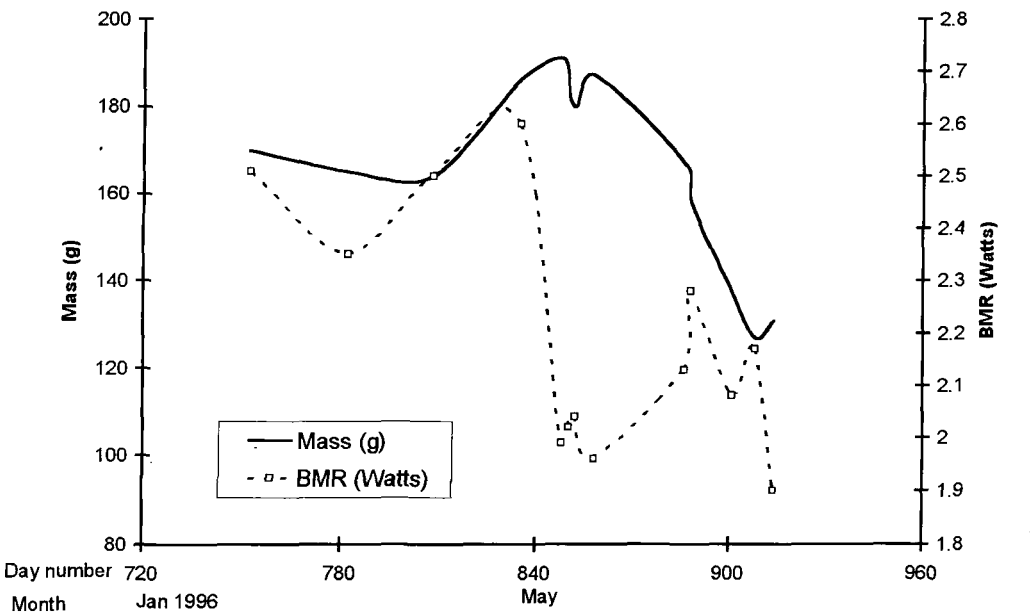


Figure I.1T: Juvenile Knot WYY



Figures I.2A-I.2T: Seasonal variation in total lean mass (predicted using TOBEC) and lean-mass-specific BMR in adult and juvenile Knot

Figure I.2A: Adult Knot LL

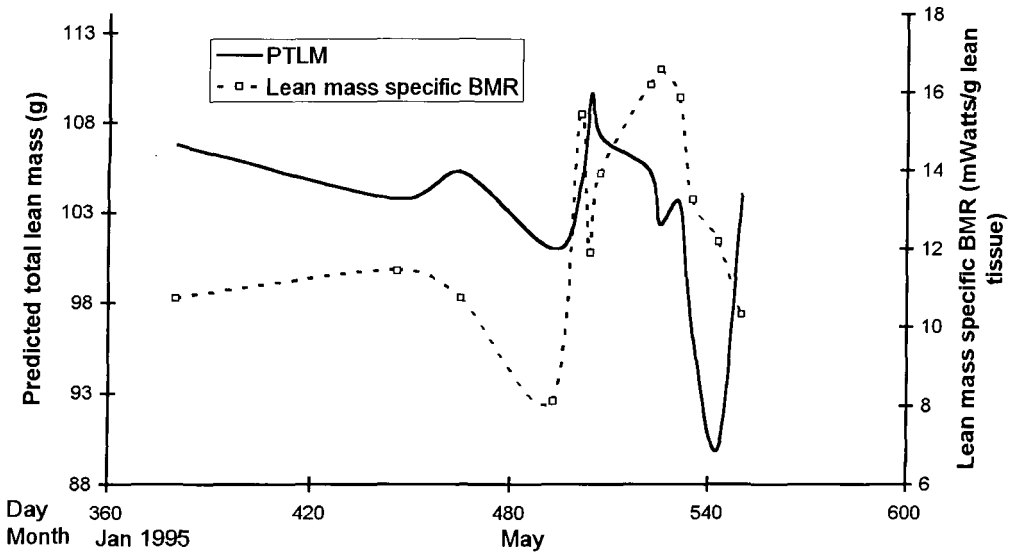


Figure I.2B: Adult Knot WW

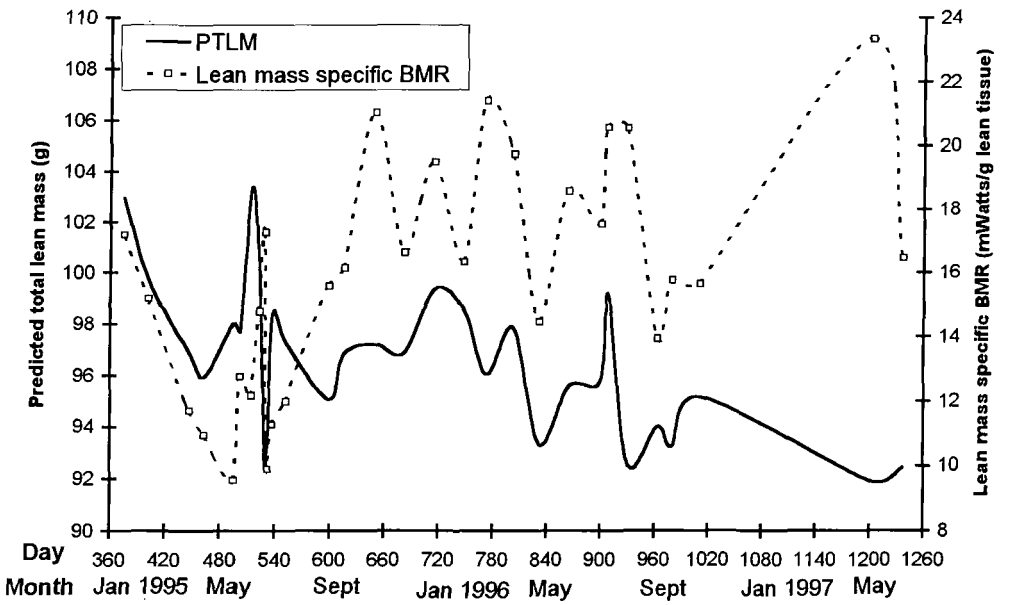


Figure I.2C: Adult Knot YY

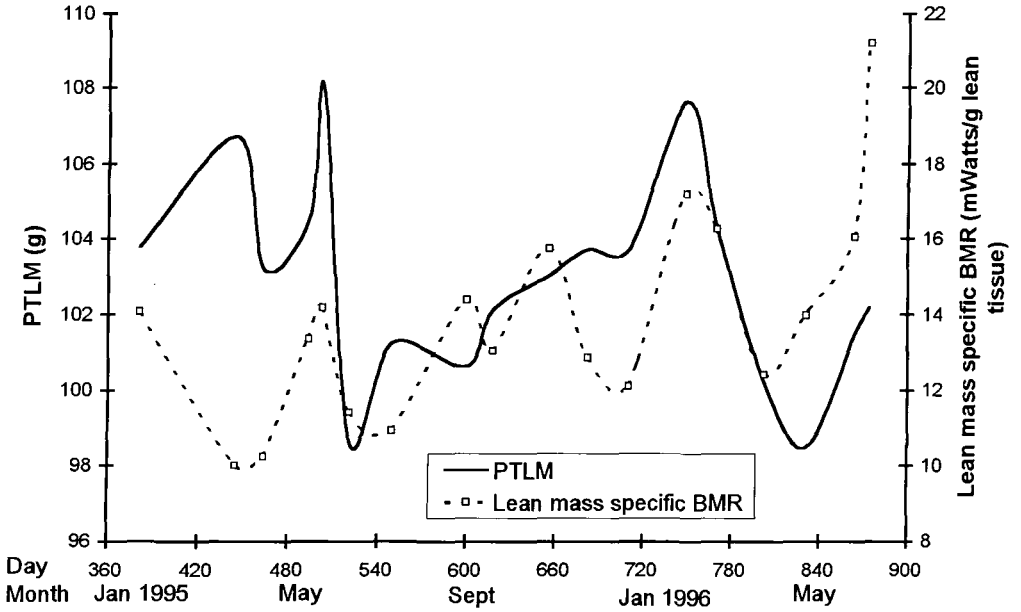


Figure I.2D: Adult Knot LY

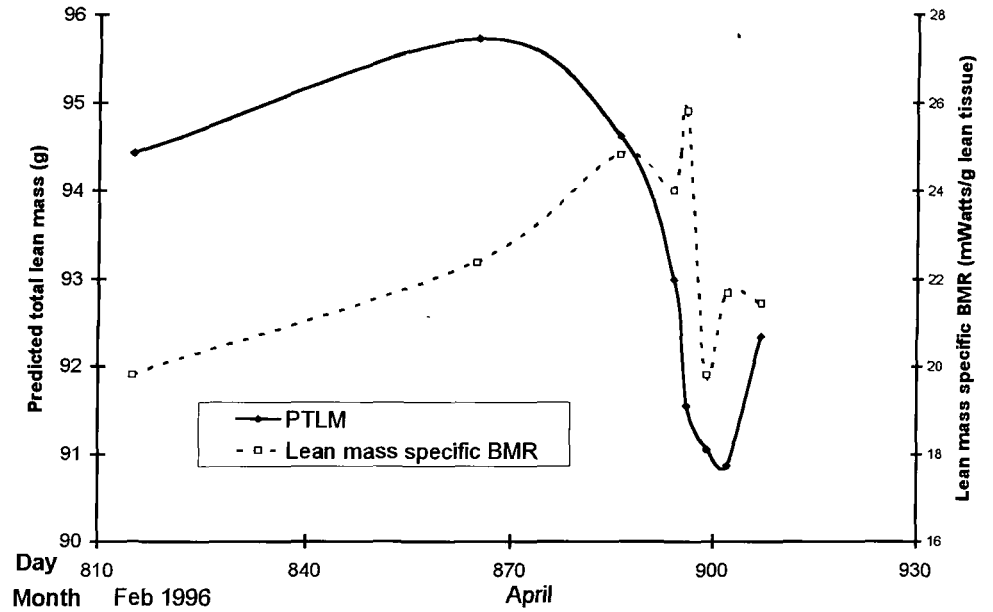


Figure I.2E: Adult Knot LG

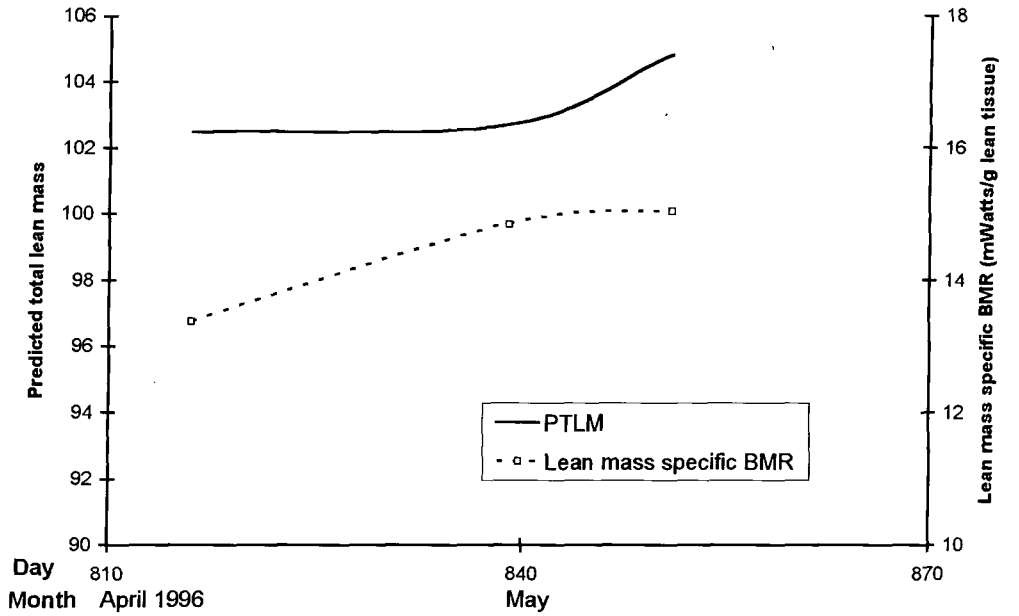


Figure I.2F: Adult Knot YG

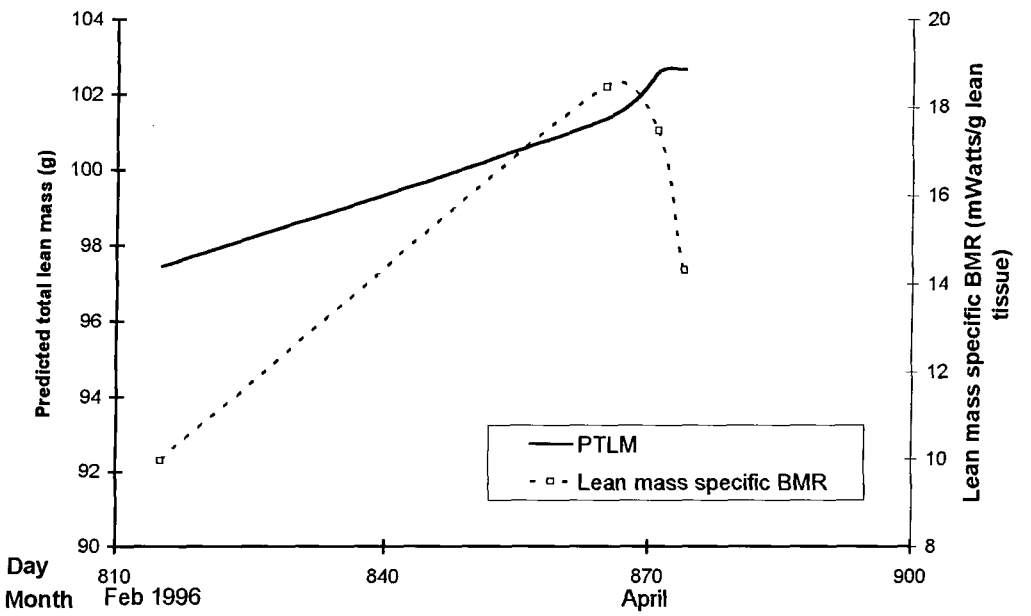


Figure I.2G: Adult Knot BW

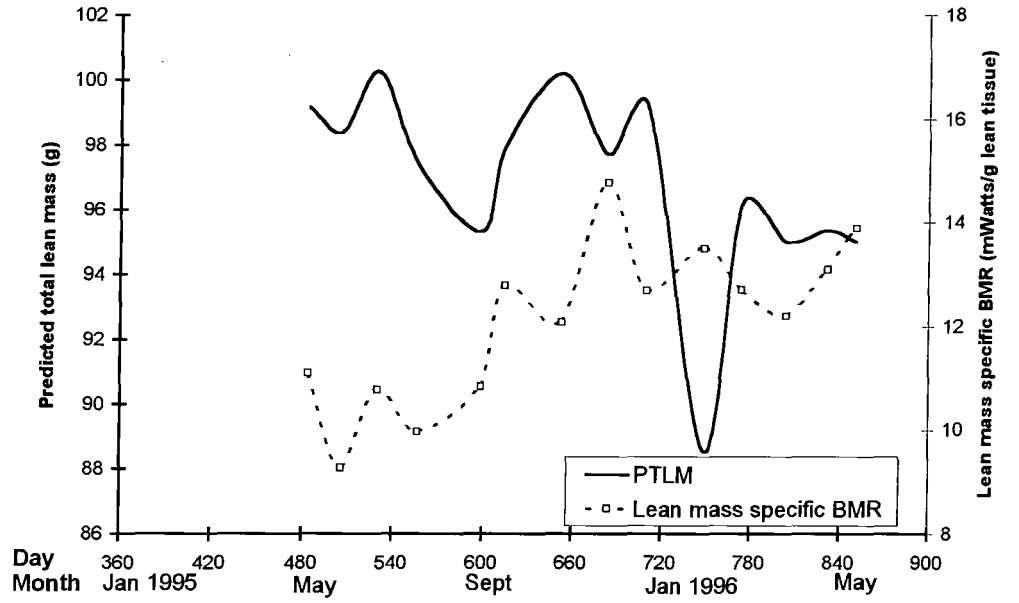


Figure I.2H: Adult Knot WH

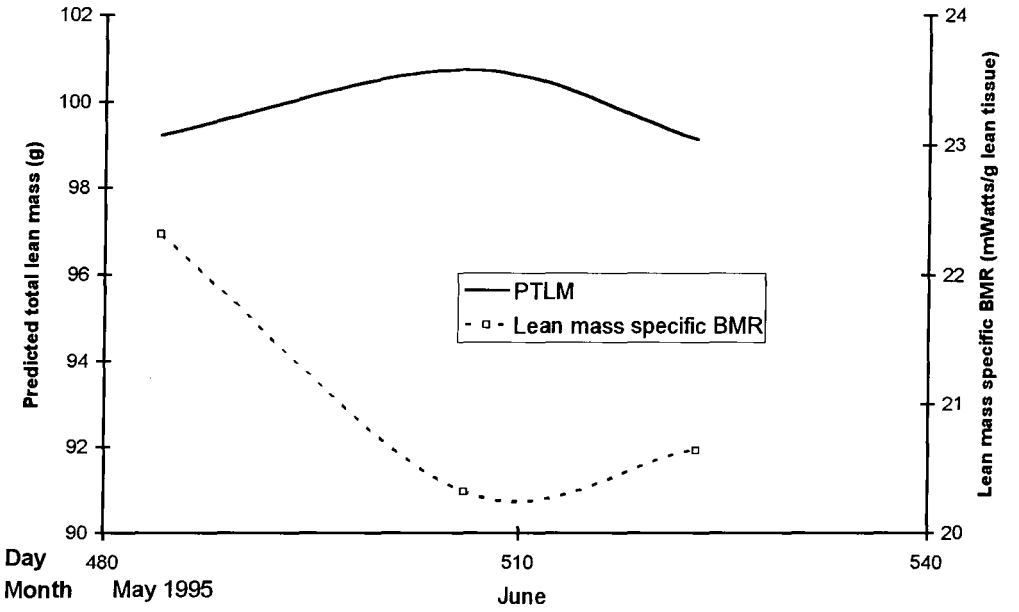


Figure 1.2I: Adult Knot Yellow

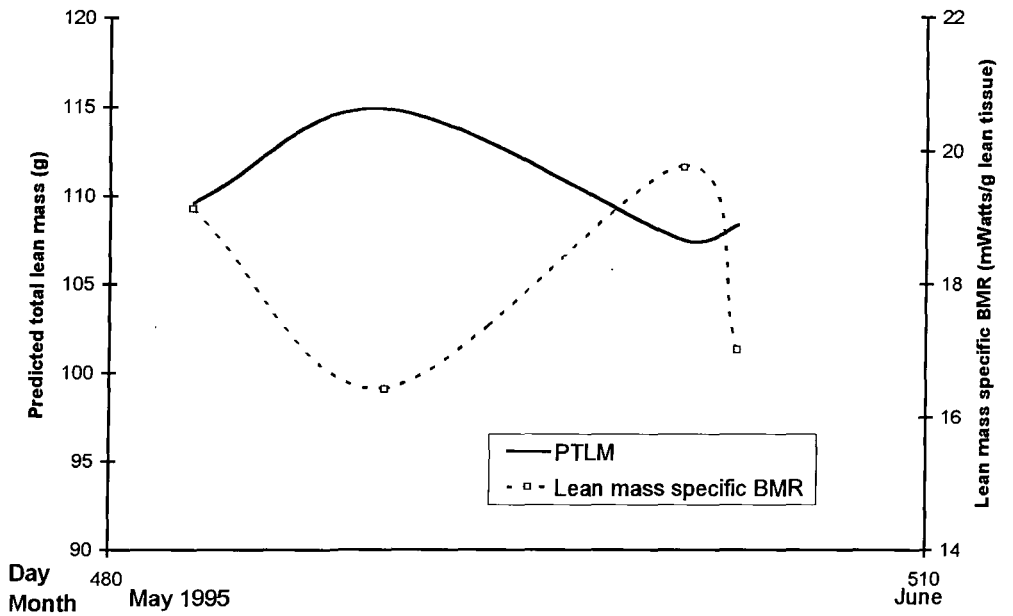


Figure I.2J: Adult Knot AA

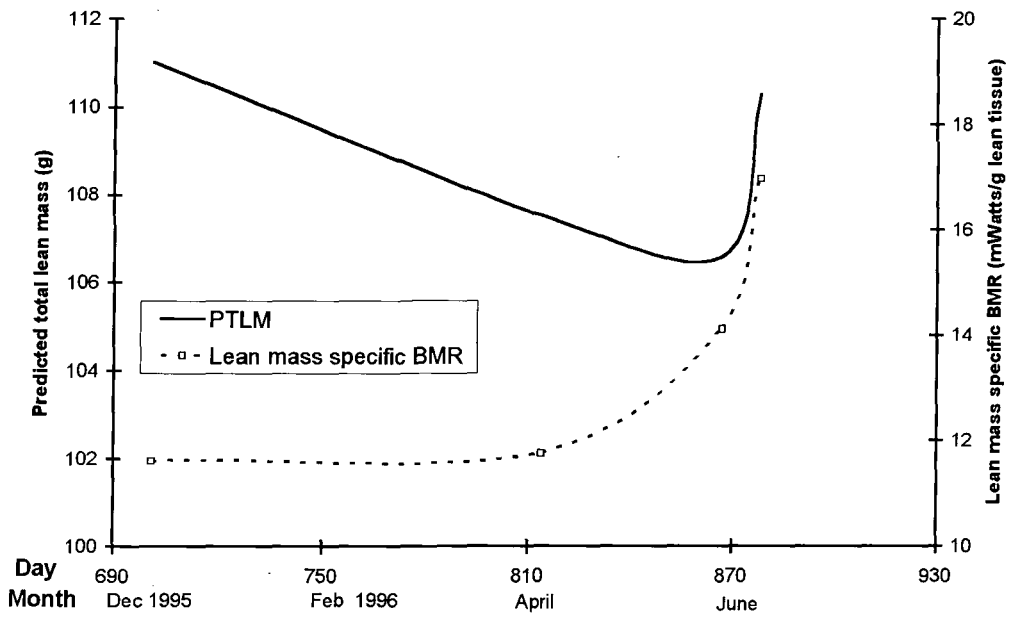


Figure I.2K : Juvenile Knot GW

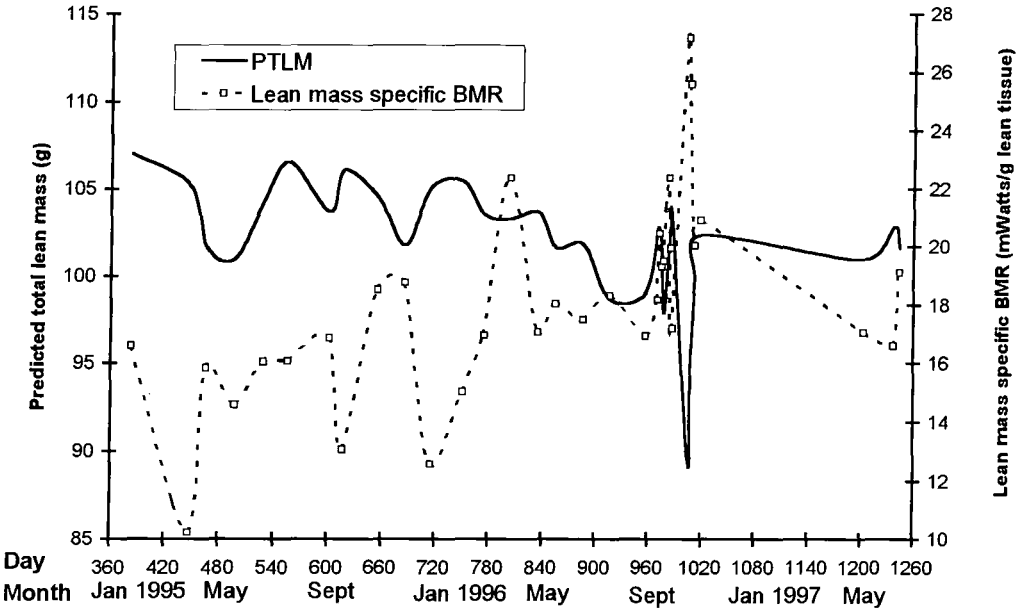


Figure I.2L : Juvenile Knot GY

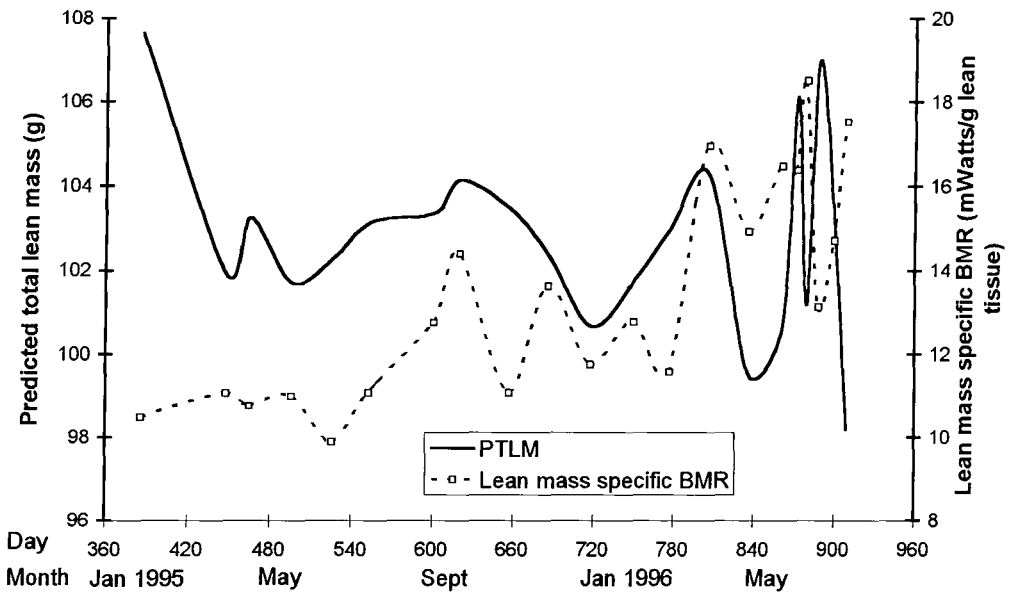


Figure I.2M : Juvenile Knot WGG

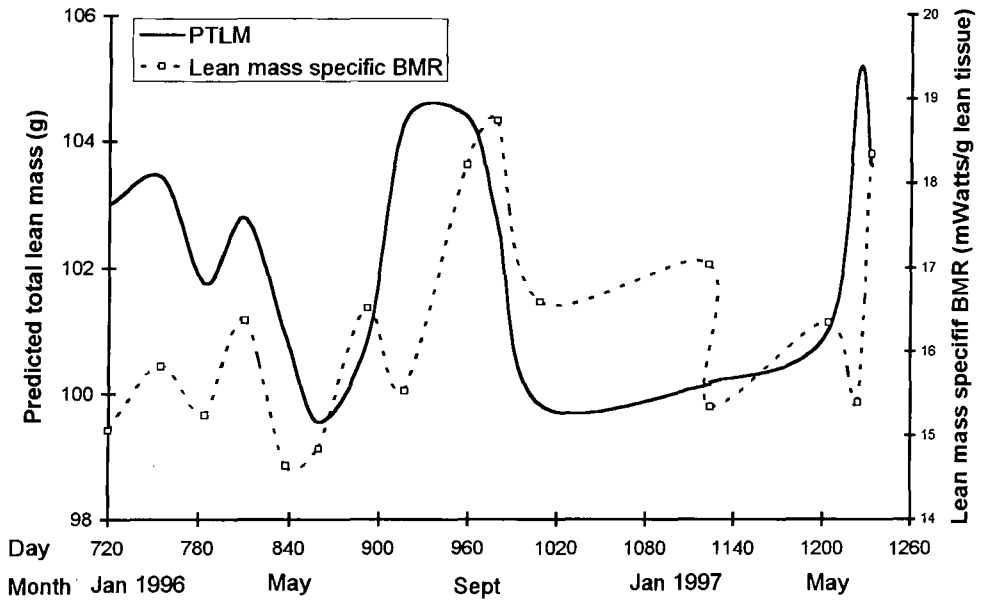


Figure I.2N: Knot WGY

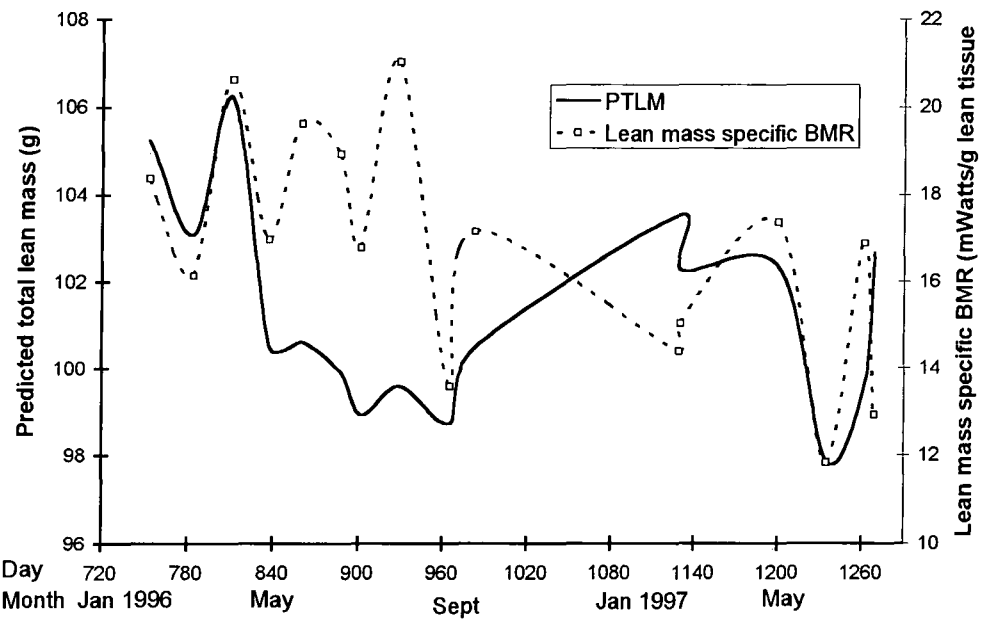


Figure I.2O : Juvenile Knot WLL

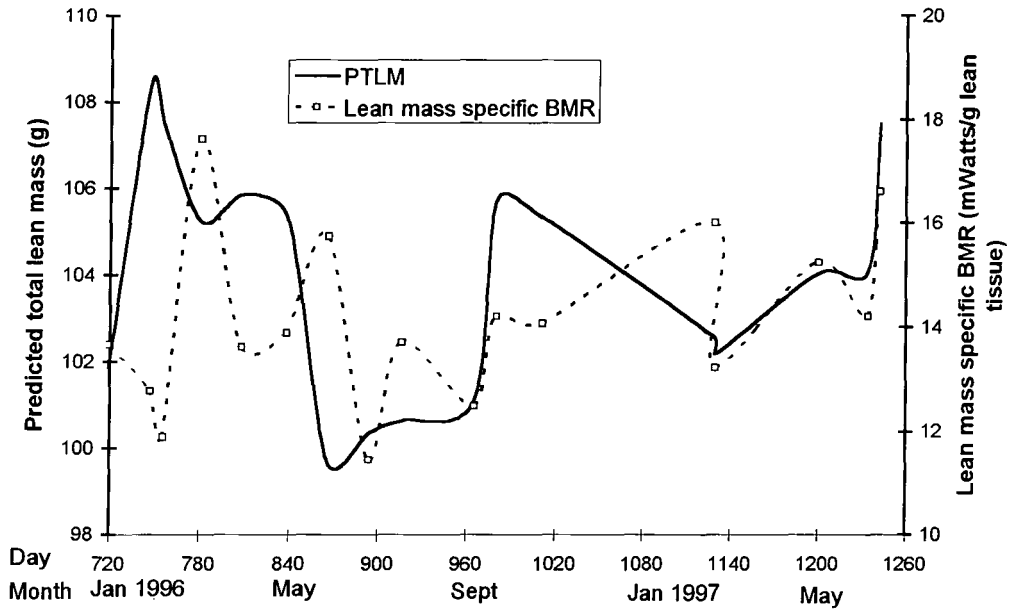


Figure I.2P: Juvenile Knot WWW

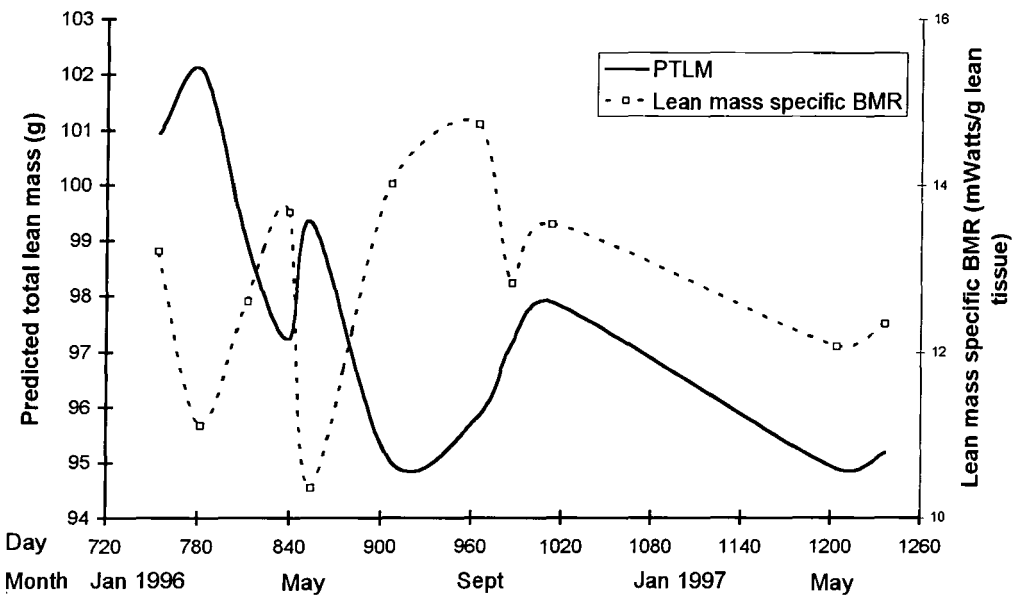


Figure I.2Q: Juvenile Knot WYG

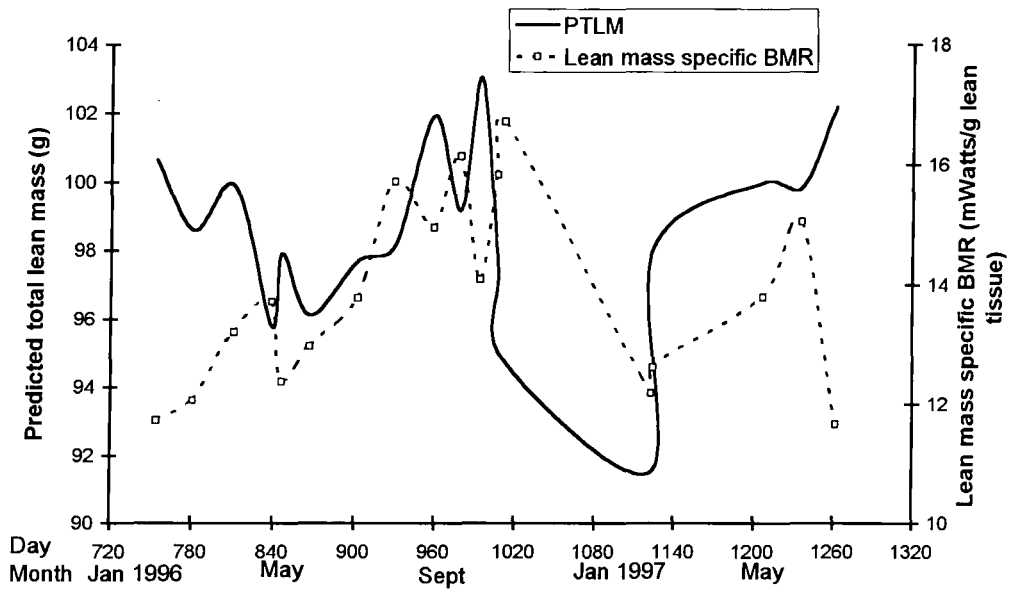


Figure I.2R : Juvenile Knot WLG

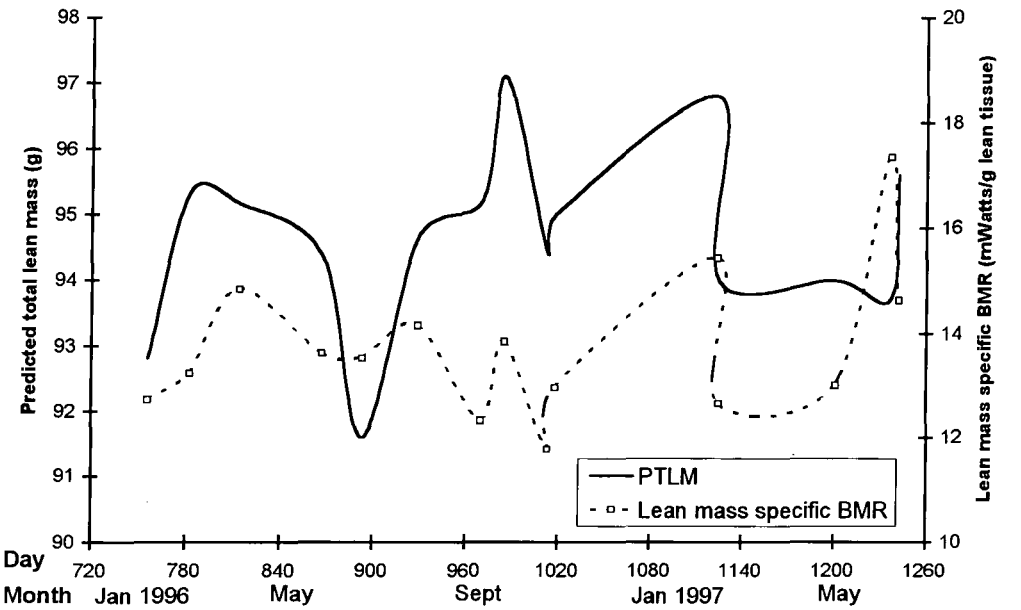


Figure I.2S : Juvenile Knot WG

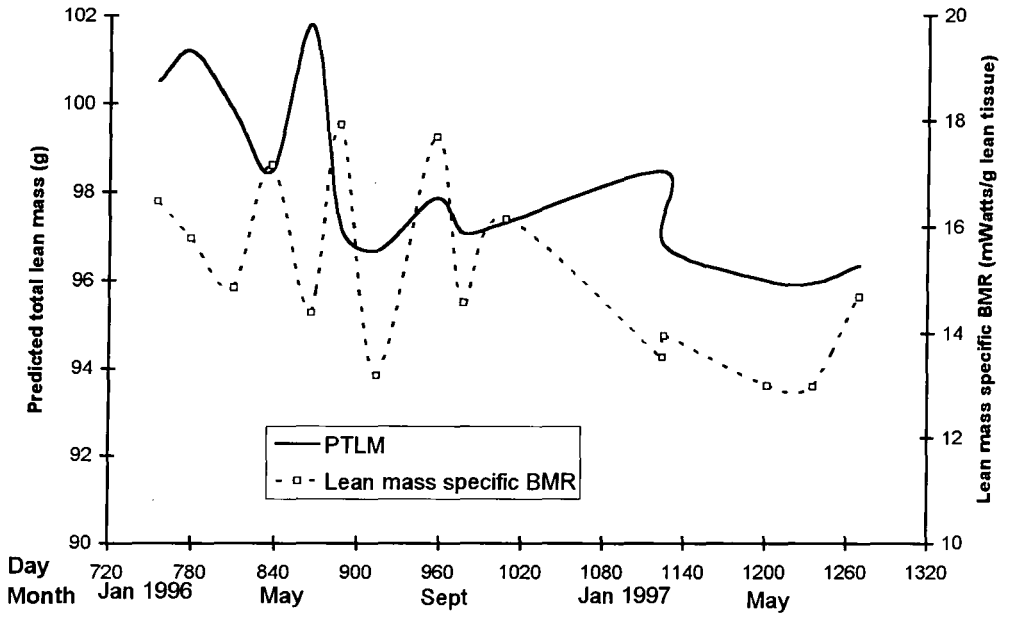
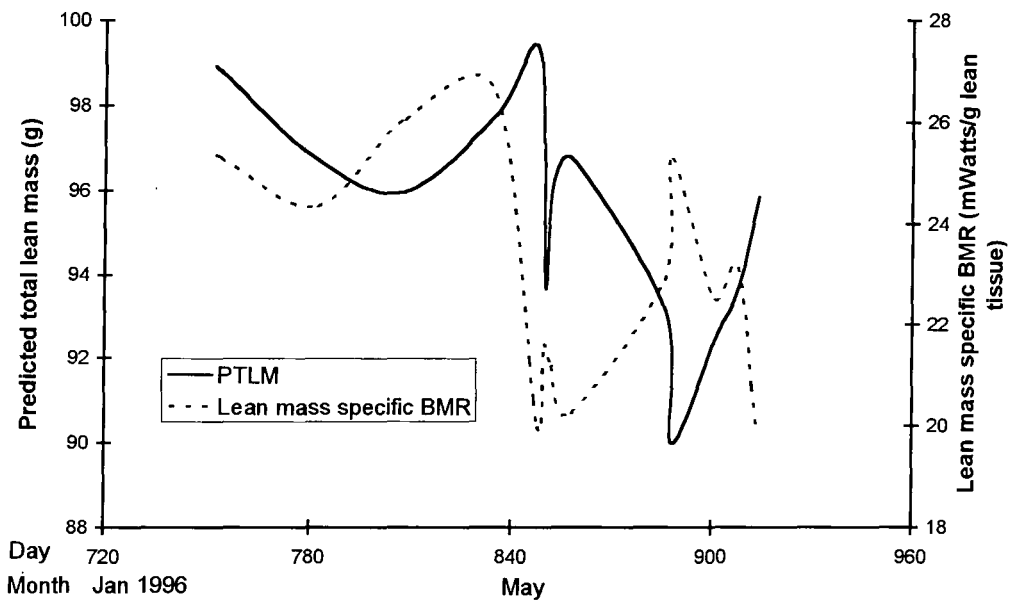


Figure I.2T: Atypical juvenile Knot WYY



Figures 1.3A-S. Scatterplots showing the intra-individual relationship between BMR and body mass in captive Knot. Different symbols denote different physiological states.

(See Chapter 3, tables 3.3.4.1 & 3.3.4.2 for regression equations produced and significance).

FigureI.3A: Adult Knot GG

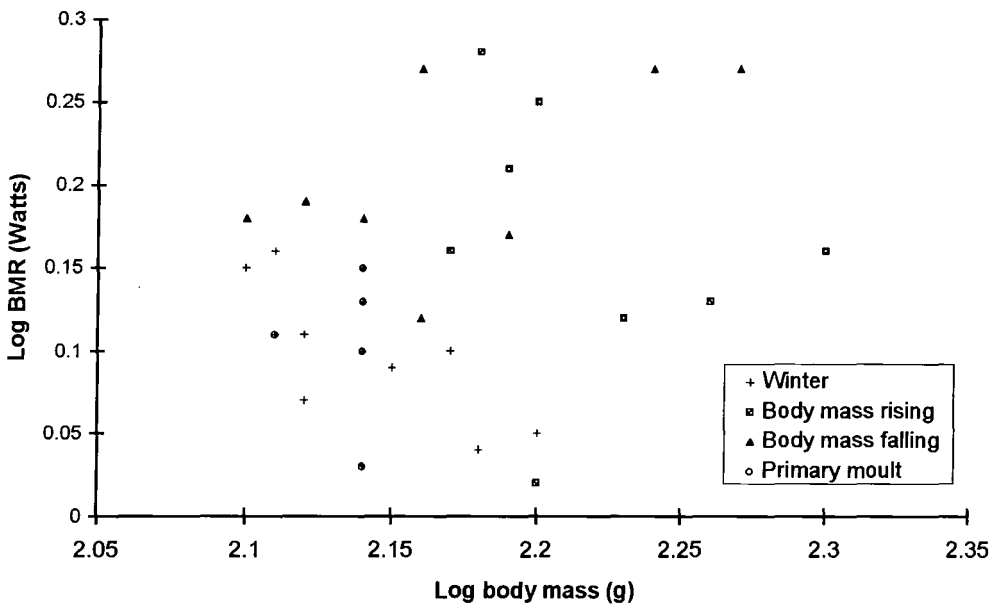


Figure I.3B: Knot LL

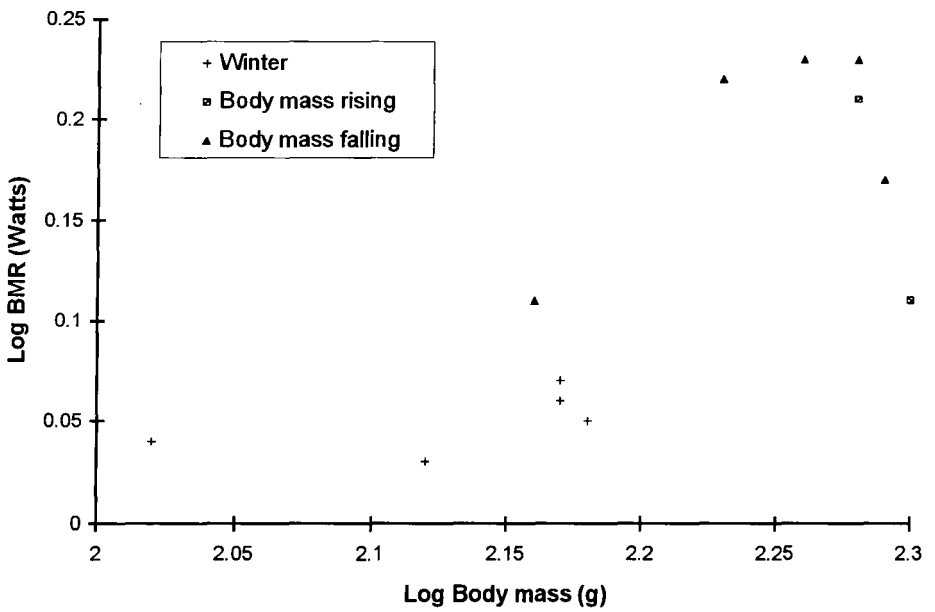


Figure 1.3C: Adult Knot WW

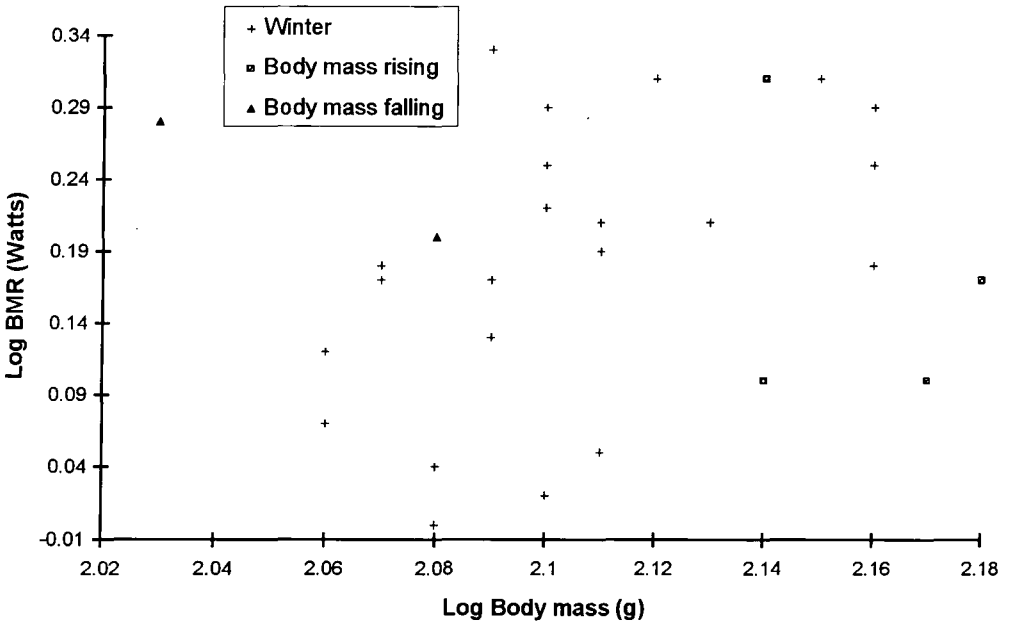


Figure 1.3D: Adult Knot YY

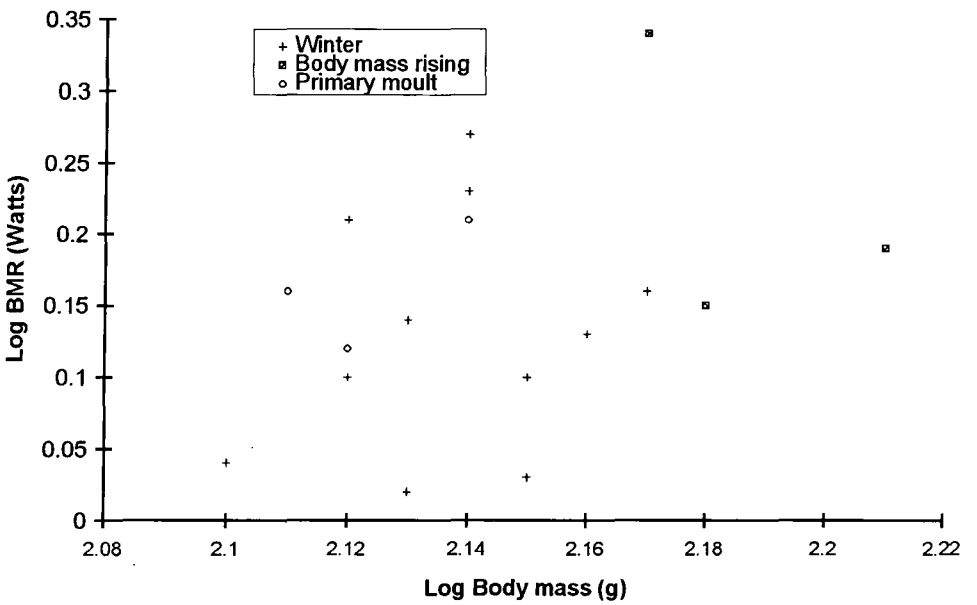


Figure 1.3E: Adult Knot BW

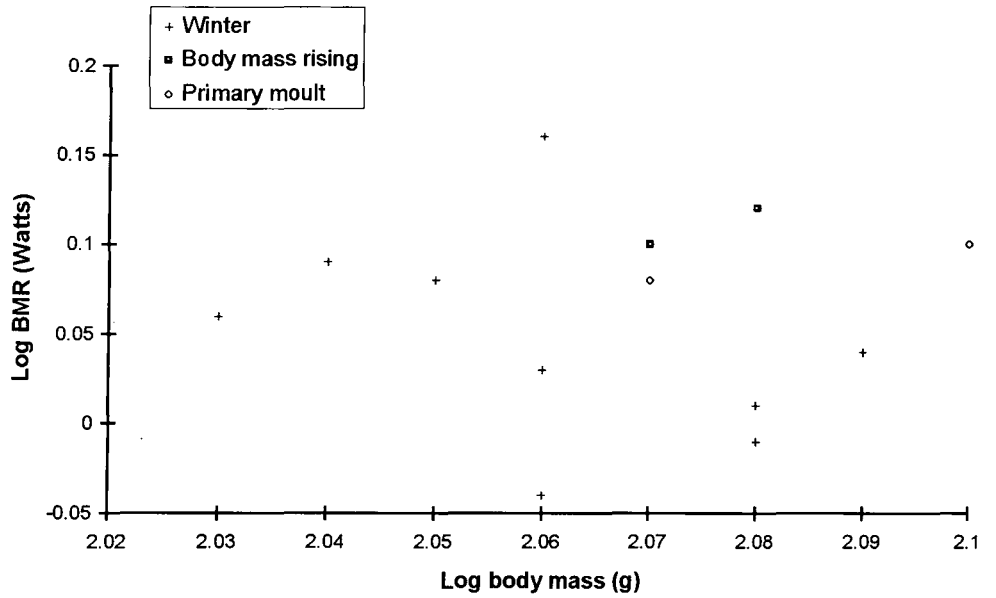


Figure 1.3F: Juvenile Knot GL

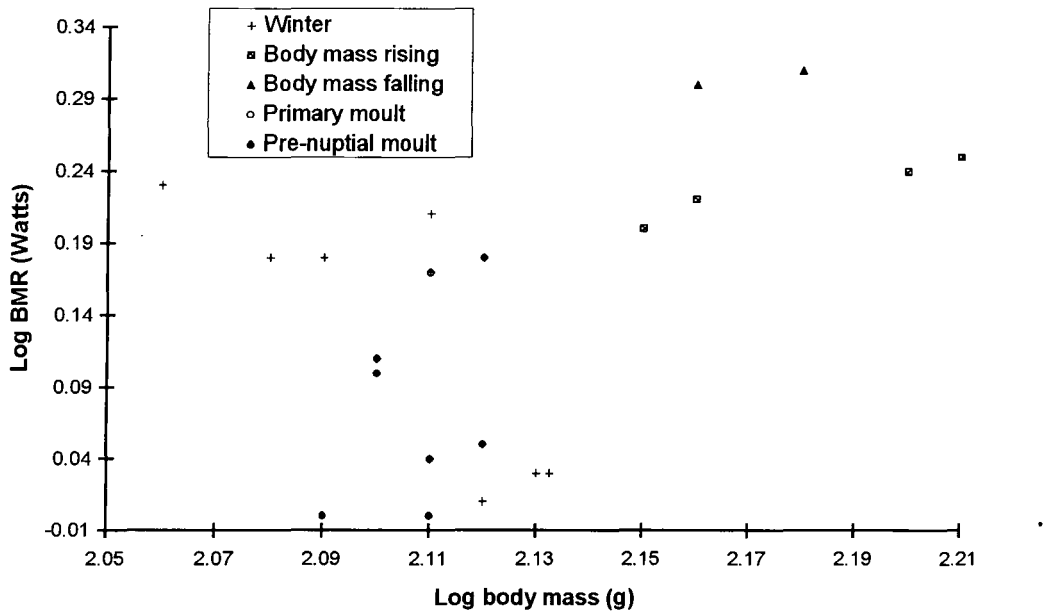


Figure I.3G: Juvenile Knot GO

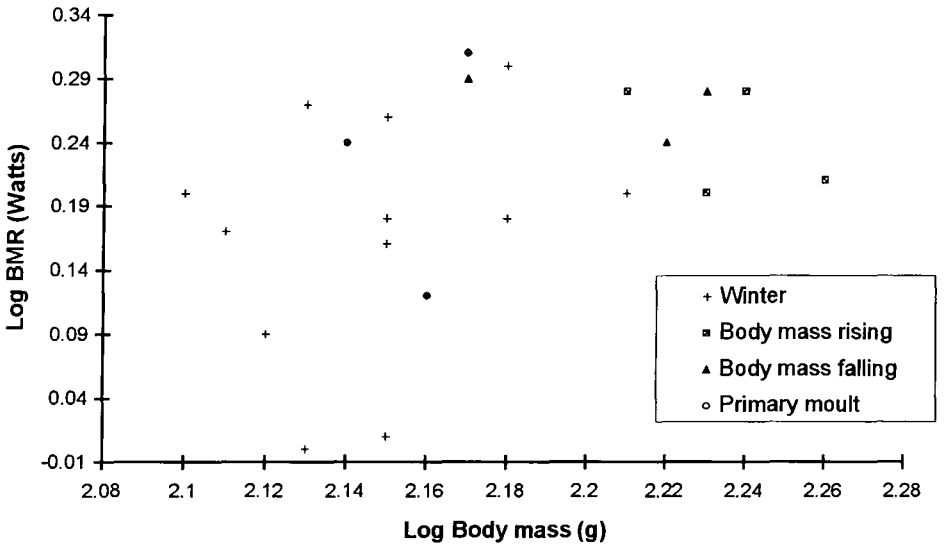


Figure 1.3H: Knot GW

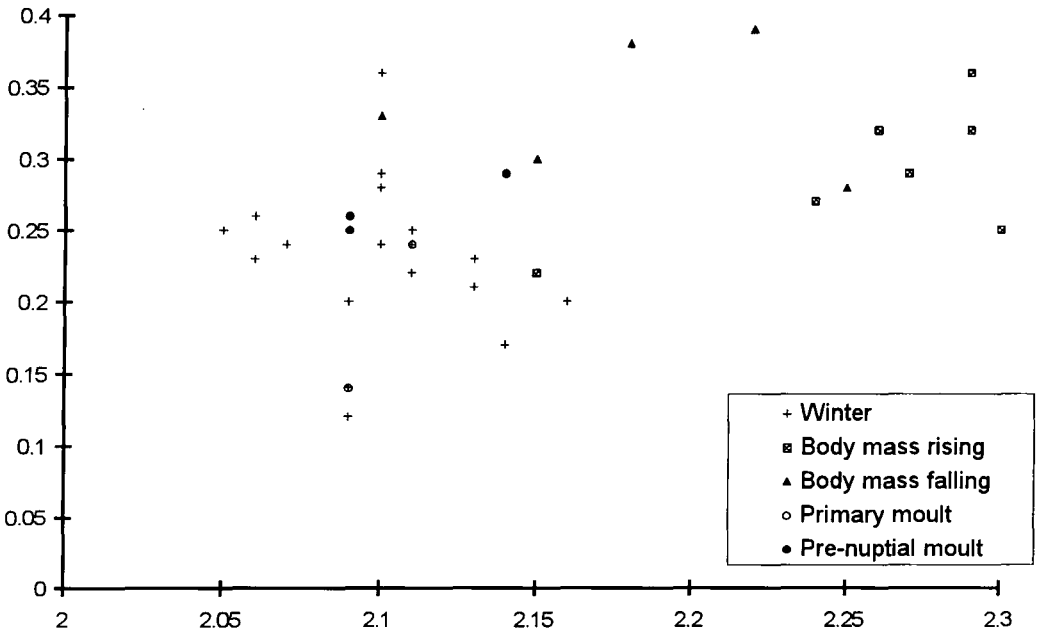


Figure 1.3I: Knot GY

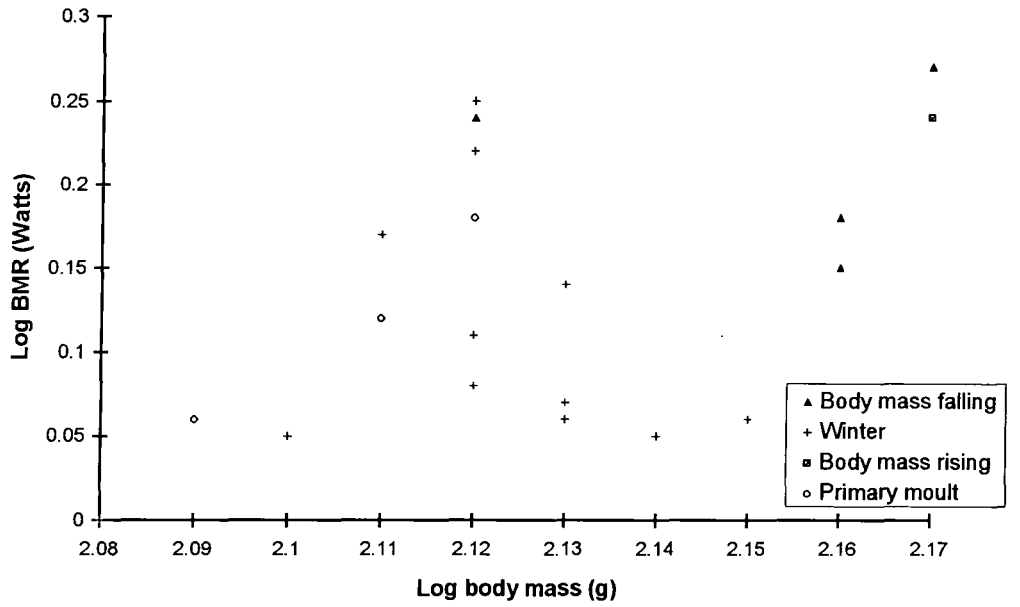


Figure I.3J: Juvenile Knot GF

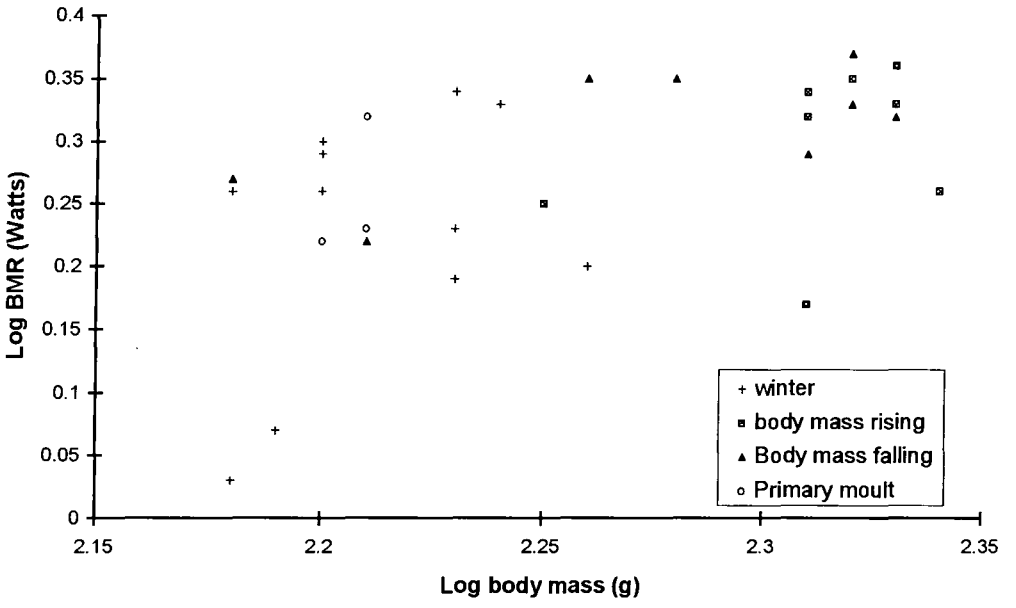


Figure I.3K: Knot WGL

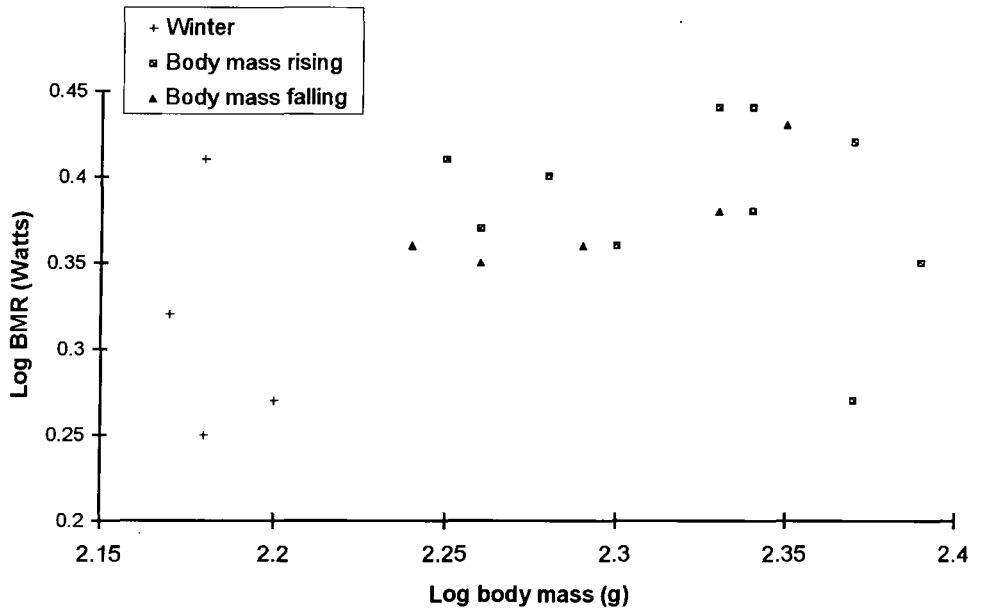


Figure I.3L: Knot WGG

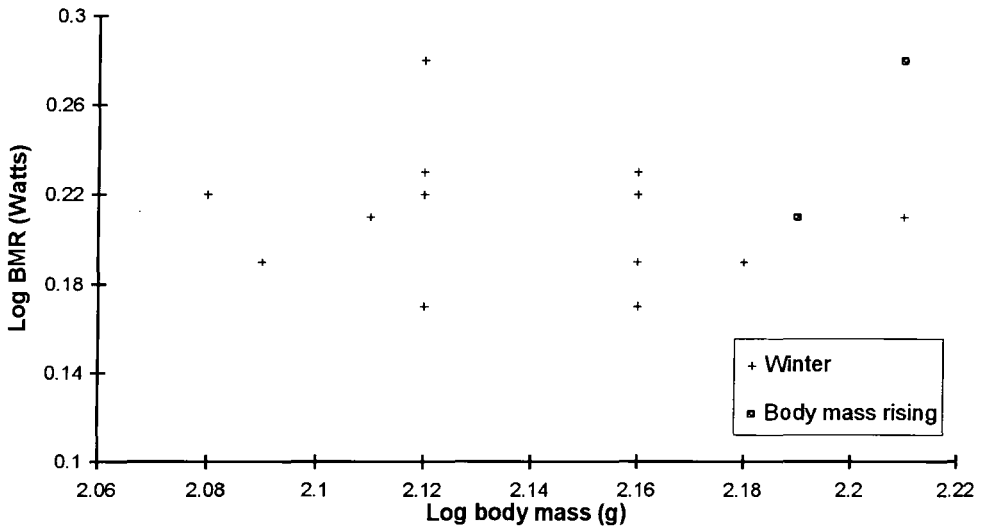


Figure I.3M: Knot WGY

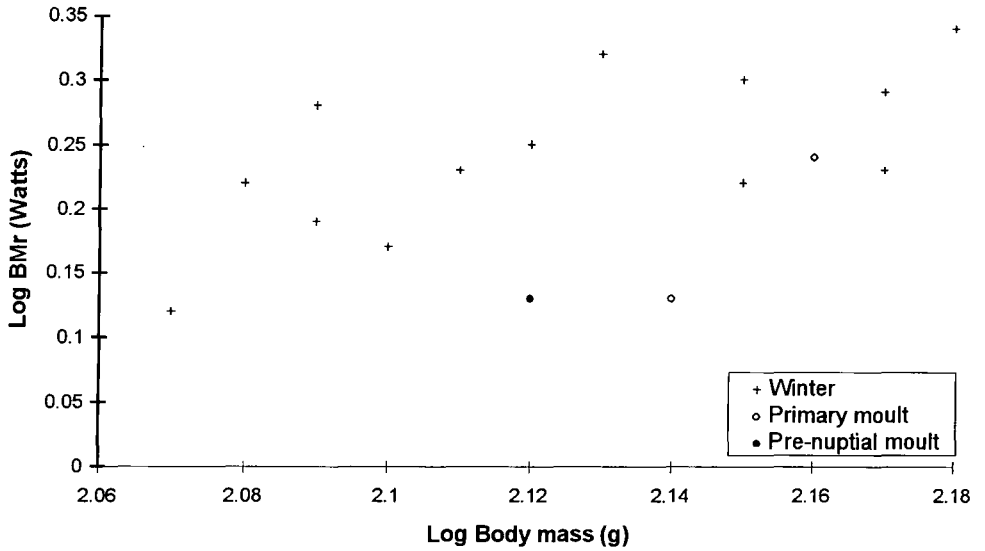


Figure I.3N: Knot WLL

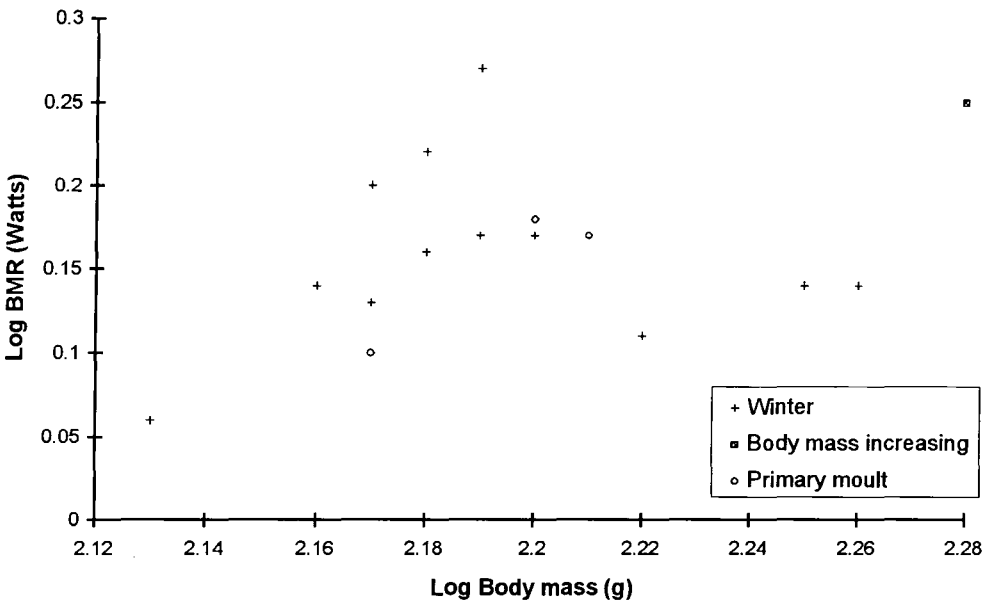


Figure I.3O: Knot WWW

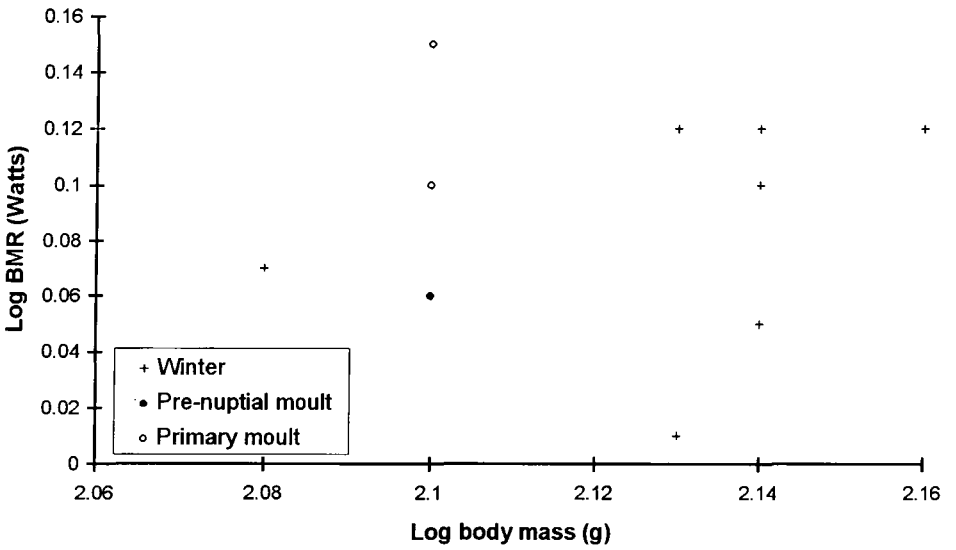


Figure I.3P: Knot WYG

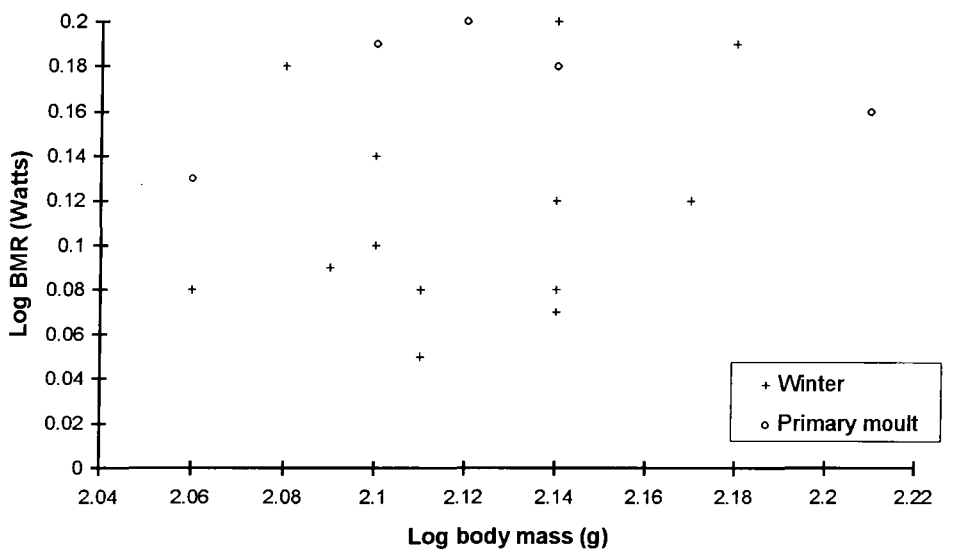


Figure I.3Q: Knot WLG

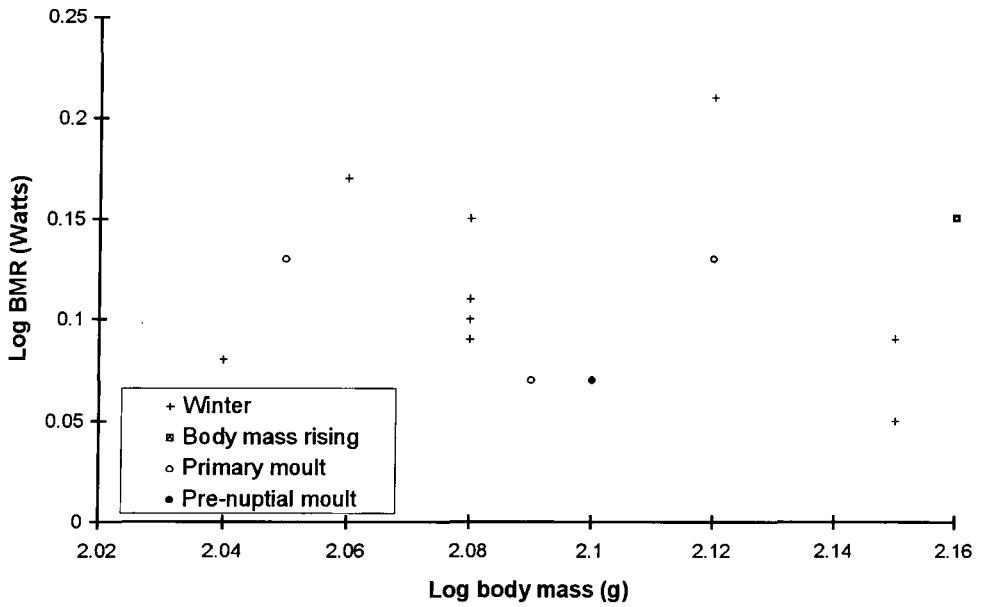


Figure I.3R: Knot WG

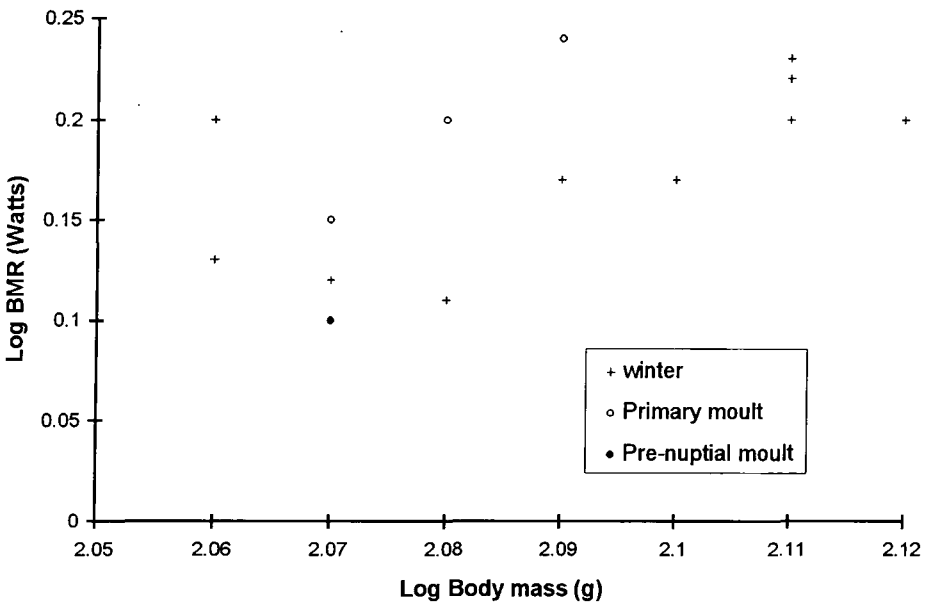
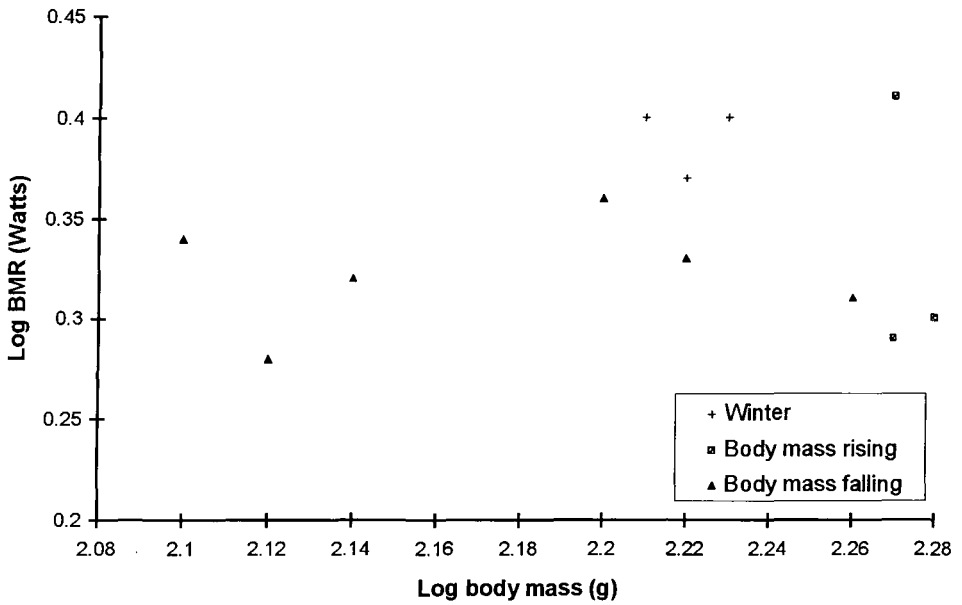


Figure I.3S: Knot WYY



APPENDIX II: Evaluation of previous studies and use of the non-invasive TOBEC (Total Body Electrical Conductivity) procedure for predicting the total lean mass and the total fat mass in live Knot

Introduction

Body composition in many species of birds and mammals is known to vary both on a daily and seasonally cycle. This variation is known to be influenced by many phases of the avian life cycle (Blem & Shelor, 1990), including periods such as reproduction and migration. The ability to measure and follow these changes in total lean mass and in the amount of stored lipids, both between and within individual birds, is central to the understanding of the ecology and physiology of these species.

Traditionally, the technique used to study variations in body composition, with particular reference to shorebirds, has used solvents to extract stored lipids from dried carcasses (Evans & Smith, 1975; Davidson, 1981a; Dobush *et al* 1985). There are however major drawbacks to traditional carcass analysis for quantifying lipid stores, which result in solvent extraction being undesirable under many circumstances. Such limitations include the fact that lipid extraction is time consuming and expensive. Solvent extraction requires the death of the individual, which means, therefore, that the temporal changes in body composition cannot be followed within that individual. A large sample size may also be necessary, which may be impossible if endangered or protected species are involved (Schoech, 1996). Such a constraint on sample size may also lead to inconclusive results.

A wide range of non-invasive techniques have been employed, with differing degrees of success, to estimate the physical condition and the energy reserves in birds. These techniques are attractive primarily because they do not require the death of the bird and therefore subsequent measurement of the same individual can be carried out (Conway *et al*, 1994). These include ultrasound (Sears, 1988), fat scoring (Scott *et al*, 1996), blood chemistry (Le Mayo *et al*, 1981), labelled water dilution space (Nagy & Costa, 1980) and determination of body mass adjusted for size by morphological measurements (Spengler *et al*, 1995).

The most widely used indirect technique to predict body composition in birds has been 'fat scoring', the visual estimation of subcutaneous fat deposits (McCabe, 1943; Conway *et al*, 1994; Scott *et al*, 1995). The important benefits of fat scoring are that it is cheap and quick but it may also be highly inaccurate in some species (Krementz & Pendleton, 1990) and may be prone to inter-observer variability on occasion. Scott *et al* (1995) reported that considerable variation existed in fat mass between individual Ringed plover *Charadrius hiaticula* assigned to the same fat score, and that a large overlap existed in the ranges of fat mass in different fat scores.

Another indirect technique employed has been the use of formulae derived from various morphological features (wing, culmen, tarsus-length), in conjunction with a measure of total body mass, to estimate total lean mass (TLM) and lipid content (Davidson, 1981a; Piersma & Van Brederode, 1990; Spengler *et al*, 1995). Such measurements have been deemed to be too imprecise for study of individual birds, in fact being deemed satisfactory for use only in comparisons between groups of individuals from the same population (Perdeck, 1985). Indeed, Castro *et al* (1990) said that the application of a formula derived by them to predict fat mass (FM) in Sanderling *Calidris alba,* was applicable only to the specific population of birds

from which the measurements were taken. These morphologically based formulae also do not take into account changes in total lean mass occurring within an individual with season, but provide only a single lean mass value estimated from body size (Mitchell, 1996).

An increasingly common method of estimating total lean mass (TLM) and fat mass (FM), is the use of Total Body Electrical Conductivity or TOBEC (Walsberg, 1988; Witter & Goldsmith, 1997). The presence of a restrained animal (Scott *et al*, 1991; Roby, 1991; Skagen *et al*, 1993; Scott *et al*, 1996), within a solenoid coil acts as a conductor and alters the electromagnetic conductance. The electrical conductivity of lipids is around 4-5% that of lean tissue, body fluids and bone (Pethig, 1979). Therefore, the primary determinant of the TOBEC signal output (I) is total lean mass. TOBEC is highly correlated with TLM (Walsberg, 1988; Castro *et al*, 1990; Roby, 1991; Scott *et al*, 1991; Skagen *et al*, 1993; Scott *et al*, 1996) and has been shown to be a reliable predictor of TLM. By subtracting the predicted total lean mass (PTLM) derived from TOBEC from the total body mass, a predicted total fat mass (PFM) of an individual can be determined (Walsberg, 1988). There is a need, however, to calibrate the TOBEC-derived PTLM against the actual TLM derived from destructive carcass analysis. Predictive models derived from single species give better estimates of TLM than those obtained from interspecific models (Scott *et al*, 1991), because body shape is species-specific. The same error is attached to PFM as well as PTLM, but this error for PFM usually represents a greater proportion of the actual FM since TLM is generally greater than FM (Morton *et al*, 1991).

Many models have used TOBEC measures to help predict total lean mass and fat mass. These range from simple linear and second order-order polynomial equations (Walsberg, 1988; Castro *et al*, 1990; Scott *et al*, 1991; Roby, 1991;

Scott *et al*, 1996; Schoech, 1996), to more complicated multiple regression models additionally employing biometric measurements (Skagen *et al*, 1993; Lyons & Haig, 1995). Multiple regression models have also been produced to predict lipid mass directly by using body fat as the dependent variable, in order to evaluate the contribution of TOBEC and body mass for predicting total fat mass (Morton *et al*, 1991; Skagen *et al*, 1993; Asch & Roby, 1995; Mitchell, 1996). These different methods will be discussed later.

Construction of calibration curve for Knot

To derive a regression equation that allows prediction of TLM from measurement of Total Body Electrical Conductivity (TOBEC), it requires a number of animals to be sacrificed immediately after a TOBEC measurement has been taken. TOBEC measurements taken on dead birds are not comparable to those from live birds, even if they are heated to normal body temperature (Scott *et al*, 1991). The actual TLM, obtained by carcass analysis, is then regressed against the electrical conductance or TOBEC index (I). Predictive equations were produced using a sample of 11 adults (see Table 2), held in conditions of captivity between 6-16 months (see Chapter 2). Individuals were weighed on a Pesola balance to the nearest gram, then a TOBEC measurement (model SA-1, EM-SCAN), taken just prior to death by cervical dislocation. Birds were then immediately dissected (following the protocol of Mitchell, 1996), sexed, the carcasses and organs weighed and put into individual sealed plastic bags and frozen at -20°C until carcass analysis could be carried out. Four individuals (WL, YL, OR, GN), had liver and pectoralis major tissue removed for electron microscopy (see Chapter 2). The liver mass was corrected for this loss, assuming that the loss in water and fat was uniform throughout the whole organ.

The overall sum of the dissected organs and the carcass of each individual bird gave the total body mass (BM) of that bird. The organs and carcass were then dried to a constant mass in a vacuum oven at 40°C, their masses then summed to give a total dry body mass (TDBM). Organs were then put back in the appropriate carcass and the carcass underwent lipid extraction using Soxhlet apparatus and petroleum ether as a solvent. Petroleum ether was chosen because it tends to remove fewer polar lipids than other solvents such as chloroform (Dobush *et al*, 1985; Conway *et al*, 1994). In some cases the organs underwent lipid extraction separately from the carcass. Once all of the lipid had been extracted the carcass and organs were dried once again to a constant mass in a vacuum oven at 40°C, and the sum of all the organs and the carcass was calculated to give the total lean dry mass (TLDM). To obtain the actual fat mass (FM), TLDM was subtracted from TDBM and to obtain total lean mass(TLM), FM was subtracted from BM. (See Table 1 for summary of terms).

Another 9 captive individuals (Table 3), were used as independent tests of the predictive powers of the various models employed. Their analyses followed a similar protocol as that above, except that the liver, brain, kidney, gut, stomach, pectoralis major (PM), supracoracoideus and the heart were all dissected out immediately after death (see Chapter 4). None of these individuals were used to provide tissue samples for either electron microscopy or enzyme assays (see Chapter 2). The organs of these 9 individuals were then dried separately and with the exception of the brains, underwent lipid extraction separately from the carcass. Once the dry weights and the lean dry weights of the organs and carcass were known for each individual, the values were summed and the TDBM, TLDM, FM and TLM calculated.

Table 1: Explanation of abbreviations used in Appendix 2.

(Adapted from Mitchell, 1996)

ID	**Bird identification number**
BM	**Total body mass**
I	**TOBEC Index**
TLM	**Total lean mass, derived from carcass analysis**
FM	**Fat mass, derived from carcass analysis BM-TLM**
TDBM	**Total dry body mass**
TLDM	**Total lean dry mass**
$PTLM_1$	**Predicted total lean mass derived from linear regression equation of TLM with I Equation 1**
$PTLM_2$	**Predicted total lean mass derived from second order polynomial curve of TLM with I Equation 2**
PFM_1	**Predicted fat mass from equation 1. TBM-PTLM[1]**
PFM_2	**Predicted fat mass from equation 2. TBM-PTLM[2]**
PFM_3	**Predicted fat mass derived from multiple regression of BM and I Equation 3**

To obtain a predicted total lean mass (PTLM) and an estimate of fat mass (PFM), linear regression and second-order polynomial models were fitted to plots of TLM and the TOBEC index (I). These models in turn gave values of $PTLM_1$, $PTLM_2$, PFM_1 and PFM_2 respectively. In order to evaluate the usefulness of TOBEC and BM for predicting FM, an estimate of fat mass (PFM_3), with an error independent of that associated with predicting TLM, was produced using a multiple regression model which had FM as the forced entry dependent variable and both BM and I as independent variables. (Morton *et al*, 1991; Skagen *et al*, 1993; Mitchell, 1996). The power of the above models to predict total lean mass from TOBEC indices were tested independently against 9 Knot that were not used to produce the equations, that is each of the 9 individuals was tested against each equation and the errors subsequently obtained. A set of truly independent individual points to test the equations is to be preferred to the procedure of cross-validation. (Skagen *et al*, 1993; Mitchell, 1996), although a larger sample size may be required to carry this out satisfactorily.

Results

The equations produced by regressing TLM on I for the 11 individuals whose body compositions are detailed in Table 2, are shown below:

Simple linear:

$$PTLM_1 = (0.199*I) + 64.929 \qquad \textbf{Equation 1}$$

Second-order polynomial:

$$PTLM_2 = (0.182*I) + (0.0005*I^2) + 64.425 \qquad \textbf{Equation 2}$$

The multiple regression equation used to predict PFM^3 from BM and I:

$$PFM_3 = (0.982*BM) - (0.182*I) - 65.5 \qquad \textbf{Equation 3}$$

Independent testing of the above 3 equations yielded mean errors (see Table 3), of 1.4g for $PTLM_1$ and 1.5g for $PTLM_2$. These mean errors represented 1.5% of mean TLM (95.6g).

The mean absolute errors for PFM_1 and PFM_2 were as for $PTLM_1$ and $PTLM_2$, but represented 10.0% and 11.1% of mean FM (22.2g) respectively. The mean error of PFM_3 derived from equation 3 was higher at 2.2g, representing 18.0% of the mean FM.

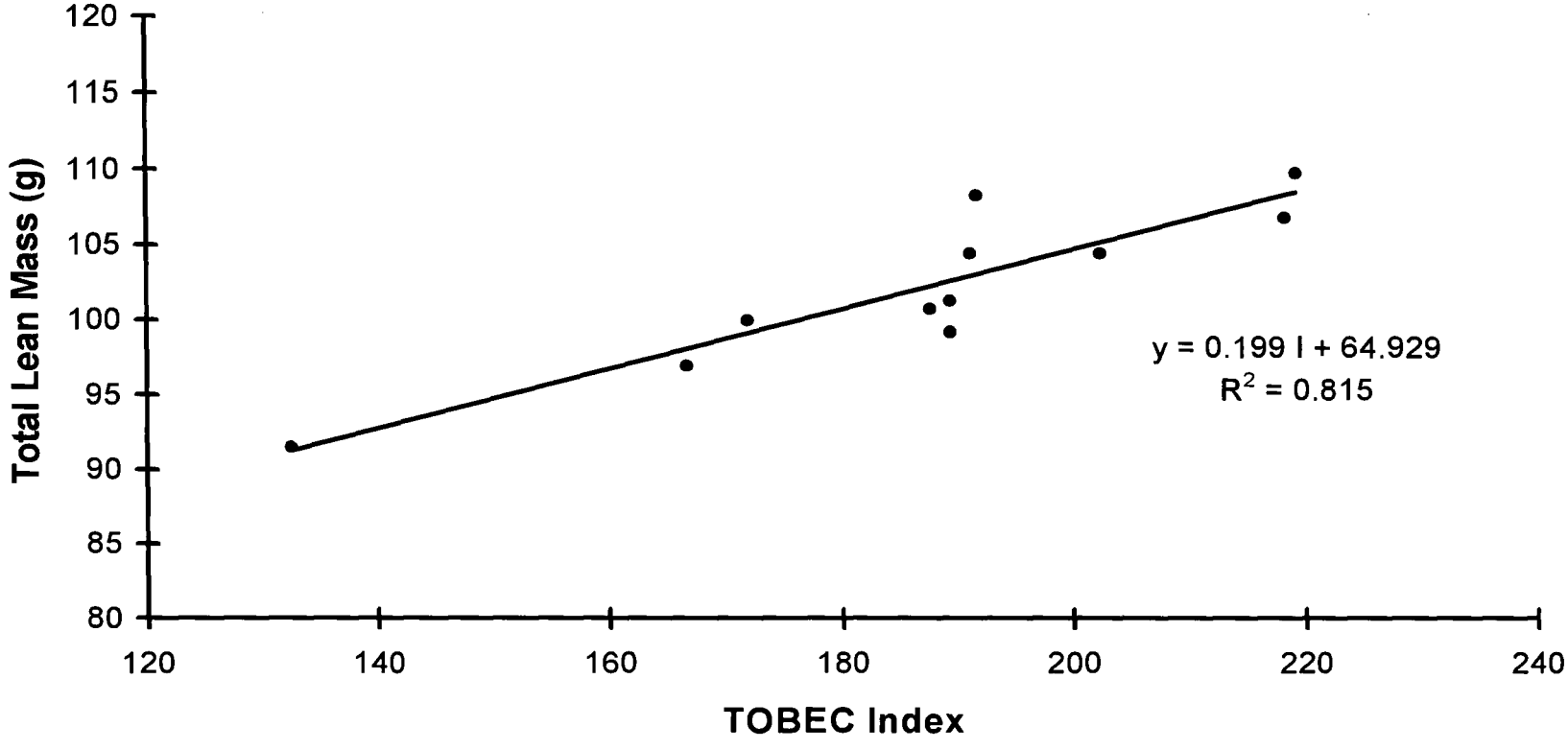
Although the mean error was almost the same for equation 1 as for equation 2, it was slightly lower in equation 1 and therefore, the simple linear regression equation (Fig 1) was adopted for calculation of predicted total lean mass (PTLM) and predicted fat mass (PFM) throughout this thesis.

Discussion

Application of TOBEC to predict total lean mass

The predictive model for Knot that gave the lowest mean absolute error for predicted total lean mass (PTLM), was produced by the linear regression of TLM and TOBEC (Equation 1). The TLM in both equations was treated as the dependent variable (see Walsberg, 1988; Scott *et al*, 1991; Mitchell, 1996). This is in direct opposition to other investigators (for review see Asch & Roby, 1995), who argue that TOBEC readings are subject to 'substantial errors related to the subjects posture and position in the measurement'. While this may be true if attention is not paid to the position in the solenoid of the restrained animal, a very high repeatability of TOBEC measurement can be obtained, as it was in this study,

Figure 1: Relationship between total lean mass (TLM) and TOBEC index



using the SA-1 TOBEC model. Therefore, as the errors from measuring TOBEC were not likely to exceed those errors produced from carcass analysis and as the predictor variable was TLM, it was decided that TLM should be the dependent variable (see Scott 1991; Mitchell, 1996).

The statistical method of choice to test the accuracy of the predictive equations has been cross-validation (Skagen *et al*, 1993; Conway *et al*, 1994; Mitchell, 1996). This procedure, in essence, removes one datum point, from the sample and its PTLM is predicted from the equation produced from the remaining data points. The procedure is then repeated sequentially for all the data points of the sample and an error of difference between the TLM and PTLM is calculated. The advantage of this technique is that it introduces a degree of independent testing, without the need to sacrifice any further individuals (Mitchell, 1996). However, the use of totally independent data points to test the predictive powers of the regression equations and determine the errors produced is the most powerful method of testing. This procedure was carried out by Scott *et al* (1991), using a sample of 5 Starlings *Sturnus vulgaris* to test an intraspecific linear equation.

In my study, I tested the powers of the intraspecific equations to predict TLM, using an independent sample of 9 captive Knot. The absolute mean error (see table 2) produced from using the linear regression model of TLM and TOBEC was 1.4±0.7g (95%CI) over a range of TLM of 91.6-104.8g. Scott *et al* (1991), for Starlings, reported an error of 0.9g over a range of TLM of 65-85g. The 2nd-order polynomial (Equation 2), of TLM and TOBEC produced an absolute mean error of 1.5±0.7g (95%CI), only marginally higher than that of the linear equation. In both predictive equations the range of absolute errors was exactly the same (3.0 - 0.2g).The finding that the lowest absolute mean error of predicting TLM from TOBEC intraspecifically was produced using a linear regression equation

Table 2: Body composition data of 11 Knot used to construct TOBEC calibration curve

ID	BM (g)	%WATER	TLM (g)	FM (g)
WL	131	67.1	108.3	22.4
GN	120	65.6	91.5	28.9
OR	126	66.5	104.9	20.9
YL	114	66.1	103.3	12.3
OG	110	64.5	96.9	12.8
YE	163	63.3	106.8	56.6
WH	153	65.1	100.0	52.9
WO	128	63.8	100.7	26.9
YYY	122	64.8	99.2	23.2
YW	148	64.8	109.8	38.0
LW	155	64.1	104.4	50.6
Mean	134	65.1	102.2	31.4
SE	5.1	0.3	1.5	4.5

Table 3: Independent comparison of the errors obtained between the actual values of total lean mass and total fat mass (TLM, FM) and the predicted values obtained using predictive models derived from using Total Body Electrical Conductivity TOBEC (PTLM, PFAT).

ID	BM g	% WATER	TLM g	TLM-PTLM$_1$ g	TLM-PTLM$_2$ g	FM g	FM-PFM$_1$ g	FM-PFM$_2$ g	FM-PFM$_3$ g	TOBEC INDEX
WG	109	63.0	97.6	+1.2	+1.2	11.3	-1.2	-1.2	-4.5	
JYG	112	63.9	92.3	+0.3	+0.2	20.0	-0.3	-0.2	-3.0	
WGY	112	63.0	98.1	-2.8	-2.9	13.7	+2.8	+2.9	-0.1	
JLL	118	64.5	94.3	-1.8	-1.9	23.8	+1.8	+1.9	-0.9	
WYG	112	61.9	104.8	+3.1	+3.1	7.2	-3.1	-3.1	-5.7	
JYY	120	66.5	96.1	-0.5	-0.6	24.2	+0.5	+0.6	-1.2	
BGG	112	64.2	91.9	+1.1	+0.9	20.5	-1.1	-0.9	-2.3	
JLW	121	65.2	93.9	+2.0	+2.1	26.6	-2.0	-2.1	-1.7	
RGG	144	65.5	91.6	+0.2	+0.2	52.3	-0.2	-0.2	-0.6	
MEAN	118	64.2	95.6	±1.4	±1.5	22.2	±1.4	±1.5	-2.2	
SE	3.4	0.5	1.3	0.3	0.3	4.1	0.3	0.3	0.6	
95% CI	±7.8	±1.2	±3.0	±0.7	±0.7	±9.5	±0.7	±0.7	±1.4	

PTLM$_1$ and PFM$_1$ Derived from linear regression equation
PTLM$_2$ and PFM$_2$ Derived from 2nd-order polynomial regression equation
PFM$_3$ Derived from multiple regression equation
% WATER (Total water content/TLM)*100

was consistent with the findings of other workers (Scott *et al* 1991; Roby 1991; Mitchell, 1996). Second-order polynomial equations are more appropriate for interspecific studies (Walsberg, 1988; Scott *et al*, 1991) when using TOBEC. It is then surprising that the absolute errors produced when independently testing data points against the 2nd-order polynomial equation are only marginally poorer. (This simply shows that the 2nd-order polynomial regression line does not have a tendency to deviate from a linear relationship). This may, in part be due to the 9 Knot used as independent tests of the equations have slightly lower TLM (range 91.6-104.8g), than the TLM of the 11 Knot (range 91.5-109.8), used to produce the equations and the deviation from the line does not occur until higher TLM encountered, i.e. until the curve begins to reach its asymptote.

The absolute error in estimating TLM (TLM-$PTLM_1$) increased as TLM increased (see Fig.2.1); that is the larger the TLM the larger the error of prediction by TOBEC. The fact that absolute error in predicting FM (Fig 2.2) is not related to actual fat mass, shows that TOBEC is reliant only on TLM. There was no significant correlation seen between the absolute error of prediction and % water content of TLM (Fig 3). No correlation was seen between absolute error of prediction and mass being predicted in either TLM or FM by Mitchell (1996).

The resolution of the linear regression model over a narrow range of TLM (91.6-104.6g), in this study was 9.1%±5.3(95%CI). This compares favourably with that found by Mitchell (1996) working on Redshank *Tringa totanus* and using the same SA-1 TOBEC, who calculated a resolution of 9.6%±5.6 (95%CI) over a wider range of TLM (97-142g). This resolution was sufficiently low for me to confidently estimate changes in lean mass both between and within individual Knot. This confidence in the predictive powers of TOBEC can be further backed

Figure 2.1 and 2.2: The absolute error in predicting total lean mass (TLM-$PTLM_1$) and the absolute error in predicting total fat mass (FM-PFM_1), when compared to the actual mass of TLM and FM respectively.

2.1) TLM Correlation = 0.742 P<0.05

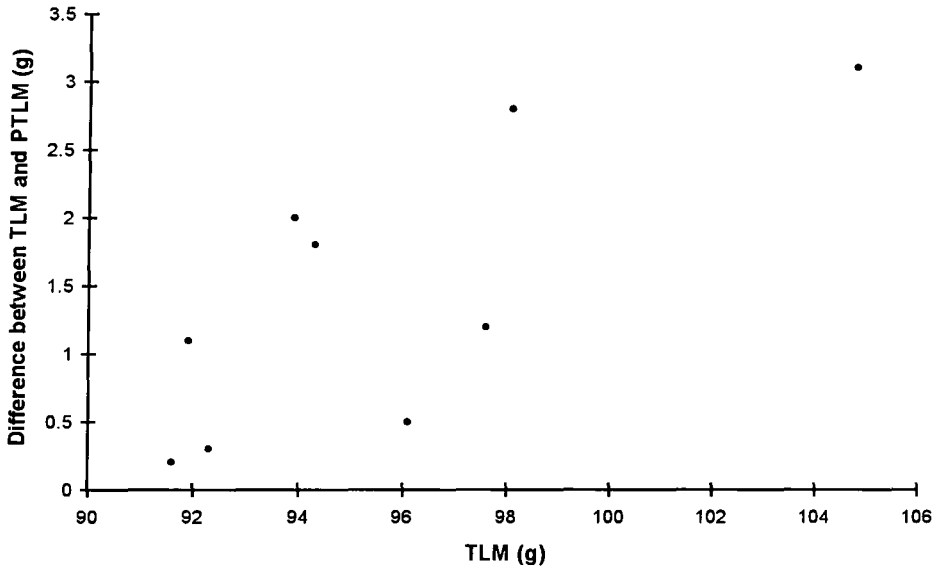


Fig 2.2) FM Correlation = -0.347 P>0.05

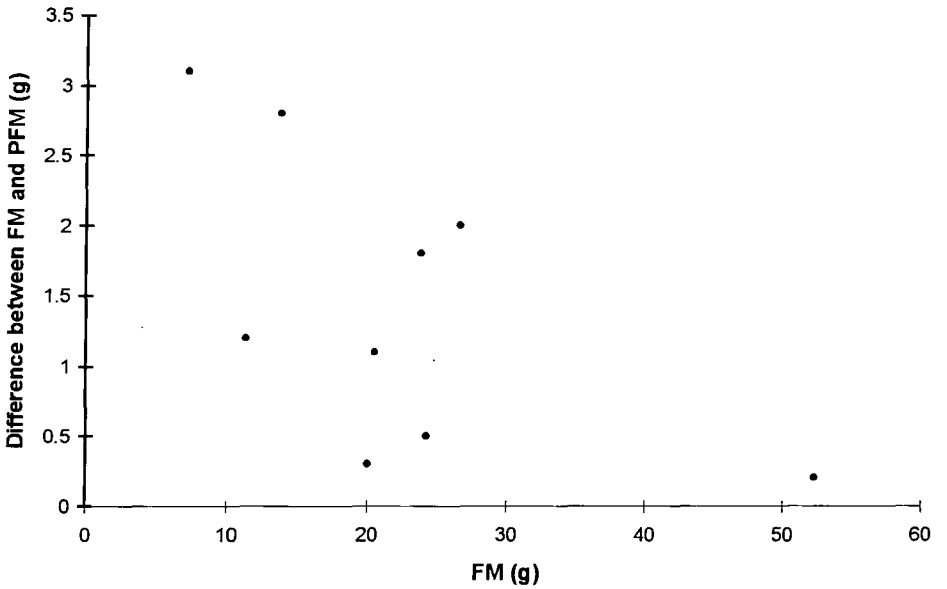
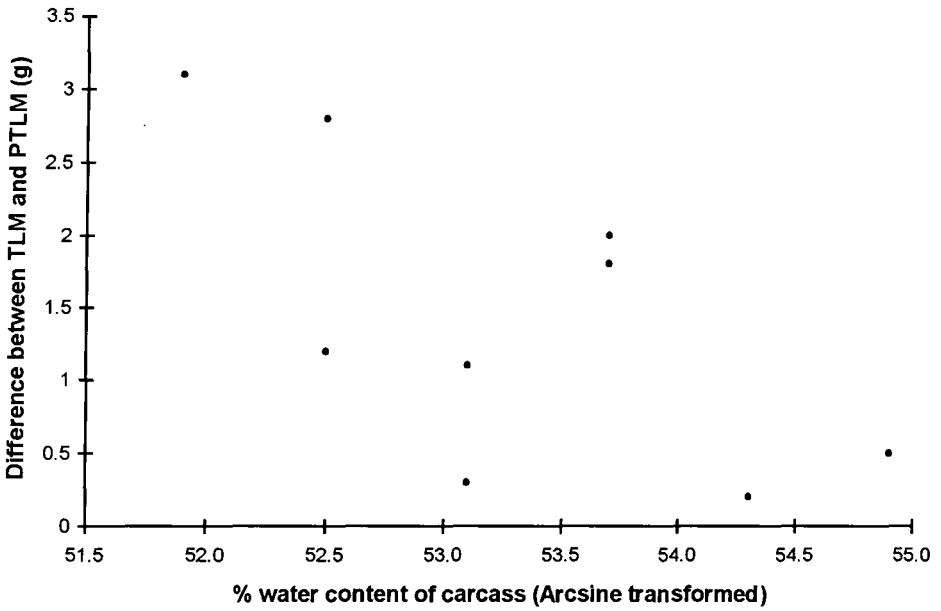


Figure 3: The absolute error in predicting total lean mass (TLM-$PTLM_1$), when compared to the water content (%) of the TLM. (Arcsine transformed)

Correlation = -0.661 P>0.05



up by referring to Appendix 1, which clearly shows that PTLM remained highly stable within individual Knot.

Application of TOBEC to predict total fat mass

The estimation of fat mass in this study was carried out by subtracting the values of TLM predicted by the linear regression ($PTLM_1$) and the 2nd-order polynomial ($PTLM_2$), from TBM to give values of PFM_1 and PFM_2. A third estimate of fat mass (PFM_3), was produced directly by multiple regression with BM and I as independent variables (equation 3). The smallest error was achieved using the linear equation that gave an mean absolute error of 1.4±0.7g, over an actual fat range of 7.2-52.3g (95%CI). This gave a level of resolution of 2.7%±1.3, which was sufficient give confidence when comparing the fat masses between and within individual Knot. The largest absolute errors in predicting FM were obtained using the multiple regression model.

During the past few years much discussion has been generated over the use of TOBEC to predict lipid mass in birds. Many studies (Morton *et al*, 1991; Skagen *et al*, 1994; Conway *et al*, 1994; Meijer *et al*, 1994; Lyons & Haig, 1995; Spengler *et al* 1995), have introduced multiple regression models utilising measures of body mass, TOBEC and various biometrics to help predict lipid mass in birds. Skagen *et al* (1993), stated that multiple regression models using fat mass as the dependent variables (type B models), yielded lower "fat-predictive" errors than models simply involving TLM and I (type A models). Both in my study and in Mitchell's (1996), the opposite was found i.e. the multiple regression approach was less successful in predicting lipid mass than a simple linear

regression model, although no skeletal measurements were included in our multiple regression models.

Much of the confusion regarding the predictive powers of TOBEC must, in part, be due to the many different approaches adopted when determining the whole-body lean and lipid content and the actual use of the TOBEC equipment. As mentioned earlier, the choice of solvent used during carcass analysis to extract fat will affect the TLM and FM obtained. The use of chloroform (Walsberg, 1988; Scott *at al*, 1991; Conway *et al*, 1994; Schoech, 1996; Mitchell, 1996), is thought to extract all lipids including phospholipids and also some non-lipids (Dobush *et al*, 1985; Blem, 1991). Petroleum ether (Meijer, *et al*, 1994; Lyons & Haig, 1995; Asch & Roby, 1995; this study), and ethyl ether (Morton *et al*, 1991), on the other hand remove triglycerides only. A mixture of chloroform and petroleum ether was used by Castro *et al* (1991). Therefore, TLM will be underestimated slightly when using chloroform and overestimated slightly when using petroleum ether and ethyl ether. Since chloroform removes some non-lipids, it was decided in this study that petroleum ether was the solvent of choice.

The temperature at which the carcasses are dried prior to and after solvent extraction may also be a source of error in calculating TLM. It has been reported by Blem (1991), that volatile lipids will evaporate in ovens at temperatures over 60-70°C (see Roby, 1991; Castro *et al*, 1990 Meijer, *et al* 1994). To prevent the evaporation of lipids during vacuum oven drying in this study and in the studies of Scott *et al* (1991) and Mitchell (1996), a temperature of 40°C was employed. Another possible source of error in calculating TLM is through not using the entire carcass during solvent extraction, other studies have used a homogenised aliquot of tissue(Walsberg, 1988; Roby, 1991; Morton *et al*, 1991; Conway *et al*, 1994) from a tissue sample as little as 1-2g (Walsberg, 1988). The likelihood of

errors in estimating TLM arising must increase when using aliquots of tissue as small as 1-2g.

Body temperature of the subject is also known to affect the TOBEC reading (Walsberg, 1988), although surprisingly this was not found by Conway *et al* (1994). Therefore, the results obtained by Meijer *et al* (1994), Schoech (1996) and Witter & Goldsmith (1997) must be treated with caution because they anaesthetised subjects prior to measurement of TOBEC to assure that all individuals were positioned uniformly within the instrument. Anaesthesia will cause a lowering of body temperature and thus reduce TOBEC for a given lean mass. This may be why their curve had a lower elevation than that produced by Scott *et al*, (1991) for Starlings *Sturnus vulgaris* (Mitchell, 1996). Many workers have also reported problems with fluctuating TOBEC readings when measuring individual birds. Conway *et al* (1994), recorded mean TOBEC readings from 16 replicate measurements on live birds. In my study, TOBEC measurements were repeated only 4 times for each subject with very little fluctuation between each reading. If the animal is properly restrained and the position of the animal is consistent within the TOBEC apparatus, there is no need for more than 4 readings to be taken for each individual.

Appendix III- The effects of captivity on body mass composition and body mass lean components

Introduction

It is well known that many species of shorebird exhibit seasonal fluctuations in body mass, caused by variations in both lean and fat mass components. Shorebirds in the wild, such as Dunlin *Calidris alpina*, Knot *Calidris canutus* and Redshank *Tringa totanus,* show fairly predictable body mass changes during the winter (see Pienkowski *et al,* 1979; Dugan *et al,* 1981; Davidson, 1981; Scott *et al,* 1994; Mitchell, 1996). A variety of shorebird species have also been studied successfully when kept captive (Scott, 1991; Cadee, 1992; Goede 1993; Melter & Bergmann, 1996; Scott *et al,* 1994; Piersma, 1994 ; Mitchell, 1996; this study), and their body masses have been seen to follow closely the seasonal pattern of changes seen in wild conspecifics, although the timing and intensity of fattening is not always exactly the same as that seen in the wild (see Goede, 1993; Melter & Bergmann, 1996).

Scott *et al* (1994) and Mitchell (1996), showed that there was no difference in the magnitude of the seasonal body mass changes occurring in wild Redshank wintering on Teesmouth and in captive Redshank taken from that estuary, and that the patterns over time of body mass change of the two groups were significantly correlated. However these workers also showed, by using Total Body Electrical Conductivity (TOBEC, see Appendix II), that although the overall body mass did not differ significantly between the two groups, the body composition did. There was a significant reduction in predicted total lean mass (PTLM) in Redshank examined after one month in captivity, balanced by a significant increase in predicted fat mass (Scott *et al,* 1994), and predicted lipid index (Mitchell, 1996). Body compositions of captives remained thereafter

significantly different from those of wild conspecifics, i.e. the former had lower PTLM and higher predicted fat mass (PFM) and predicted lipid index (PLI).

The reduction in lean mass following introduction of wild birds into captivity has been well documented in Knot (Piersma *et al*, 1995), and in Redshank (Mitchell, 1996). These workers compared the masses of various organs in wild and captive birds. They showed that this reduction in total lean mass in captivity was caused primarily by reduction in the masses of the liver, kidney, gut, and stomach. These organs have been given the term the 'digestive organs' (Piersma 1994) or 'nutritional organs' (Piersma *et al*, 1995). A large decrease in the cross-sectional area of the gizzard was reported in captive Knot (Piersma 1994), when compared to wild conspecifics. They reported that gizzard mass could be modified in two ways:

1) Mechanically due to endurance training or disuse atrophy and/or
2) Chemically due to endocrine and/or neural mechanisms.

This reduction in the mass of the gizzard is probably why even 'hungry' captive Knot took time to re-adapt to eating hard-shelled prey after being fed on soft artificial food (Piersma *et al*, 1993). Mitchell (1996) also postulated that the reduction of intestine mass and length in captive Redshank may be due in part to the provision of softer prey in captivity than eaten in the wild. A soft food diet is likely to be easier to absorb through the gut than hard-shelled molluscs and this may lead to a shortening of the gut because a large area for absorption is not necessary. There is evidence that wild birds can adjust gut morphology to suit food type, availability, quality and feeding rate (Ankney, 1977; Heitmeyer, 1987), and therefore 'control' the rate of nutrient absorption and metabolism as and when required (Scott *et al*, 1994). It has been shown that House wrens *Troglodytes aedon*, that underwent forced exercise and exposure to subzero temperatures increased their stomach and intestine mass by 10% and 35% respectively (Dykstra & Karasov, 1992).

Changes in body composition (lean mass and fat mass), as mentioned above, are known to take place at some time within a month of introduction of Redshank into captivity (Mitchell, 1996), but it is not known how quickly these changes take place. Such changes in body composition have very important implications for studies that measure metabolic rate, since it is imperative to know how quickly body composition stabilises. The following work was carried out primarily to answer this by measuring total body electrical conductivity (TOBEC) of two groups of Knot at intervals during their first month in captivity. A comparison of various organ masses was also carried out to see what differences, if any, developed between wild and captive Knot.

An additional study was also carried out on two groups of Knot, caught in different years that had significantly different mean predicted total lean masses (PTLM) at capture, to check whether they maintained these differences in PTLM in captivity. Convergence in organ size and overall lean mass in captivity was reported between captive Knot of the geographical races *islandica* and *canutus* (Piersma *et al*, 1995) and is quoted as an example of metabolic flexibility in birds.

Methods

General

All birds were caught under licence and kept under the conditions described in section 2.1. The protocol for the measurement of Total Body Electrical Conductivity (TOBEC) followed that of section 2.2 and Appendix II.

Body composition

13 birds (7 juveniles & 6 adults) were captured at Teesmouth on 6/11/95 (Group A) and 10 all adults 7/1/97 (Group B). A TOBEC measurement of each was taken in the field within 2 hours of capture. No further measurements of TOBEC were taken until birds had spent 14 days in captivity because it was deemed necessary to give them a period to adjust to the conditions of captivity without suffering any handling stress. A TOBEC measurement of each individual was then taken weekly thereafter.

Organ mass

The protocol for the weighing of dissected organs and muscle blocks followed that of Section 2.4. Fifteen wild Knot in total, caught on 6/11/95 (n=5), 01/3/95 (n=5) and 04/3/96 (n=5), were sacrificed in the field and then brought back to Durham University where they were dissected. Intestine length was measured to the nearest millimetre using a ruler. Three captive Knot from group A were sacrificed in mid-December 1995 and another 10 also from group A, at different dates throughout May and June 1996. 8 captive Knot from group B were sacrificed during June 1997. They were dissected and organ sizes measured as before.

Results

Effects of captivity on body mass and body composition

Body mass, PTLM, PFAT and PLI were compared between entry into captivity (week 0) on 6/11/97 (Group A) or 7/1/97 (Group B) and at the start of weeks 2 (14 days), 3 (21 days) and 4 (28-30 days). Sizes of body mass components at weeks 2, 3 and 4 were expressed as percentages of the values recorded at week 0 for each individual bird, and mean percentages calculated for each group of birds.

Group A

From the graphs (A1-A13), it can be seen that each bird's body mass decreased rapidly after entry into captivity on 6/11/97 (week 0) till week 2 (exceptions being juvenile Knot WGG and WLG). Body mass then tended to increase by week 4 to levels similar to or exceeding levels seen on entry into captivity. The mean total body mass of the 13 birds was significantly less (ANOVA, $F_{3,48} = 4.063$, $P<0.05$), during week 2 (91%) and week 3 (93%), than during week 0 (100%) or week 4 (100%), (Student-Neuman-Keuls test [SNK]), $P<0.05$). Week 0 and week 4 were not significantly different from each other (SNK, $P>0.05$).

Predicted total lean mass (PTLM) decreased markedly in all individuals within two weeks of entry into captivity. The mean PTLM was significantly less (ANOVA, $F_{3,48} = 128.395$, $P<0.0001$) during week 2 (88%), week 3 (86%) and week 4 (87%) than at week 0 (100%), (SNK<0.05). Predicted fat mass (PFAT) however was significantly higher (ANOVA, $F_{3,48} = 6.079$, $P<0.01$) during week 4 (172%), than during week 1 (100%), week 2 (103%) and week 3 (133%). Lipid

Figures A1 to A13 : The changes exhibited in body mass and body composition seen over a 4-8 week period in 13 Knot brought into captivity on 6/11/95 (Group A).

(Day 0 = day of capture in the field)

Fig A1: Knot LG

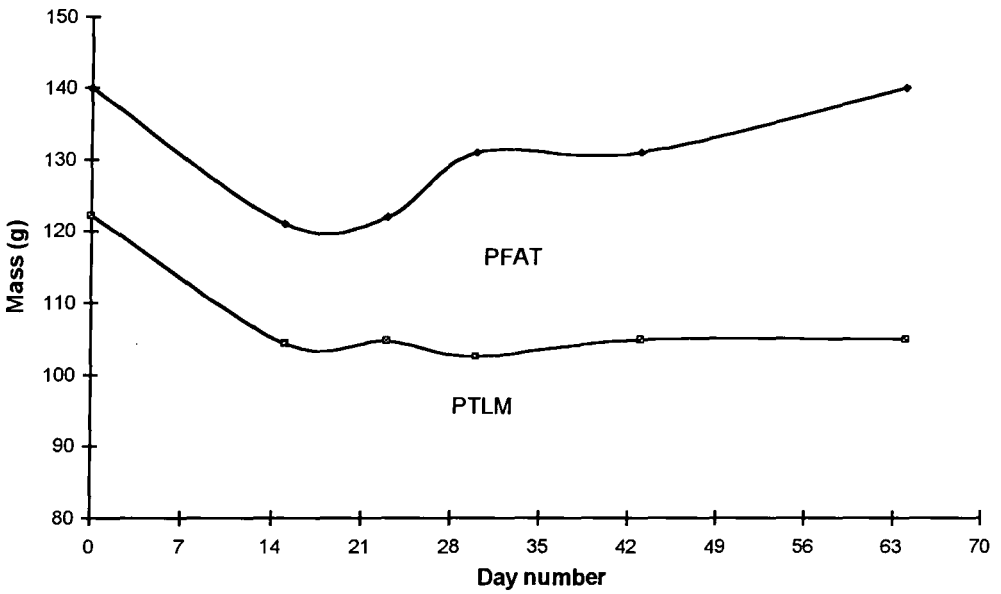


Fig A2: Knot LY

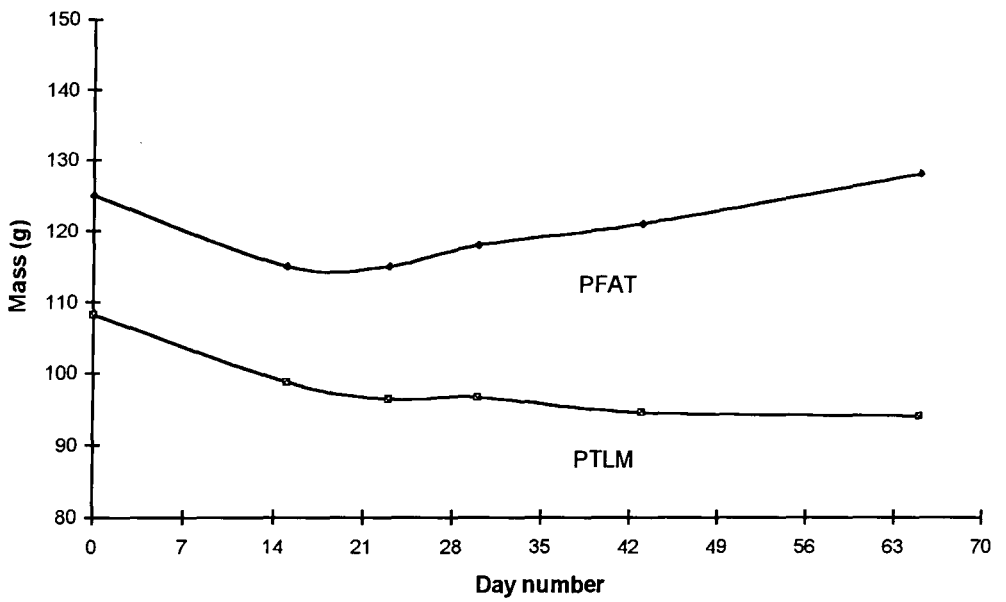


Fig A3: Knot WO

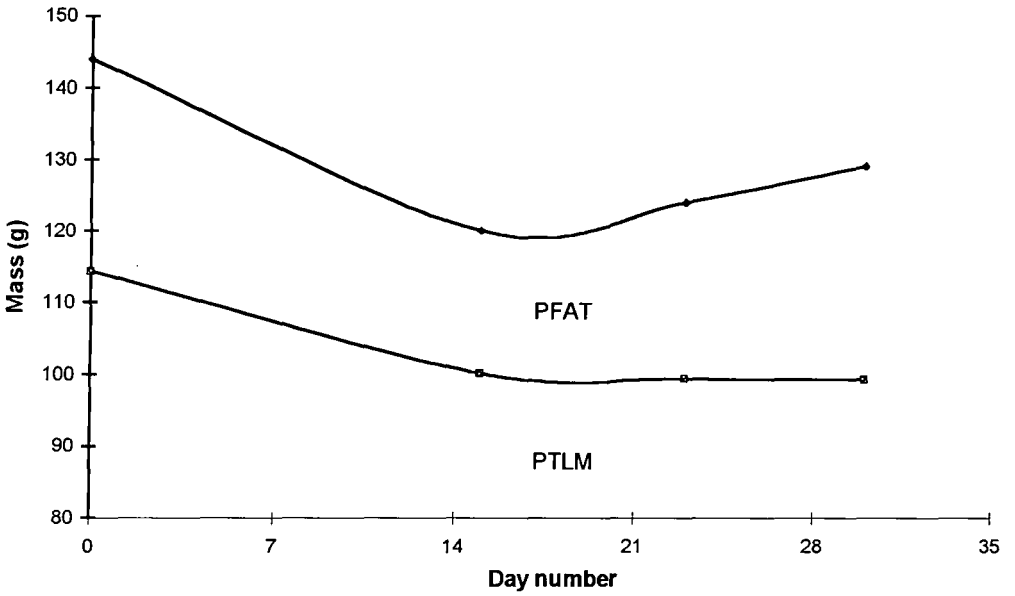


Fig A4: Knot YG

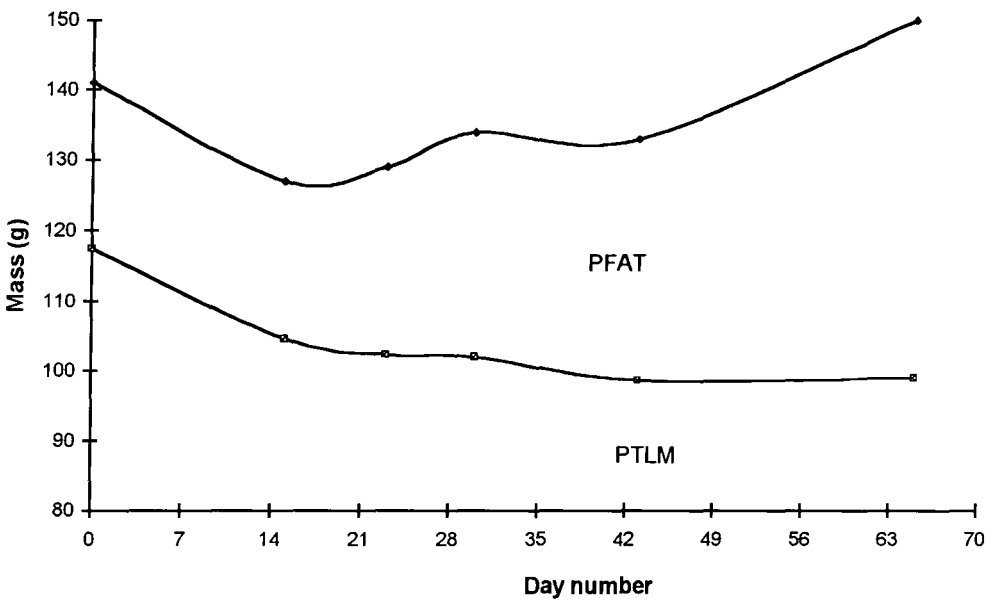


Fig A5: Knot YYY

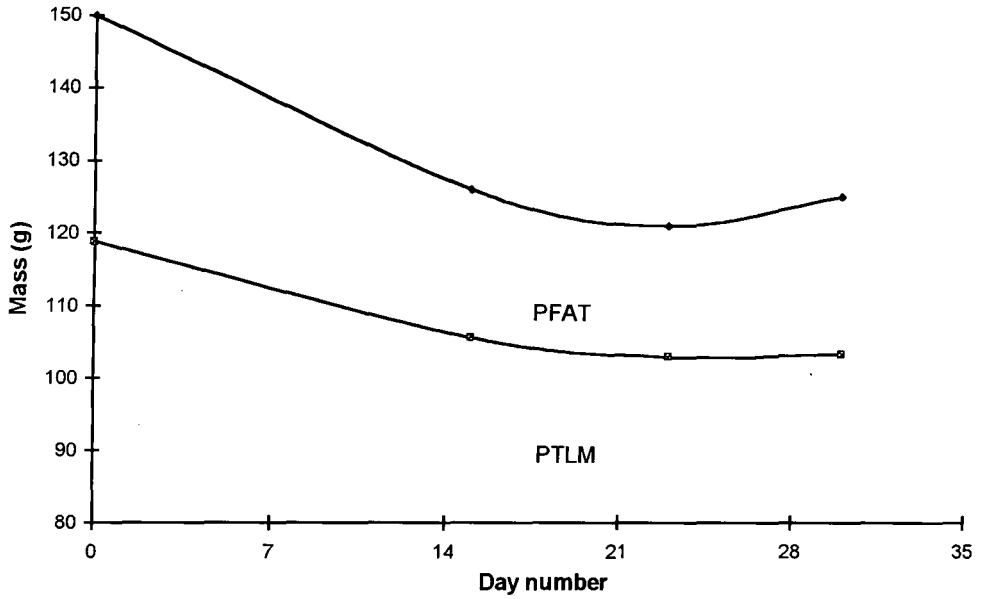


Fig A6: Knot YW

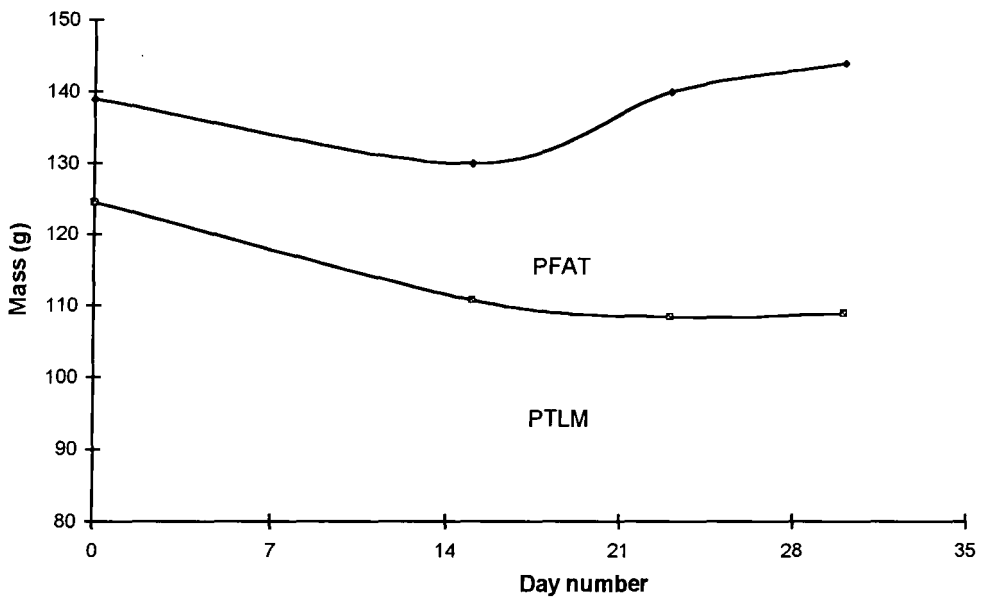


Fig A7: Knot WGG

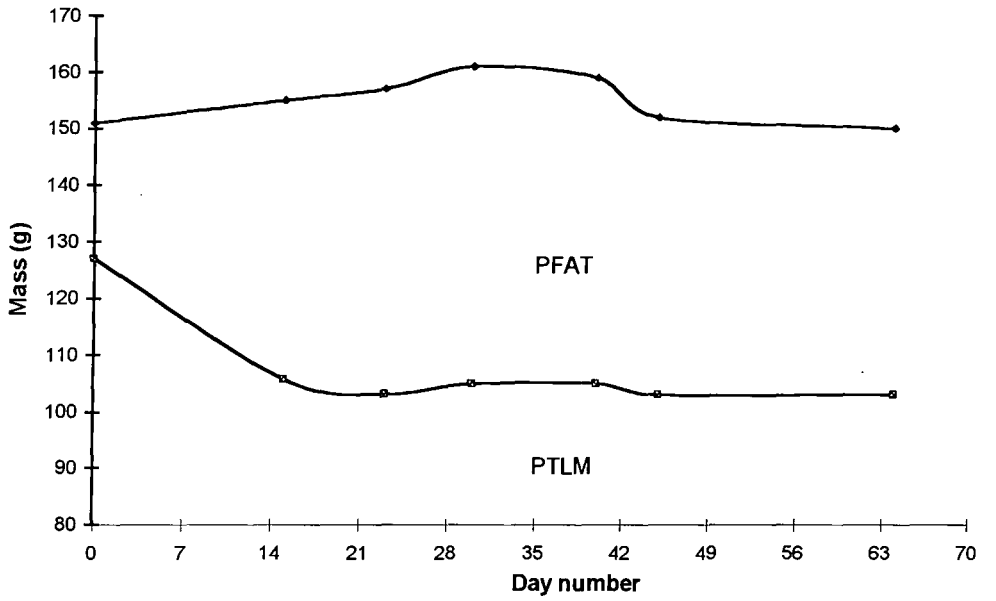


Fig A8: Knot WGY

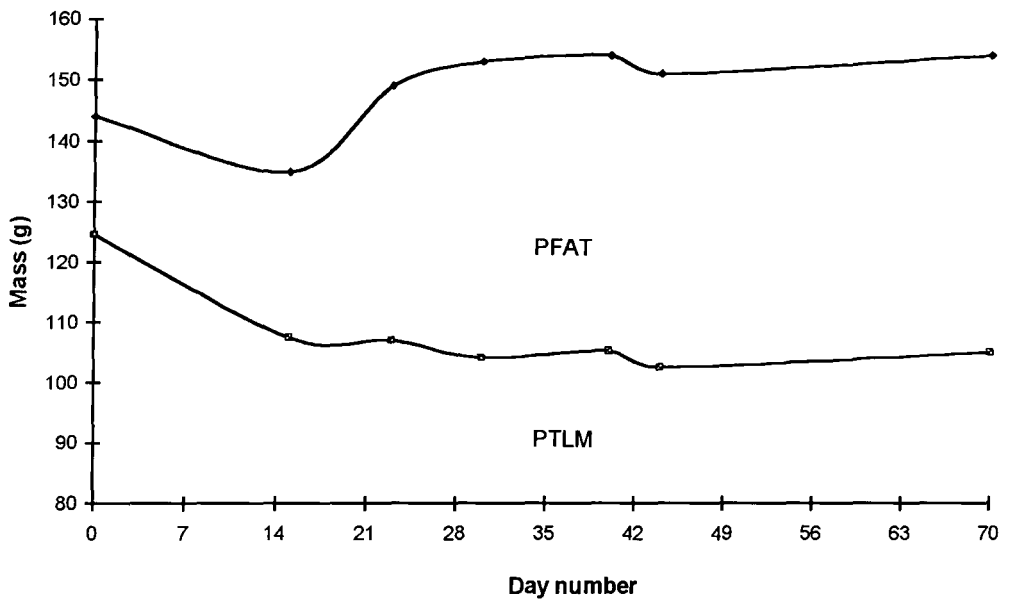


Fig A9: Knot WLG

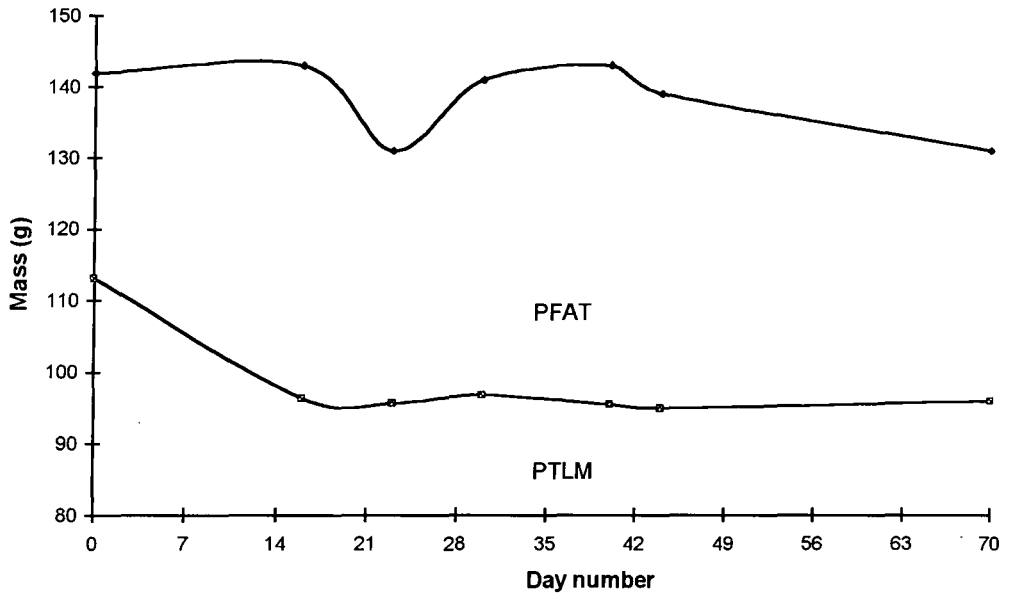


Fig A10: Knot WLL

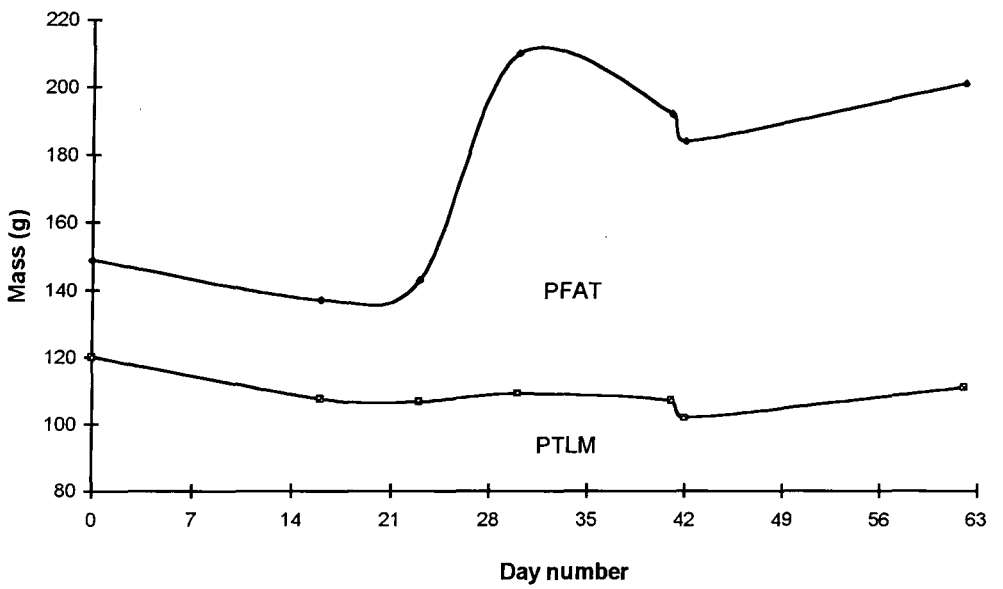


Fig A11: Knot WWW

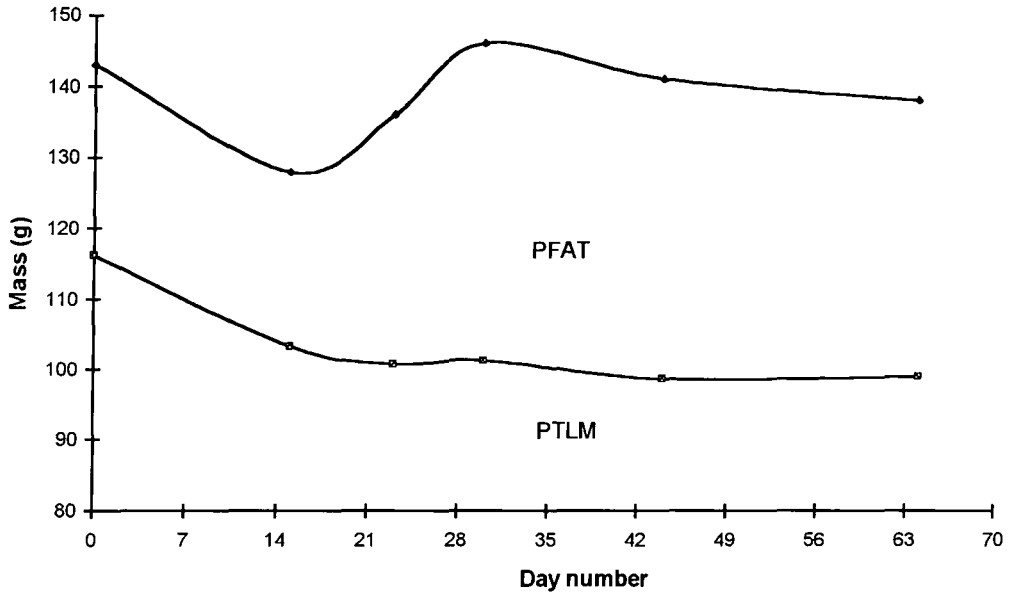


Fig A12: Knot WYG

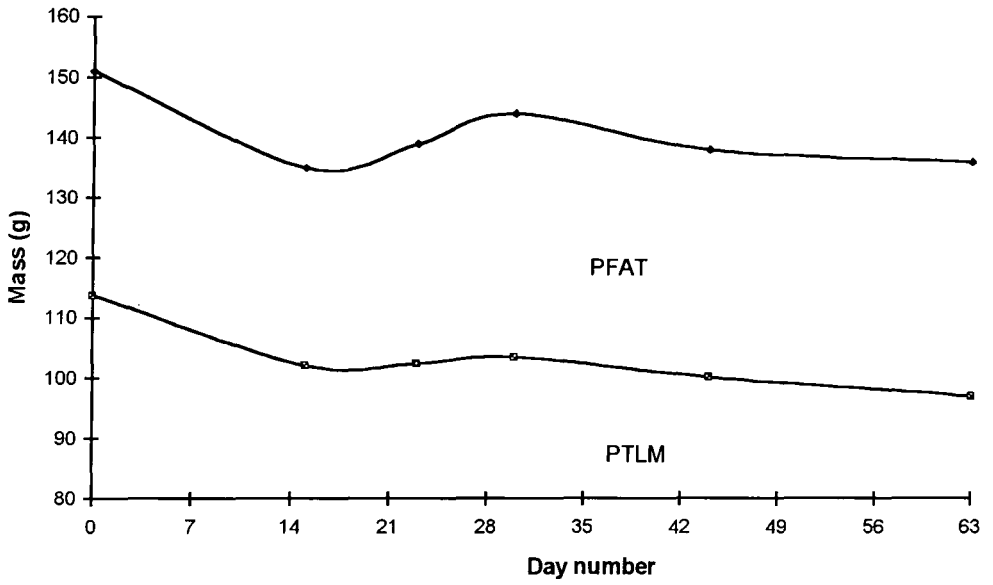
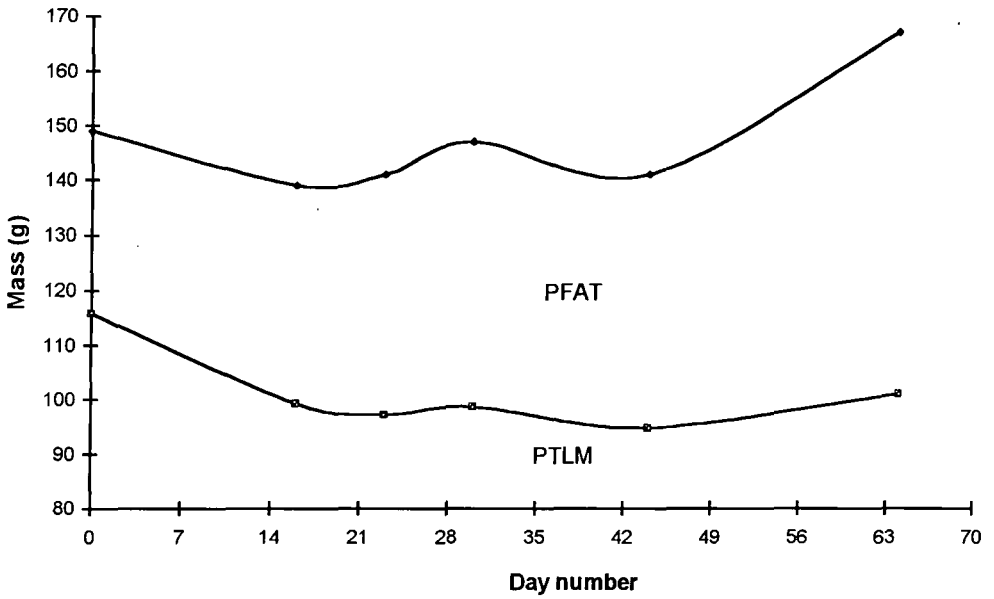


Fig A13: Knot WYY



indices also increased significantly with time (ANOVA $F_{3, 48} = 7.029$, $P<0.001$). Lipid index at week 4 (127%) was significantly higher than that at week 0 (100%) and week 2 (109%). The lipid index at week 3 (117%) was also significantly higher than at week 1 (SNK<0.05). (See Table 1 for comparison of body mass components between week 0 and week 4).

There was no significant difference in mean body mass between 22 adult Knot and 20 juvenile Knot caught in the wild on 6/11/97, from which a sub-sample (Group A) were taken into captivity (Log transformed $T_{40}=0.339$, $P>0.05$). The range and the variance (S^2) in body mass seen in juvenile Knot on this date was greater than that seen in the adults (range =118-168g, $S^2 = 171$; and range =125-154g, $S^2 = 64$ respectively). There was also no significant difference in PTLM ($T_{40} =0.579$, $P>0.05$) and PFAT (Log transformed $T_{40} =0.035$, $P>0.05$), although again the ranges and variances in the juvenile Knot (PTLM range= 104-131g, $S^2 =48$; PFAT range= 8-46g, $S^2 = 79$) were greater than those seen in adults (PTLM range= 106-124g, $S^2 =25$; PFAT range= 15-35g, $S^2 = 37$).

Group B

Graphs A14-A23 show that the body mass of each bird (except RGG) in this group also decreased between entry into captivity on 7/1/97 and week 2. As with group A, body mass then increased to levels in week 4 similar to those seen at week 0. The mean mass of the 10 birds was significantly lower (ANOVA $F_{3, 36} = 10.570$, $P<0.0001$), during week 2 (88%) and week 3 (92%), than during week 4 (99%) and week 1 (100%). Week 1 and 4 were not significantly different to each other (SNK, $P<0.05$). Mean PTLM was significantly higher (ANOVA $F_{3, 36} = 18.393$, $P<0.0001$) at week 0 (100%) than at entry to weeks 2 (95%), week 3 (93%) and week 4 (93%). Mean PFAT was also significantly higher (ANOVA $F_{3, 36} = 8.416$, $P<0.001$) during week 4 (129%), than week 0 (100%), week 2 (63%)

Figures A14 to A23 : The changes exhibited in body mass and body composition seen over a 4 week period in 10 Adult Knot brought into captivity on 7/1/97 (Group B).

(Day 0 = day of capture in the field)

Fig A14: Knot BGG

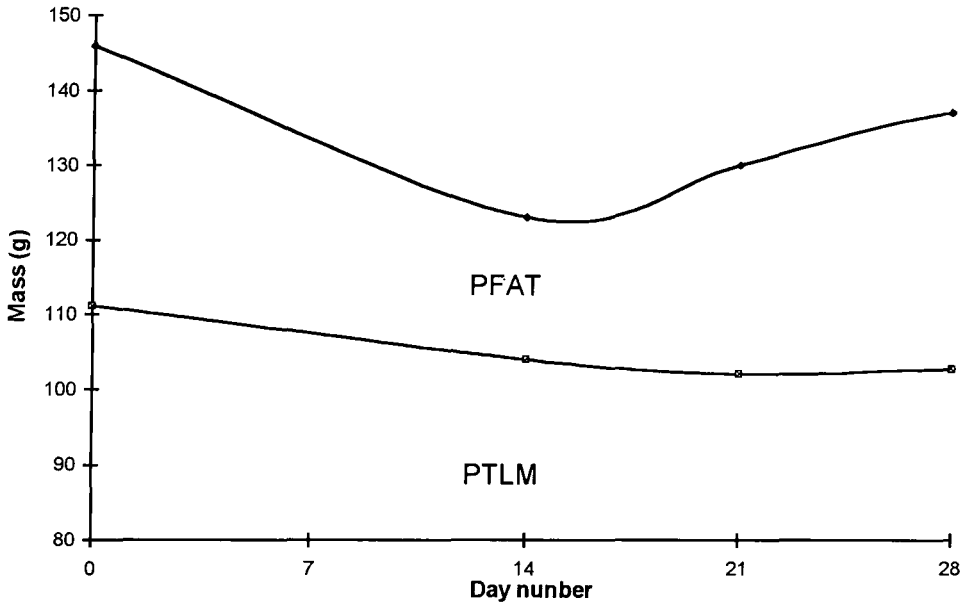


Fig A15: Knot BYY

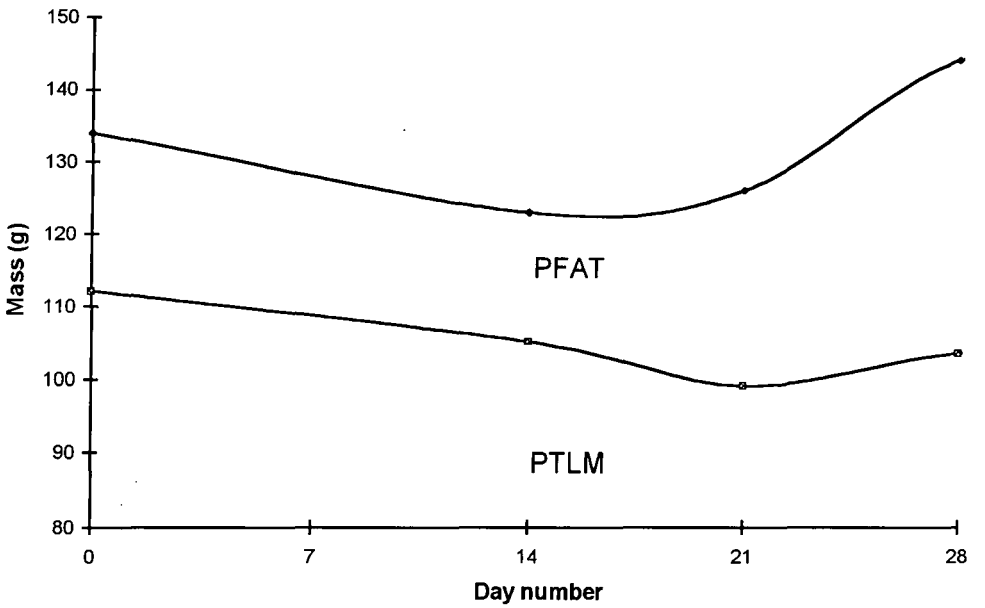


Fig A16: Knot JLL

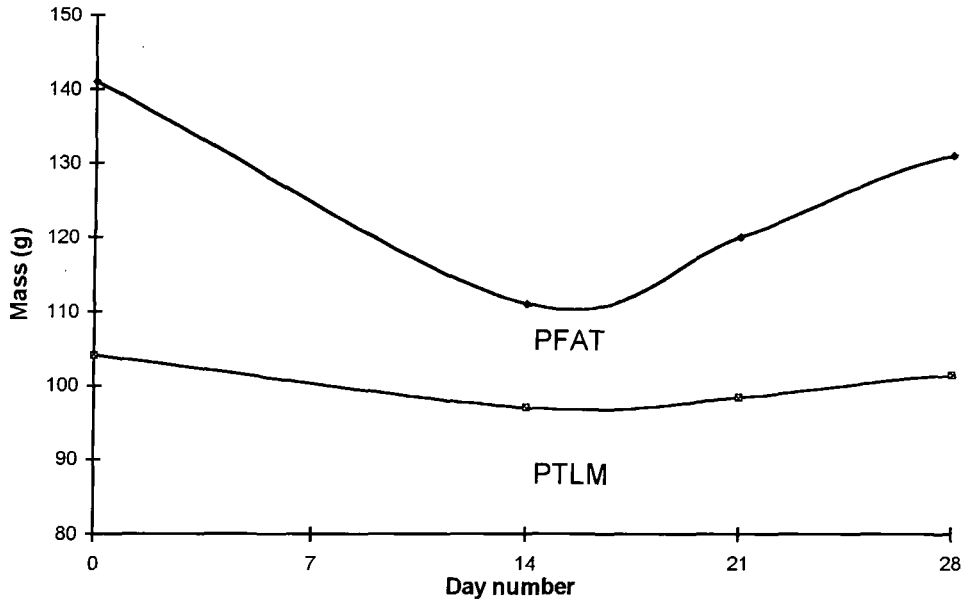


Fig A17: Knot JLW

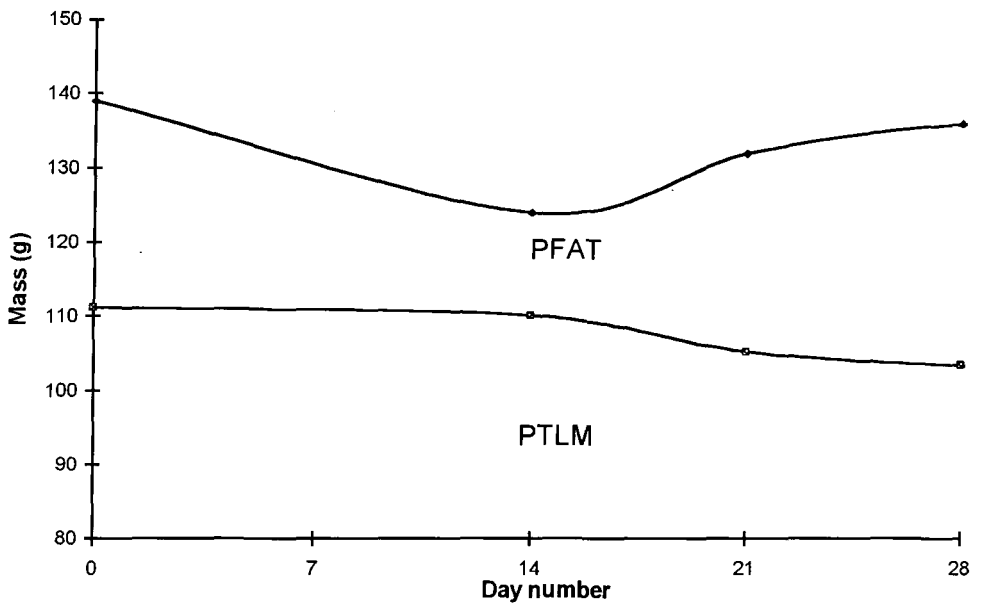


Fig A18: Knot JWG

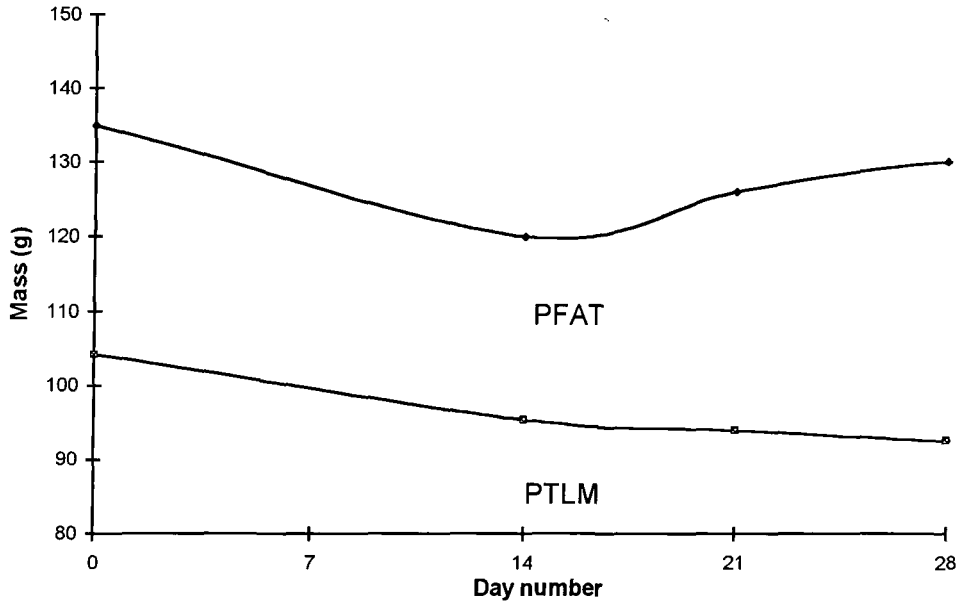


Fig A19: Knot JWW

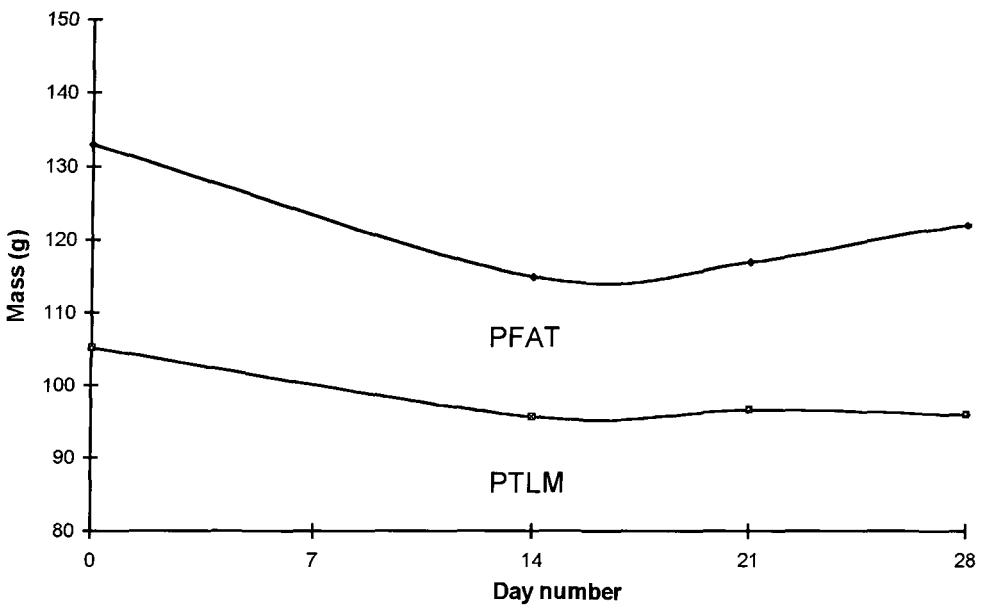


Fig A20: Knot JWY

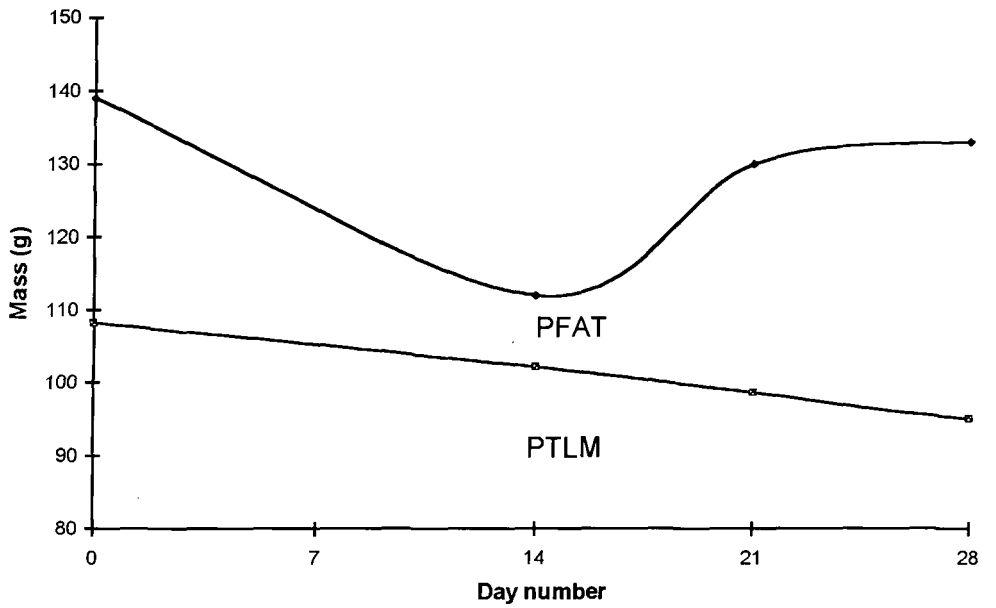


Fig A21: Knot JYG

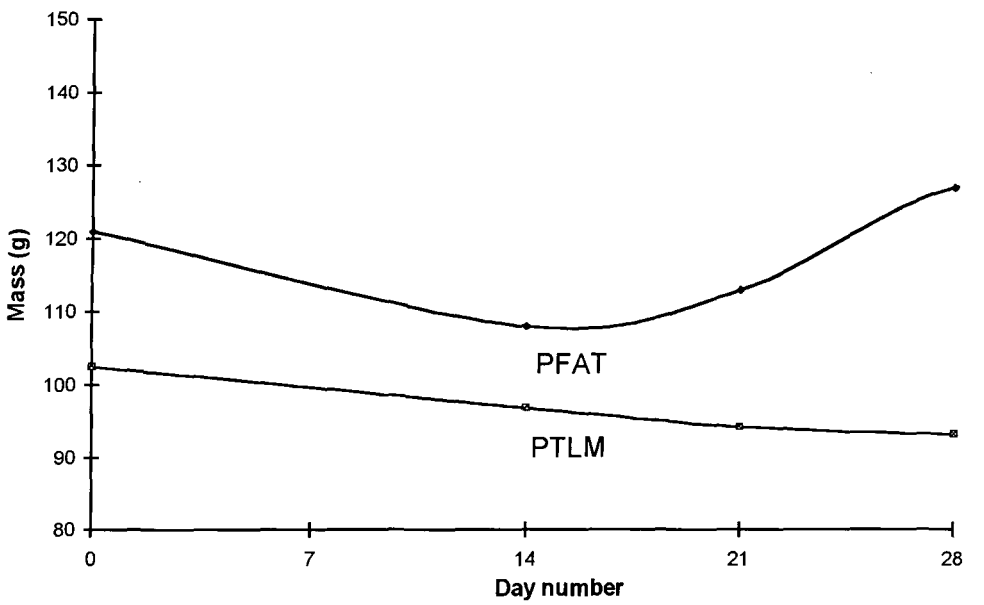


Fig A22: Knot JYY

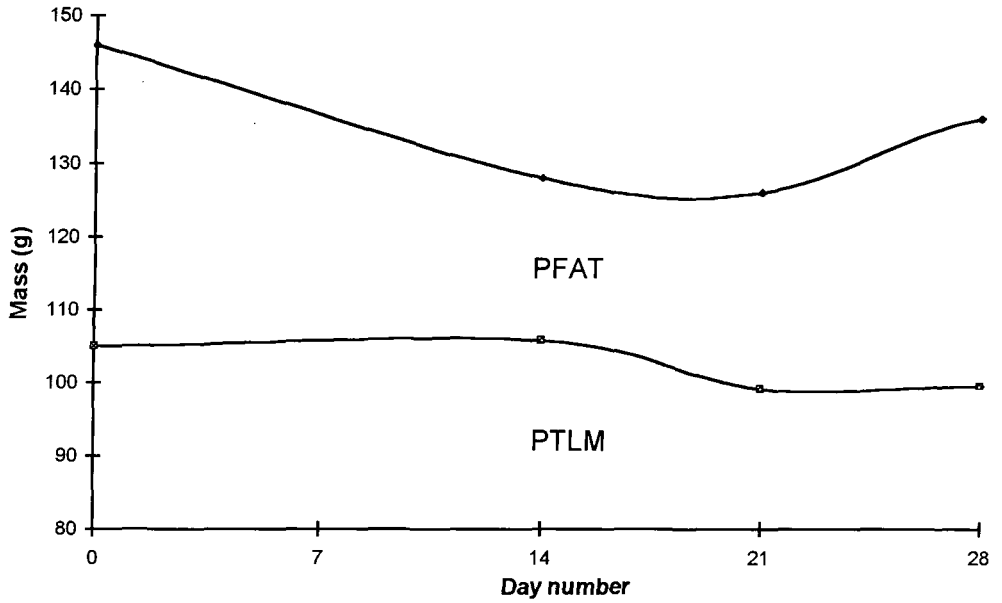


Fig A23: Knot RGG

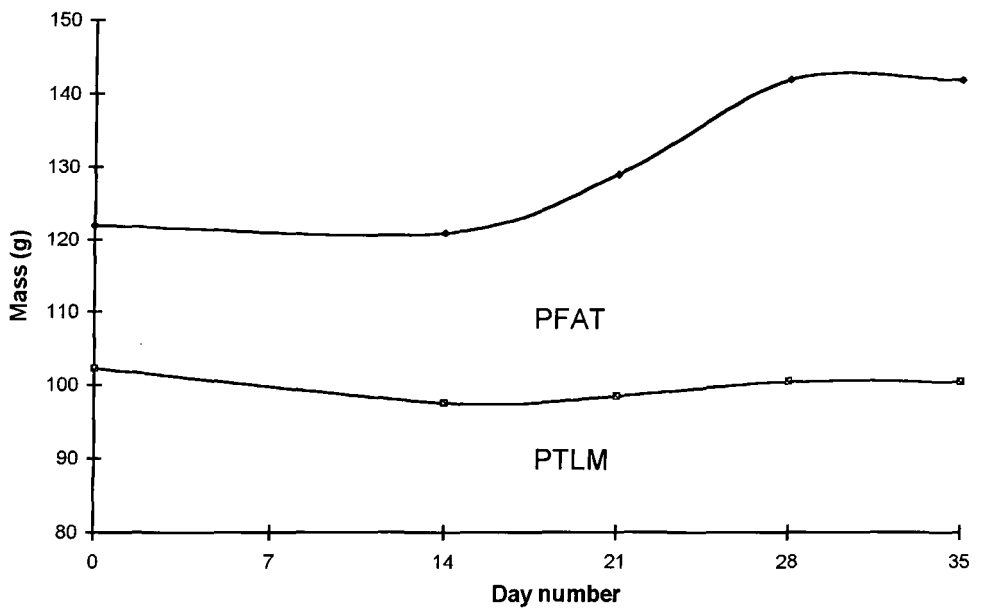


Table 1: Changes in body mass and body composition between capture in the field on 6/11/95 (week 0) and week 4 of captivity in 13 Knot (Group A).

ID	Mass (g) at week 0	Mass (g) at week 4	PTLM (g) at week 0	PTLM at week 4	PFAT at week 0 (PLI%)	PFAT at week 4 (PLI%)
LG	140	131	122	103	18(36)	28(47)
LY	125	118	108	97	17(37)	21(43)
WO	144	129	114	99	30(45)	30(48)
YG	141	134	117	102	24(41)	32(49)
YYY	150	125	119	103	31(46)	22(42)
YW	139	144	124	109	15(32)	35(49)
WGG*	155	161	127	105	24(40)	56(59)
WGY*	144	153	125	104	19(37)	49(57)
WLG*	142	141	113	97	29(45)	44(56)
WLL*	149	210	120	109	29(44)	101(69)
WWW*	143	146	116	101	27(43)	45(55)
WYG*	151	144	114	102	37(50)	41(53)
WYY*	149	147	116	99	33(47)	48(57)
Mean	144	145	118	102	25(42)	43(53)
SE	2.0	6.1	1.4	1.0	1.8(1.4)	5.5(2.0)

*	**Juvenile Knot on 6/11/95**
PTLM	**Predicted total lean mass derived from TOBEC**
PFAT	**Predicted total fat mass derived from TOBEC**
PLI	**Predicted lipid index (Predicted fat mass/ total body mass*100) derived from TOBEC**

Table 2: Changes in body mass and body composition between capture in the field on 7/1/97 (week 0) and week 4 of captivity in 10 adult Knot (Group B).

ID	Mass (g) at week 0	Mass (g) at week 4	PTLM (g) at week 0	PTLM at week 4	PFAT at week 0 (PLI%)	PFAT at week 4 (PLI%)
BGG	146	137	111	103	35(24)	34(25)
BYY	134	144	112	104	22(16)	40(28)
JLL	141	131	104	101	37(26)	30(23)
JLW	139	136	111	103	28(20)	33(24)
JWG	135	130	104	93	31(23)	37(29)
JWW	133	122	105	96	28(21)	26(21)
JWY	139	133	108	95	31(22)	38(28)
JYG	121	127	102	93	19(15)	34(27)
JYY	146	136	105	100	41(28)	36(28)
RGG	122	142	102	100	20(16)	42(29)
Mean	136	134	106	99	29(21)	35(26)
SE	2.6	2.0	1.1	1.3	2.2(1.3)	1.4(0.8)

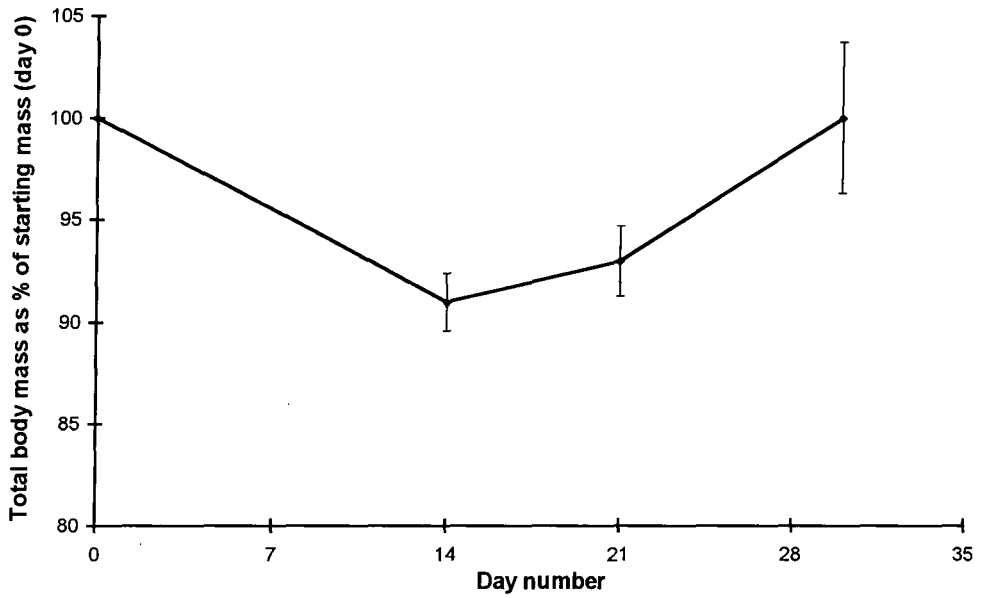
and week 3 (96%). Mean PFAT was also significantly higher by week 3 and 4 than week 2 (SNK, $P<0.05$). Mean PLI also increased significantly by week 4 (130%) from week 1 (100%), 2 (72%) and 3 (105%). Mean PLI was also significantly lower during week 2, than week 1 and 3.

There was no significant difference in body mass (Paired T-test t_{10} =0.35, $P>0.05$), PTLM (Paired T-test t_{10} = 0.90, $P>0.05$), or in PFAT (Paired T-test t_{10} = 0.52, $P>0.05$) between week 4 and week 8 of captivity in individuals of group A. From the graphs A1-A13, it can be seen that PTLM did appear to stabilise during this time-period, although from these graphs it would appear that body mass and hence PFM did not stabilise in all individuals. The lack of significance between week 4 and week 8 in body mass and PFM is because in some individuals these parameters increased during this time (e.g. Knot LG, WYY) but in other individuals they decreased (e.g. Knot WLG, WLL). It was not possible to compare the body composition of bird's in group B over this time period (see Appendix IV).

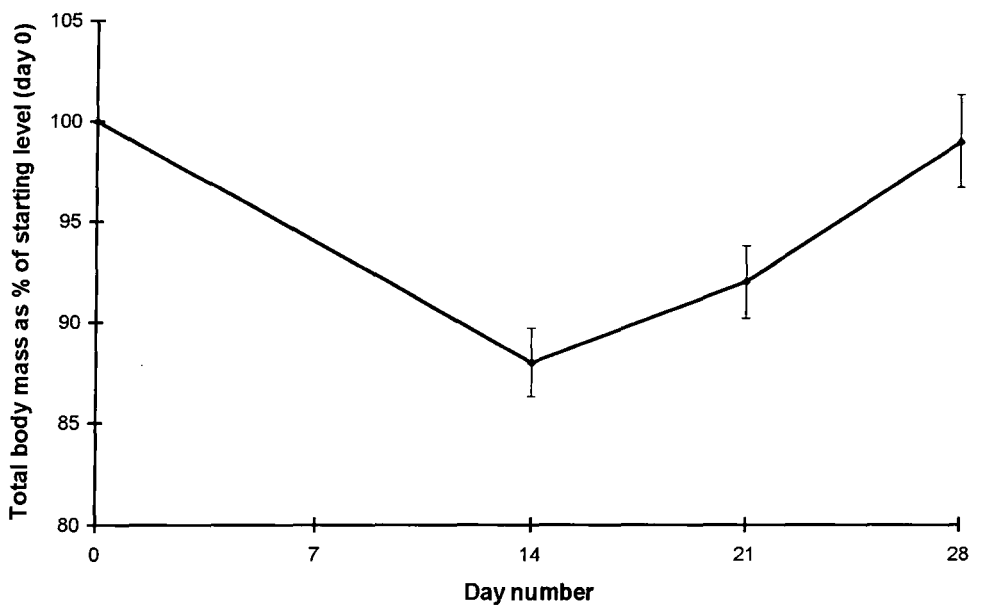
The % change (Graphs B1-B6) in body mass and PFAT in group A and group B between week 0 and week 4 were not significantly different between the two groups (T_{21} = 0.19, $P>0.05$ and T_{21} = 1.56, $P>0.05$, respectively). However, the % decrease seen in PTLM between week 0 (100%) and week 4 was significantly greater in group A (13%) than in group B (7%), (T_{21} = 4.98, $P<0.001$). The mean mass of individuals in group A when brought into captivity on week 0 were significantly greater (T_{21} = 2.49, $P<0.05$) than that of group B, as was PTLM (T_{21} =5.79, $P<0.010$) but not PFAT (T_{21} =1.21, $P>0.05$). The mean mass of PTLM at week 4 was still significantly higher in group A individuals (T_{21} =2.12, $P<0.05$), although there were no significant differences in body mass or PFAT between the two groups after 4 weeks in captivity (T_{21} = 1.46, $P<0.05$ and T_{21} =1.12, $P>0.05$ respectively.

Figures B1 to B6: The changes exhibited in mean body mass and body composition over a 4 week period in group A (caught on 6/11/97) and group B (7/1/97) after entry into captivity. Error bars indicate 1 x SE (Day 0 = day of capture in the field)

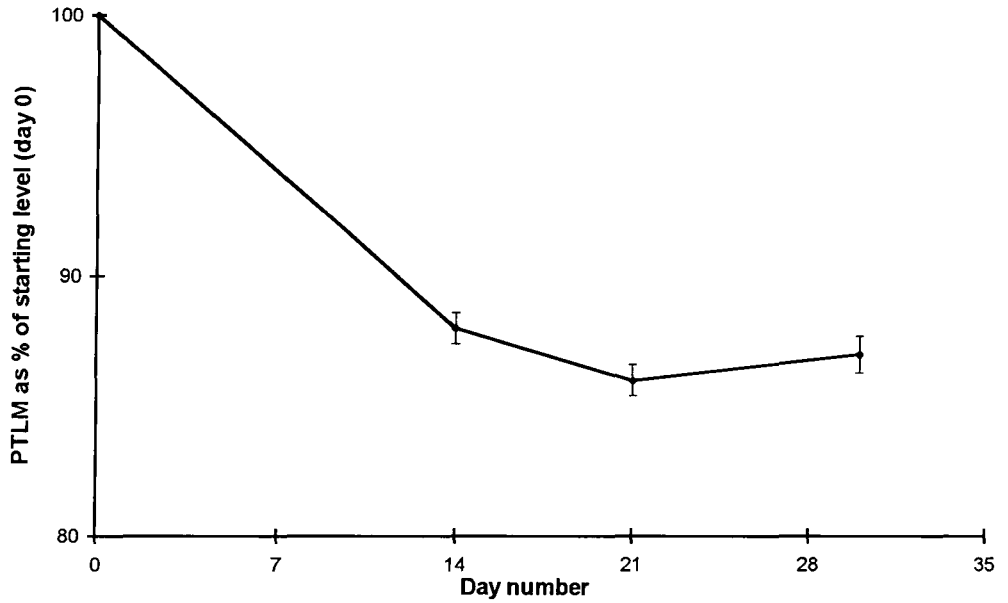
B1: Group A (Total body mass)



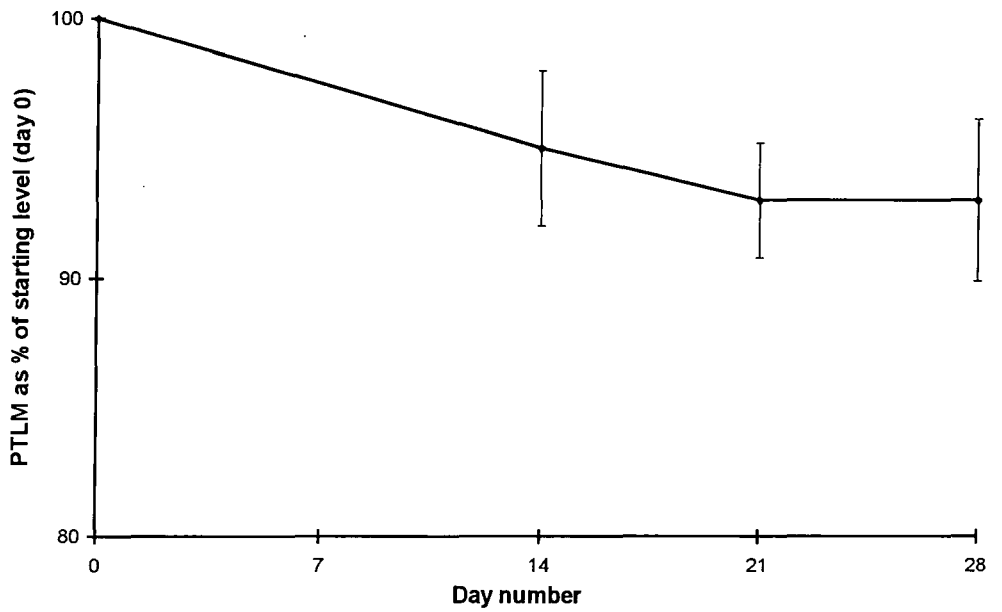
B2: Group B (Total body mass)



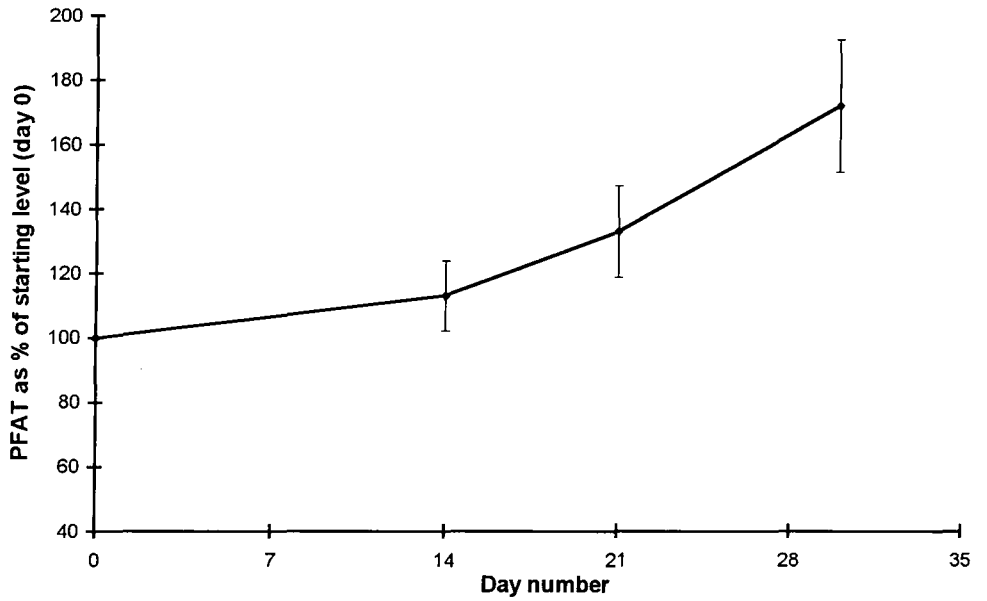
B3: Group A (PTLM)



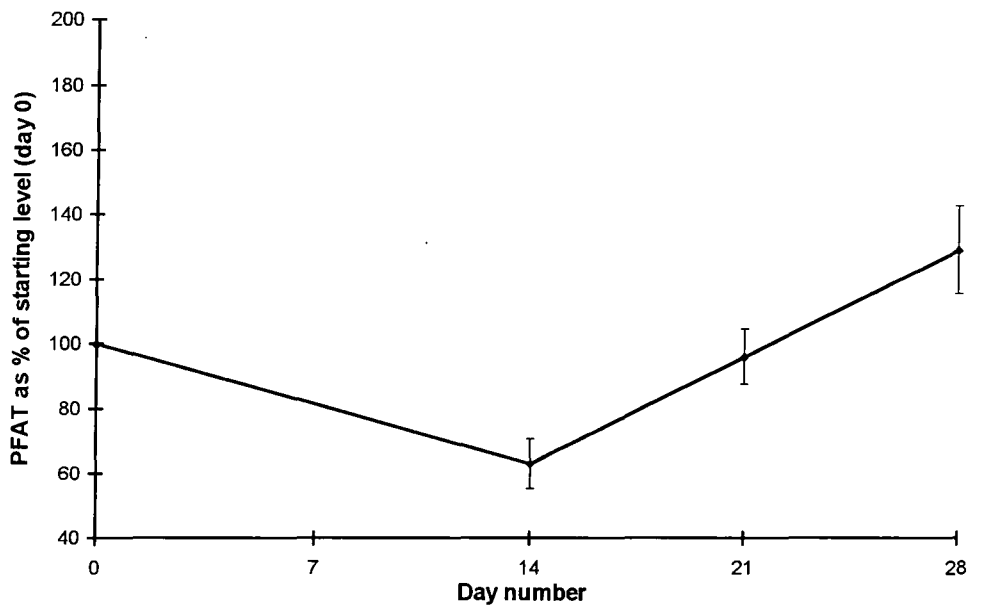
B4: Group B (PTLM)



B5: Group A (PFAT)



B6: Group B (PFAT)



There were no significant differences in the % changes in BM (T_{11}=2.15, P>0.05), PTLM (T_{11}=0.50, P>0.05), PFAT (T_{11}=1.7, P>0.05) or PLI (T_{11}=1.39, P>0.05) between adult and juvenile Knot by week 4 of captivity (Group A). However, PFAT had increased by over 100% in juveniles and by only 36% in adult Knot. This suggests that although the differences in mean BM and PFAT were not significant, juvenile Knot were variable, as individuals, in their response to captivity.

Effects of captivity on organ masses

Only individual Knot from group A were used in the comparison between organ masses of wild and captive Knot. This was because there was no significant differences between body mass (Mann-Whitney U-test, U_{45}=171.5, P>0.05), PTLM (U_{45}=204.0, P>0.05) and PFAT (U_{45}=160.5, P>0.05) between the 15 wild birds sacrificed (30/1/95, 1/3/95, 4/3/96) and the individuals in group A brought into captivity on 6/11/95. There was however, a significant difference between the individuals of group B brought into captivity on 7/1/97 and the 15 wild Knot. The PTLM of group B was significantly lower (U_{46}=76.0, P<0.001) and PFAT was significantly higher (U_{46}=128.0, P<0.05) when compared to the 15 wild Knot, although body mass was not significantly different (U_{46}=224.5, P>0.05).

Table 3 shows the comparative difference between wild and captive birds (group A) in certain lean mass components. The mean liver mass was over 70% lower in captive birds, and the mean gut mass (stomach + intestine), had decreased by over 60%. The mean length of the intestine, had also decreased by some 40% in captivity when compared to wild birds. There was no significant difference in the pectoral muscle mass between captive and wild birds or in the mean values of standard muscle index (SMI).

Table 3: Comparisons of wet organ mass and gut length between wild and captive Knot in group A. (n= sample size)

Values are means with standard errors in parentheses

Organ	Wild	Captive	% Reduction	t	P
Liver (g)	8.63(0.43) **n=15**	2.46(0.26) **n=10**	71	11.22	<0.001
PM (g)	14.42(0.45) **n=10**	13.92(0.48) **n=10**	3	1.18	>0.05
Standard muscle index (SMI) *	0.246(0.06) **n=10**	0.226(0.07) **n=10**	8	2.07	>0.05
Gut mass (g) (Stomach + intestine mass)	23.73(0.64) **n=10**	8.46(0.78) **n=10**	64	14.71	<0.001
Intestine length (mm)	651(23) **n=10**	390(19) **n=9**	40	8.74	<0.001

*	Arcsine transformed
SMI	Mass of left lean dry pectoral muscle mass/ standard muscle volume (Evans & Smith, 1975)
% Reduction	Difference between mean organ mass of wild Knot and mean organ mass of captive Knot, as % of wild Knot mass

Tables 4A-4D show different organ masses as percentages of Total Lean Mass (TLM) between wild and captive Knot and also between captive Knot sacrificed at different times and in different physiological states. The differences seen between captive and wild Knot will be discussed later but it should be pointed out that the organ masses as % of TLM between captive Knot in different physiological states (Figures C1-C4), is surprisingly homogenous. This enabled the comparison in organ mass of wild Knot and captives Knot of group A to be undertaken even though individual Knot in group A may have been sacrificed at different times of the year and under differing physiological states.

Discussion

Knot that were bought into captivity on two different dates (Group A and Group B) appear to follow the changes that were found in Redshank (Mitchell, 1996), after one month in captivity. The decline seen in predicted lean mass is due primarily to the loss in the mass of intestine, stomach and liver primarily. As with Redshank (Mitchell, 1996), this loss in lean mass is compensated by an increase in fat mass, so that the difference in total body mass between entry into captivity and one month later is not significant.

As summarised in graphs B1-6 TOBEC measurements showed that the decrease in lean mass occurs within two weeks of entry into captivity and that PTLM does not return thereafter to the levels measured in the field on day of capture. Total body mass and fat mass also fell during the first two weeks in captivity in eleven of the thirteen Knot of group A and in nine out of the ten Knot in group B but body mass returned to levels seen in the field by week 4 and by that time fat mass and lipid index generally exceeded levels measured in the field. The initial decrease in body mass, fat mass and lipid index is likely to be due to stress caused by the adaptation to a new diet and new conditions in captivity.

Tables 4A, 4B, 4C and 4D: Organ masses as % of Total Lean Mass(g)

Table 4A:

ID	DATE CAPTURED	DATE SACRIFICED	LIVER %	PM %	GUT %	HEART %	KIDNEY %	PHYSIOLOGICAL STATE
BGG	7/1/97	1/7/97	3.1	12.9	7.7	2.2	0.31	3
JLL	7/1/97	27/6/97	3.0	12.9	7.3	2.0	0.22	3
JLW	7/1/97	3/7/97	2.4	13.0	6.2	1.7	0.24	3
JWG	7/1/97	1/7/97	3.4	13.4	6.5	2.0	0.22	3
JWW	7/1/97	25/6/97	3.8	12.9	6.6	2.0	0.18	3
JYG	7/1/97	27/6/97	3.9	12.3	10.0	2.0	0.21	3
JYY	7/1/97	28/6/97	3.8	13.5	7.8	2.1	0.22	3
RGG	7/1/97	3/7/97	2.5	11.7	8.6	1.8	0.22	3
Mean(SD)			3.2(0.5)	12.8(0.5)	7.6(1.2)	2.0(0.1)	0.23(0.03)	

Physiological state: 1= Body mass rising during spring/summer (Pre-migratory increase)
2= Body mass falling during summer (Post-migratory decrease)
3= Body mass stable (outside migratory period in the wild)

PM= One single pectoral muscle block

Table 4B:

ID	DATE CAPTURED	DATE SACRIFICED	LIVER %	PM %	GUT %	HEART %	KIDNEY %	PHYSIOLOGICAL STATE
LY	6/11/95	26/6/96	2.3	13.5	6.0	2.3	-	2
YG	6/11/95	24/5/96	1.6	13.2	6.4	1.8	-	1
WGG	6/11/95	19/5/97	3.7	12.6	10.3	2.6	0.21	1
WGY	6/11/95	24/6/97	2.6	13.6	5.7	1.8	0.18	3
WLG	6/11/95	28/5/97	3.1	12.3	9.1	1.9	0.15	1
WLL	6/11/95	28/5/97	2.9	13.1	10.6	1.7	0.21	1
WWW	6/11/95	29/5/97	2.0	11.8	9.6	2.0	0.18	1
WYG	6/11/95	17/6/97	3.3	12.9	6.1	1.8	0.16	3
WYY*	6/11/95	3/7/96	2.8	14.9	9.0	2.4	-	3
WGL*	6/11/95	25/6/96	2.1	14.1	8.6	1.7	-	2
Mean(SD)			2.6(0.6)	13.2(0.8)	8.1(1.8)	2.0(0.3)	0.18(0.02)	

* Sacrificed as juveniles

Table 4C:

ID	DATE CAPTURED	DATE SACRIFICED	LIVER %	PM %	GUT %
WILD 16	1/3/95	1/3/95	7.6	13.4	19.0
WILD 17	1/3/95	1/3/95	6.5	11.9	23.5
WILD 18	1/3/95	1/3/95	6.6	12.6	17.1
WILD 19	1/3/95	1/3/95	6.5	12.1	18.4
WILD 20	1/3/95	1/3/95	6.8	12.8	20.9
WILD 01	6/11/95	6/11/95	6.0	13.3	-
WILD 02	6/11/95	6/11/95	8.4	14.1	-
WILD 06	6/11/95	6/11/95	8.7	11.4	-
WILD 07	6/11/95	6/11/95	7.4	11.3	-
WILD 21	6/11/95	6/11/95	9.1	12.3	-
WILD A	4/3/96	4/3/96	4.0	-	19.1
WILD B	4/3/96	4/3/96	9.0	-	18.9
WILD C	4/3/96	4/3/96	7.1	-	19.1
WILD D	4/3/96	4/3/96	7.4	-	21.7
WILD E	4/3/96	4/3/96	8.3	-	23.2
Mean(SD)			7.3(1.3)	12.5(0.9)	20.1(2.0)

Table 4D:

ID	DATE CAPTURED	DATE SACRIFICED	LIVER %	PM %	GUT %	HEART %	PHYSIOLOGICAL STATE
BW	2/12/94	1/5/96	2.8	13.0	7.6	2.0	1
OO	2/12/94	6/7/96	2.3	12.1	9.0	1.7	2
GG	2/12/94	4/7/96	3.2	13.0	9.0	1.7	2
GL	2/12/94	28/6/96	2.4	12.9	6.5	2.2	2
GY	2/12/94	28/6/96	2.5	12.5	5.7	2.3	2
GO	2/12/94	12/6/96	3.0	12.5	7.2	2.0	2
GF	2/12/94	7/6/96	2.5	12.4	4.7	2.1	2
YY	2/12/94	24/5/96	2.7	11.7	8.3	2.0	1
GW	2/12/94	30/5/97	2.9	12.5	7.1	2.2	2
Mean(SD)			2.7(0.3)	12.5(0.4)	7.2(1.4)	2.0(0.2)	

Figures C1 to C4: Comparison of organ mass as % of Total Lean Mass (TLM) between wild Knot and captive Knot. Captive Knot sacrificed during different physiological states. (Error bars indicate 1 x SE)

Physiological state 1	Body mass rising during spring/early summer (Pre-migratory increase)
Physiological state 2	Body mass falling during summer (Post-migratory decrease)
Physiological state 3	Body mass stable (Outside migratory period in the wild)

Figure C1: Liver mass as % of TLM (n=sample size)

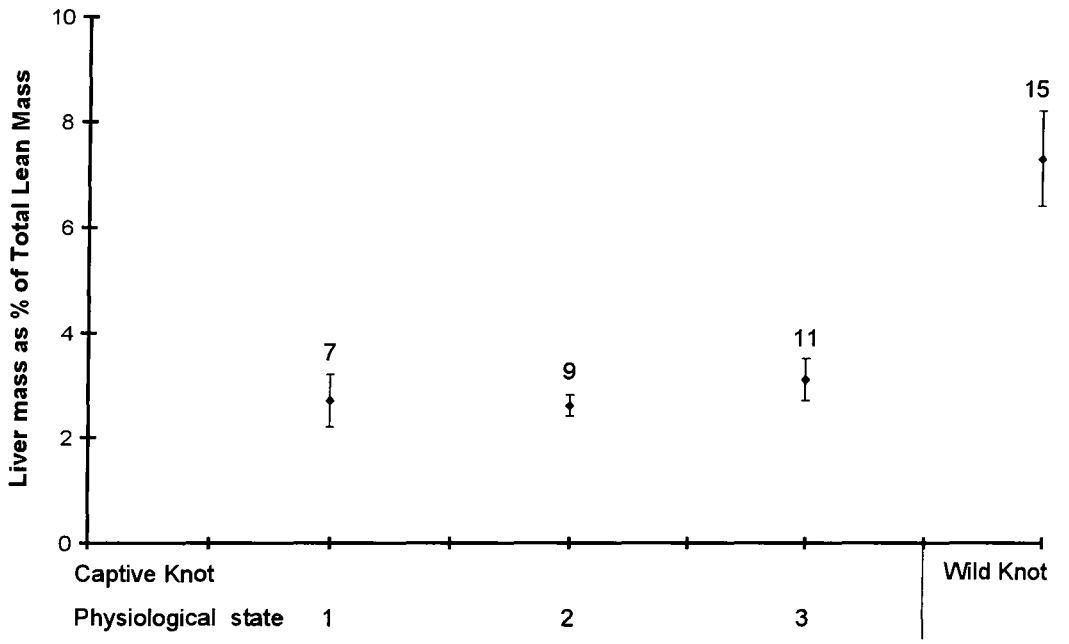


Figure C2: Pectoralis major mass

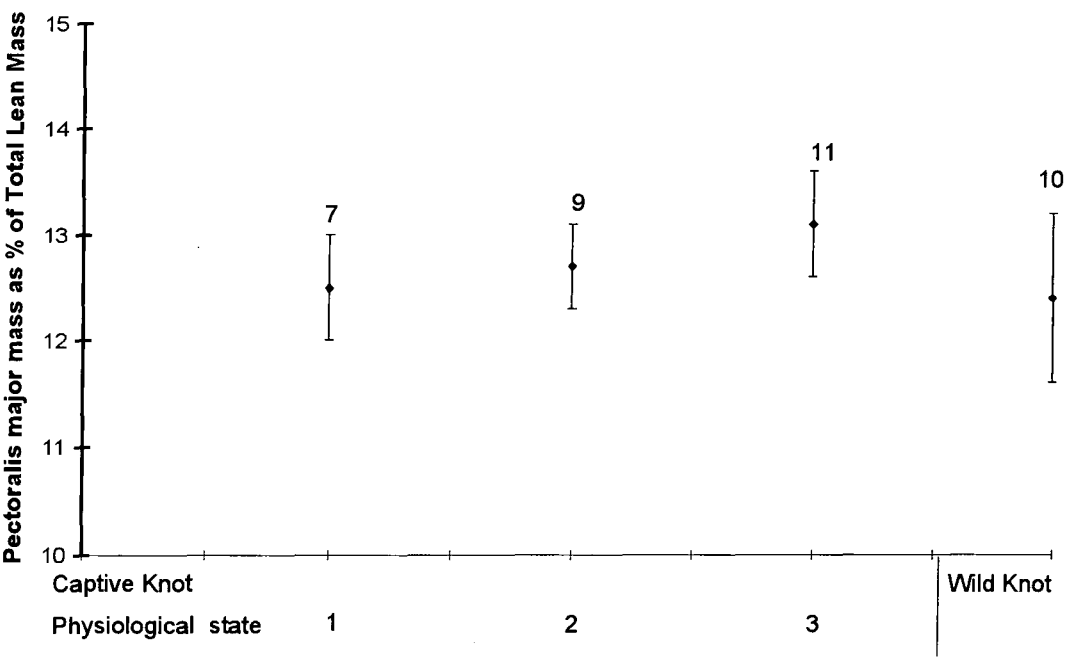
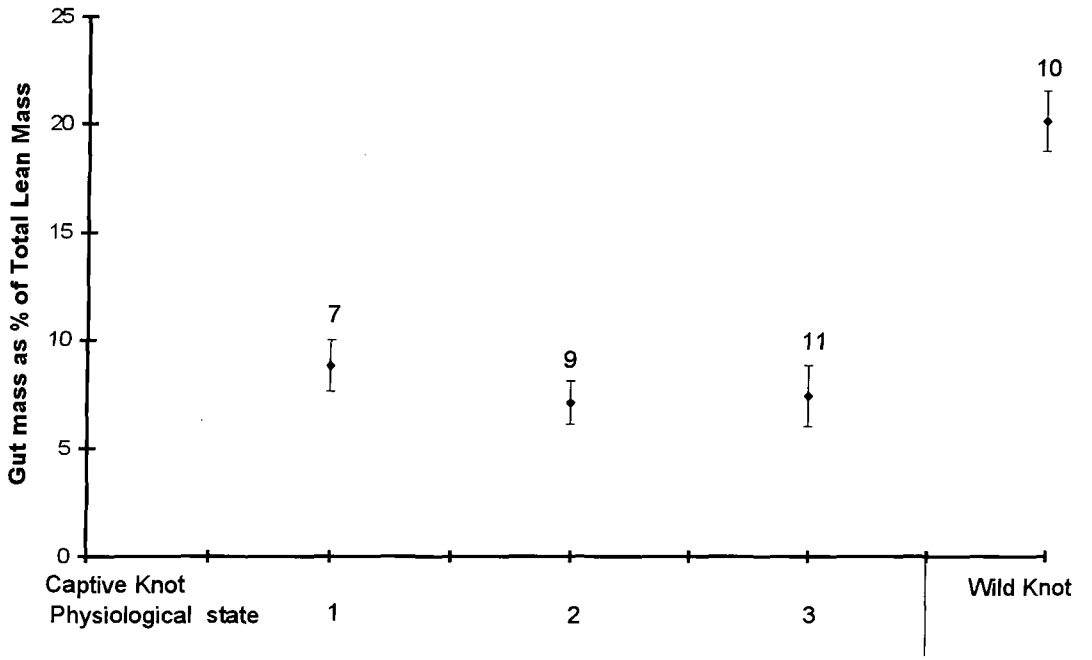
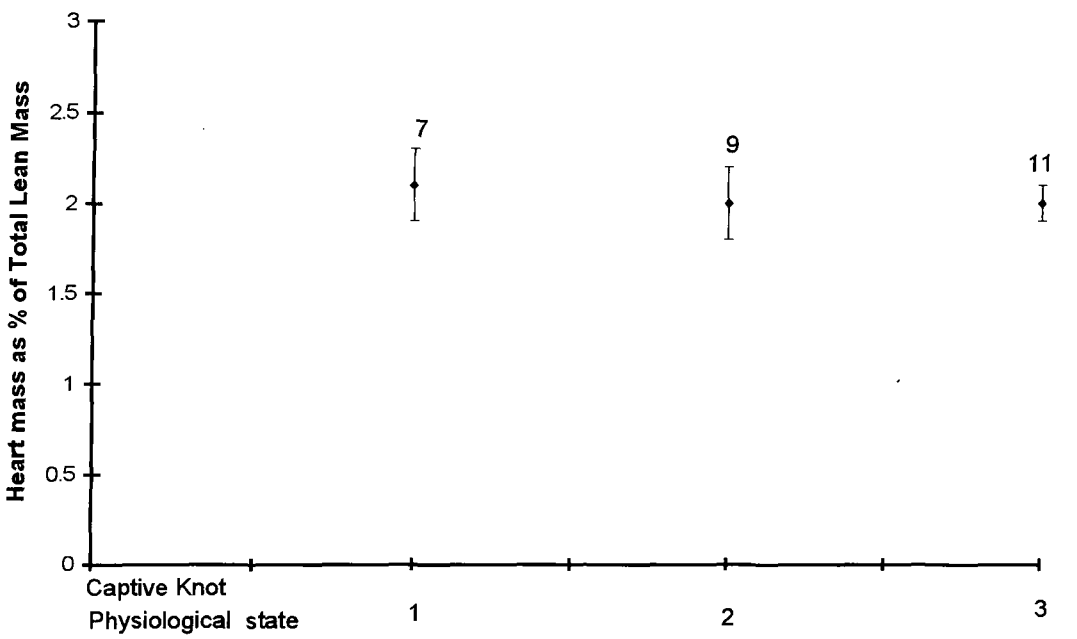


Figure C3: Gut mass



C4: Heart mass (Captives only)



Knot in group A entered captivity with a greater mean PTLM (118g) than those individuals of group B (106g) and although group A individuals lost a significantly greater percentage of PTLM after entering captivity than those in group B, at week 4 they still maintained a significantly higher mean PTLM (93g) than that of group B (87g). This finding appears to contradict those of Piersma *et al* (1993; 1996), who postulated that 'all birds' possessed considerably flexibility in their lean tissue and organ masses because two subspecies of Knot (*islandica* and *canutus*), which could be distinguished on the basis of organ masses and overall lean mass in the wild, converged to similar body composition and organ size in captivity. The fact that the Knot in the two groups (A & B), under exactly the same captive regime maintained significantly different levels of PTLM even after 4 weeks of captivity further backs up evidence (see Chapter II) that Knot can alter their metabolic rate, by both altering the mass of metabolically active lean tissues and by altering the output of these lean tissues.

The comparison of organ masses between captive and wild Knot follows on from the work of Piersma (1994) and Mitchell (1996), and again shows that captive shorebirds are able to exhibit considerable flexibility in lean tissue in the face of altered living conditions. The significant reduction in gut mass (stomach mass + intestine mass) seen in this study is probably due to disuse atrophy, because captive birds are generally fed on soft food pellets and therefore do not need a muscular gizzard to break up hard-shelled mollusc prey (Piersma *et al*, 1993). The food in captivity is also less fibrous that that eaten in the wild and this reduction in fibre content has been shown to lead to a decrease in gizzard size (Dubowy, 1985). The significant decrease in intestine length in this study and in that of Mitchell (1996), is likely also to be due to disuse atrophy The assimilation through the gut of artificial, soft food pellets is probably easier and quicker than that of hard-shelled molluscs prey in the wild, and this in turn causes a reduction in the surface area needed to adequately absorb food. The difference between mean mass of liver and gut (stomach + intestine) between wild and captive Knot (Group A) was 21.6g. Only two Knot (WGG and WGY) showed a reduction in PTLM close to this value (22g and 21g respectively) after 1 month in captivity. The

discrepancy seen with other individuals may simply be because wild birds had a greater bulk of food in their stomach and intestine at time of death, or that their gizzards contained grit, both giving an increase stomach and intestine wet mass. The captive birds may also increase another component of their lean mass in captivity. It is well known that during periods of the year when certain species of waterfowl rely solely on walking and swimming for locomotion, there is hypertrophy of the leg muscles (DuBowy, 1985; Jehl Jr, 1997). Perhaps captive Knot undergo leg muscle hypertrophy in captivity because they rely on walking more than flight.

The reason for the significant reduction in the mass of liver in captivity is more difficult to explain satisfactorily. If the costs of thermoregulation and activity are decreased in captivity, the masses of metabolically important organs such as the liver and kidney could be decreased and thereby reduce total energy expenditure (Piersma, 1994). The costs of thermoregulation in captivity in this study must still have been an important contributor to overall metabolism as the indoor aviaries followed ambient temperature closely, and certainly the costs of thermoregulation in the study by Piersma *et al*, (1993) would have still been considerable as the aviaries were outdoors. The fact that these aviaries of Piersma *et al*, (1993) would have undoubtedly given protection from the wind may have decreased the energy required to maintain body temperature. It would be interesting to know whether the mass of other metabolically important organs decreases in captivity. However, it is difficult to be sure that the organs decreased in size solely due to the lower metabolic costs encountered in captivity. The kidney and liver masses may simply decrease in size because the homeostatic pressures encountered in captivity are not as demanding as those in the wild. The liver is associated with lipid and glycogen storage and synthesis. It is also important in the synthesis of protein and may also be a major source of labile protein (Raveling, 1979). Perhaps the storage and the synthesis of these compounds are less important in captivity, leading to a decrease in the mass of the liver. One perhaps would expect the mass of the liver to increase in captivity during the period of fat deposition in spring, due to fat

synthesis and deposition. From the graph C1, this apparently does not seem to happen. Individuals that were undergoing pre-migratory fattening tended to be sacrificed very soon after body mass started to increase, perhaps liver hypertrophy occurs at a later period of pre-migratory fattening and which was simply missed in this study. The kidney itself is important in water reabsorption and the decrease in kidney mass reported by Piersma *et al* (1993) may simply be due to the fact that in captivity *ad lib* fresh water is available all the year round, the need for water reabsorption may be less critical.

Fresh pectoral muscle mass did not differ between wild and captive Knot. The fact that pectoral muscle mass did not differ would appear to indicate that the physical activity being carried out in captivity is sufficient to retain pectoral muscle mass. However, probably a more useful indicator of pectoral muscle size is the standard muscle index (SMI), which takes into consideration skeletal size and gives a measure of available protein reserves. Mitchell (1996), found no significant difference between the SMI of wild and captive Redshank, although SMI in wild Redshank were higher than in captives. In this study, mean SMI was higher in wild Knot than the captive Knot of group A, although this was not significant. The SMI in this study for wild Knot was fairly similar to the level found in wintering Knot by Davidson & Evans (1990).

So, from this study it can be seen that captive Knot lose around 7-13% of their total lean mass (predicted by TOBEC), within two weeks of captivity. Overall body mass tended to stabilise within four weeks of captivity to levels comparable to those seen in the field. This maintenance of body mass in captivity to levels similar to wild conspecifics in birds, particularly shorebirds appears peculiar to birds, as many other species of animals tend to maintain higher body mass in captivity (Kirkwood, 1991). So it can be seen that a time period of at least one month must be allowed for body composition to stabilise in captivity, although the metabolically active lean tissues would appear from this study to reduce and

stabilise within two weeks of captivity. Therefore, a period of at least two weeks and preferably four weeks should be allowed for an individual to adapt to the conditions of captivity before any metabolic rate measurement can be carried out with any confidence.

Appendix IV- The effect of weight manipulation on the body mass, body composition and Basal Metabolic Rate (BMR) of captive Knot

The work presented in this appendix describes the changes in total body mass (BM), body composition (lean mass and fat mass, predicted from TOBEC measurements) and in BMR of captive Knot *Calidris canutus*, after attaching an artificial weight to their backs. The hypothesis being tested was that captive Knot regulate their total BM and total lean mass independently, so that the fat mass carried is regulated only indirectly by difference. I predicted that if a known mass is attached to the back of a Knot, it should decrease its BM through a reduction in its fat mass by an amount similar to that of the applied weight. This test assumes that during the time-scale of this experiment, no seasonal variation would occur in either BM or body composition of individuals.

The second aim of the work was to investigate the effect that fat mass has on an individual's BMR. Avian adipose tissue is known to have a low *in vitro* metabolic activity per gram (Scott & Evans, 1992). From the work described in Chapters 3 & 4, it appears likely that the metabolic costs of fat to an individual's BMR are primarily indirect, from carrying and heating this fat mass, rather than from direct respiration by the adipocytes. Therefore, if an individual's fat mass does decrease after attachment of a weight, the prediction would be that the individual's BMR would not alter significantly since the indirect costs of carrying the inert weight are still present. This test assumes that the total lean mass (TLM), relative composition of tissues/organs that make up TLM and the metabolic activity per gram of these lean tissues do not alter during the experiment.

Many species of bird, including shorebirds, exhibit seasonal variations in BM, that follow predictable patterns from year to year (see Scott *et al*, 1994). Most of this

seasonal variation (particularly during winter) is due to the deposition and utilisation of fat stores (Evans & Smith, 1975; Scott, 1991), although the lean components of BM may also vary, particularly during preparation for long-distance migration (Davidson & Evans, 1990; Piersma, 1990; Evans, 1992; Piersma, 1994; Piersma & Gill Jr, 1998). Within a species, birds are often fatter and heavier during winter than summer and, within a winter, are often fatter and heavier during the mid-winter period (Dugan *et al*, 1981; Davidson, 1981a; Scott *et al*, 1994). The most widely acknowledged benefit of storing fat is that it liberates more chemical energy per unit weight when metabolised than any other storage material and therefore can act primarily as an insurance against starvation during periods of negative energy balance (Witter & Cuthill, 1993; Mitchell, 1996). Indeed, McNamara & Houston (1990) suggest that the risk of starvation decreases approximately exponentially with increasing fat reserves. Therefore, if the only fitness consequence of carrying a fat load is a benefit, i.e. the reduction in the risk of starvation, then fat levels should be maintained at their maximum. This is not the case, with birds tending to maintain optimal BM throughout the year and not maximal.

Both wild and captive birds appear to regulate their BM around a sliding or seasonally varying set-point during different times of the year, e.g. mid-winter and during pre-migratory fattening (see Scott *et al*, 1994). Mortensen & Blix (1985) reported evidence of BM regulation in captive Svalbard rock ptarmigan *Lagopus mutus hyperboreus*. They found that individuals that were deprived of food for 7 days lost a considerable amount of fat, but when re-fed they deposited fat and increased in BM back to levels similar to control birds. A similar phenomenon has been reported in several species of wader wintering on Teesmouth, north-east England (Dugan *et al*, 1981; Davidson, 1981a). These various wader species decreased in BM, primarily due to a decrease in fat mass, during periods of severe winter weather. After the severe weather, BM increased back to levels typical for that particular time of year. Evans (1992) also suggested that Knot using

Balsfjord, north Norway, as a staging post during spring migration had to achieve a certain pre-set level of BM before they continued with northward migration. Probably the best evidence that internal regulation of BM takes place has been reported for Knot *Calidris canutus* (Piersma 1994; Piersma *et al* 1996, this study) and Redshank *Tringa totanus* (Scott *et al*, 1994; Mitchell, 1996). Captive individuals of both species, brought into captivity and given access to *ad libitum* food, maintained seasonal patterns in BM similar to wild conspecifics. Differences occurred in body composition between the two groups, however, (see Appendix III). Waders in captivity tend to decrease in lean mass, primarily due to decreases in the masses of the liver and alimentary tract, but increase in the fat carried, thereby maintaining very similar body masses to wild birds. Scott *et al* (1994) and Mitchell (1996) showed that highly significant correlations existed between the seasonal changes seen in wild and captive Redshank in BM, even though captive birds were maintained on *ad lib* food. This suggests that captive birds maintain optimal rather than maximal body masses and suggest that limits in food supply do not cause the lower BM seen after the mid-winter peak (Scott *et al*, 1994). These findings are in direct contrast to Davidson's (1981a) suggestion that Redshank were unable to regulate their BM in the wild during and after mid-winter. Scott *et al's* (1994) results are consistent with the hypothesis that birds regulate their BM, and thereby their fat mass. This regulation of BM appears to work on a sliding-scale, with different optimal BM occurring at different times of the year.

It therefore appears that fat mass is maintained at optimal, rather than maximal levels, despite it acting as an insurance against starvation. This indicates that there must be costs associated with being fat, relative to an individual's body size (reviewed by Witter & Cuthill, 1993). The most widely accepted cost of being fat is that it may increase the risk of predation. The acquisition of fat reserves may require increased foraging effort, thereby leading to a higher risk of predation (Houston *et al*, 1997). There is evidence that mass-dependent predation costs may be important. Gosler *et al* (1995) reported that a population of Great tits *Parus major* were significantly heavier over a period of years during which their main

predator, the Sparrowhawk *Accipiter nisus* was absent, than during subsequent years when Sparrowhawks became re-established. Kullberg *et al* (1996) also suggested that the ability of heavier blackcaps *Sylvia atricapilla* to escape an artificial predator was reduced, because during take-off their angle of ascent was lower and their take-off velocity reduced. A high fat load may also affects an individual's ability to out manoeuvre a predator, probably due to increased wing-loading (Hedenstrom, 1992; Witter *et al*, 1994; Metcalfe & Ure, 1995).

The energetic costs to an individual Knot were considered in Chapter 3 of this thesis. If the metabolic expenditure of an individual increases with an increase in its fat mass, (particularly but not exclusively due to increased flight costs), this may necessitate an increased foraging time, leading to an increased risk of predation. The indirect costs of a fat mass to an individual's metabolism are also present when an individual is not flying, simply due to the maintenance and support of these fat tissues. As mentioned earlier the direct costs of the fat masses to an individual's BMR are likely to be minimal (see Chapter 4), because avian adipose tissue having a low metabolic activity per gram (Scott & Evans, 1992). Therefore, it can be seen that there is good circumstantial evidence that individual birds can regulate and maintain an optimal seasonal BM and the work reported in this chapter aimed primarily to investigate whether they achieved this through a process of internal-weighing.

Materials and methods

All Knot *Calidris canutus* used in this study were captured under licence on 7/1/97 and kept in captivity under the conditions described in section 2.1. The individuals used in this weight manipulation experiment were termed group B in Appendix III. The protocol for the measurement of body composition using TOBEC and BMR follow those in sections 2.2 & 2.3 respectively. These 8 adult

Knot were kept in captivity for 2 months to allow their total body mass (BM) and body composition (as predicted by Total Body Electrical Conductivity, TOBEC) to stabilise (see Appendix III). Individuals were then matched, as closely as possible, into pairs depending on body mass, predicted total lean mass (PTLM), predicted total fat mass (PFAT) and various biometric measurements (head-bill and tarsus-toe length). No individual was undergoing premigratory fattening or feather moult during the course of this experiment, with all individuals being kept in the same aviary

The artificial weights used in this study were cut from thin lead plates (approx. 5 millimetre in depth) into pieces approximately 40mm in length and 10mm in width. All sharp corners were rounded off and the lead weights were then coated in Araldite (RS 850-956, RS Components, Corby, Northants, UK) to prevent the oxidation of the lead. A small piece of Velcro was then attached to the lead weights using Araldite (see diagram 1). A string harness, containing a piece of Velcro, was then attached to the back of each individual Knot, with string restraints passing around both wings (diagram 2). Velcro enabled the artificial weights to be removed easily prior to the taking of a TOBEC measurement. One bird in each pair (experimental bird) was then picked at random and a lead weight (lead weight + Araldite + string harness) was attached (Day zero). The other individual in each pair (control bird) had only a string harness plus Velcro attached to its back (weight approx. 0.2 grams). The total mass of the artificial weights varied between 11.8- 13.7g (see tables 1 and 2).

Diagram 1: Side view of lead weight

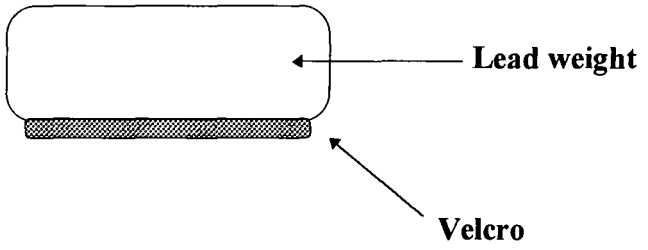
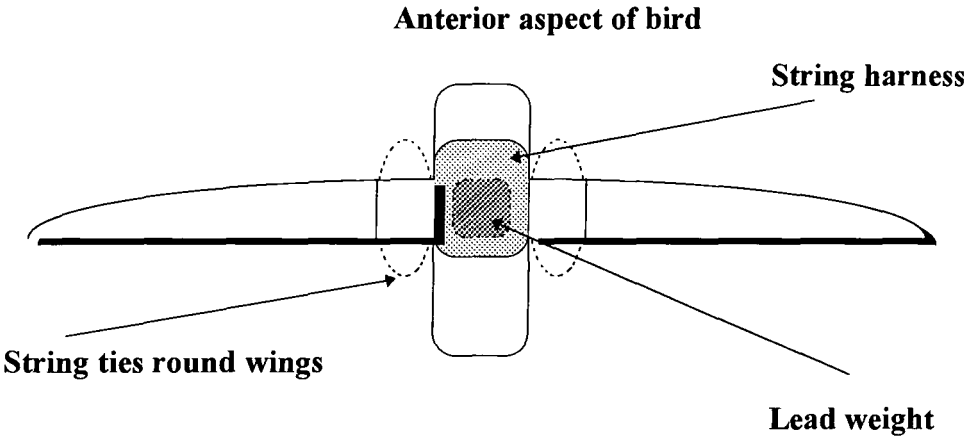


Diagram 2: Plan view of lead weight attachment



On day zero, before any weights and/or harnesses were attached, each individual in a pair underwent a TOBEC and subsequent BMR measurement. The weights and harnesses were then attached to each experimental and control bird in each pair, and the birds returned to their cage. Body mass (BM) and body composition (using TOBEC) changes were then followed in both experimental and control birds at two day intervals, until BM appeared to stabilise, i.e. showed little change (approximately 12 days later). Once BM had stabilised, a TOBEC and BMR measurement was taken and the weights and harnesses removed. The BM and body composition of each individual was then followed at two day intervals again until they stabilised some 7-8 days later. The experiment was then repeated

(Experiment 2), as before, except that the control birds in Experiment 1 were now used as experimental birds and vice versa (see below). The weights and harnesses were replaced after experiment 1 due to the attached Velcro becoming very dirty.

Table 1: ID of Knot used in Experiment 1

	Experimental bird ID	Control bird ID	Mass of artificial weight (g)
Pair 1	JYY	RGG	12.0
Pair 2	JWW	JYG	13.0
Pair 3	JWG	JLL	12.2
Pair 4	JLW	JWY	11.8

Table 2: ID of Knot used in Experiment 2

	Experimental bird ID	Control bird ID	Mass of artificial weight (g)
Pair 1	RGG	JYY	13.7
Pair 2	JYG	JWW	12.7
Pair 3	JLL	JWG	11.9
Pair 4	JWY	JLW	12.1

Results

Mean total body mass (BM), mean predicted total lean mass (PTLM) and mean predicted total fat mass (PFAT) of the 8 Knot (both experimental and control birds) at the start of experiment 1 (commenced 4/3/97) were significantly greater than those measured in the same 8 individuals one month later, prior to the start of experiment 2 (commenced 4/4/97) (see Table A1). There was no difference however between the experimental and control groups of birds, in the starting BM (T-Test, T_{14} =0.11, P>0.05), PTLM (T-Test, T_{14} =0.76, P>0.05) or in the PFAT (T-Test, T_{14} =0.24, P>0.05) when the birds from experiment 1 and experiment 2 were combined. A classic repeated measure ANOVA could not be used in this study because the time dimension was not a fixed treatment effect, i.e. duration of experiment was not the same in all pairs (see Sokal & Rohlf, 1969).

Table A1: Comparison of mean (±SE) body mass, predicted total lean mass (PTLM) and predicted fat mass (PFAT) measured in the 8 captive Knot at the start of experiment 1 and start of experiment 2.

All tests were paired T-tests.

	Experiment 1 (Grams)	Experiment 2 (g)	T Statistic	P
Body mass (Mean±SE)	132±2.6	120±2.5	10.25	<0.001
PTLM (Mean±SE)	96±1.4	93±1.0	2.47	<0.05
PFAT (Mean±SE)	36±2.0	27±2.0	7.39	<0.001

To remove the effects of individual variation in BM and body composition, both within and between groups, the difference in BM and in body mass composition that existed within individuals in each group between the start and finish of the experiment was calculated; i.e. finishing mass minus starting mass.

Graphs A1-A3 show the mean (±standard error) changes in BM and body composition (PTLM and PFAT) that occurred during this time in the experimental (n=8) and control (n=8) groups of birds. Graph A1 shows that experimental birds decreased in BM on average by a greater amount 5±3.1grams (mean±SE) during the course of the weight manipulation experiment than the control group (2±1.6g). However, the mean reduction in BM seen in the experimental birds was not significantly greater than the reduction seen in the control group (T-Test, T_{14} =0.91, P>0.05). The mean PTLM measured (Graph A2), actually increased in both the experimental (95 to 97g) and control groups (94 to 96g) during the experiment. Graph A3 shows that the experimental birds lost a greater amount of fat (7±2.4g) than that lost by the control group (4±1.9g), although this difference was once again non-significant (T-Test, T_{14} =1.01, P>0.05). The mean PFAT at the start of the experimental birds was 31g and in the control birds was 32g, but by the finish had fallen to 24g in the experimental birds and to 28g in the control birds. The above results therefore show that although mean BM did decrease during the weight manipulation experiment in the experimental birds to a greater degree than in control birds, this reduction in BM was not significantly greater. The reduction seen in mean BM in both groups, however was due exclusively to a decrease in the fat component of BM, with mean predicted total lean mess actually increasing in both groups.

Graphs B1-B16 show the changes that occurred in BM, PTLM and PFAT within individual experimental and control birds during experiment 1 and experiment 2. Graphs B1-B8 and Table A2 show that the experimental birds JWG, JYY and JWW exhibited considerable and rapid reductions in BM after the attachment of the artificial lead weights. The majority of this loss in BM in these individuals was due to a reduction in PFAT. The other experimental bird in experiment 1 (JLW) decreased slightly in BM over the first 2 days after the attachment of the weight but by the time it was removed (12 days later) this individual had actually increased in BM by 2g. The increase in BM seen in Knot JLW during this time was due largely to a 5g increase in PTLM. The control birds in experiment 1, all

Figures A1 to A3: Comparison of the mean (+SE) changes seen in (i) Total body mass, (ii) Predicted total lean mass and (iii) Predicted total fat mass in both experimental and control Knot from the start to finish (approx. 12 days) of the weight manipulation experiments 1 and 2 (data combined). (n=8 in both cases).

Fig. A1: Total body mass

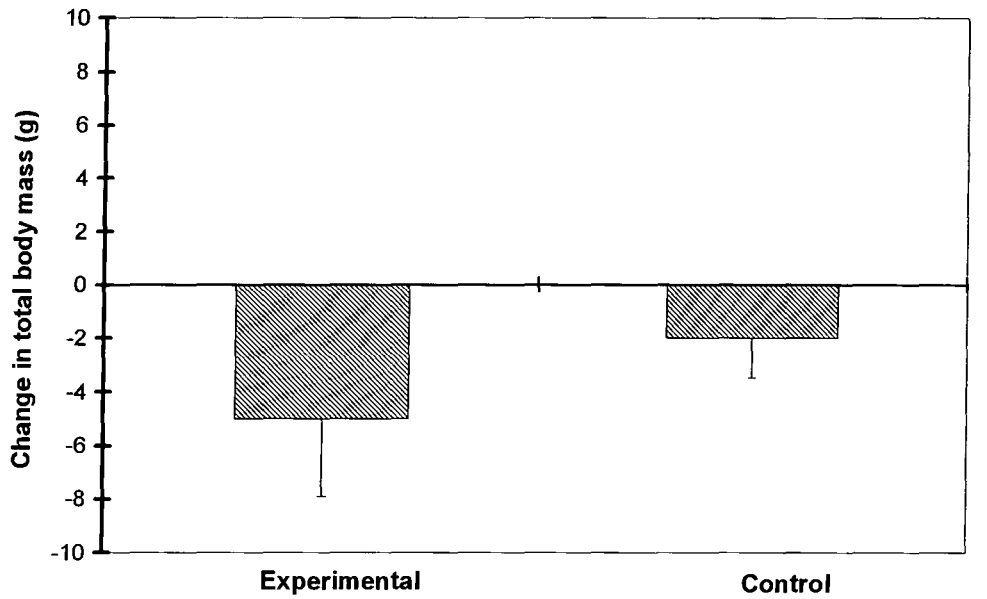


Fig. A2: Predicted total lean mass

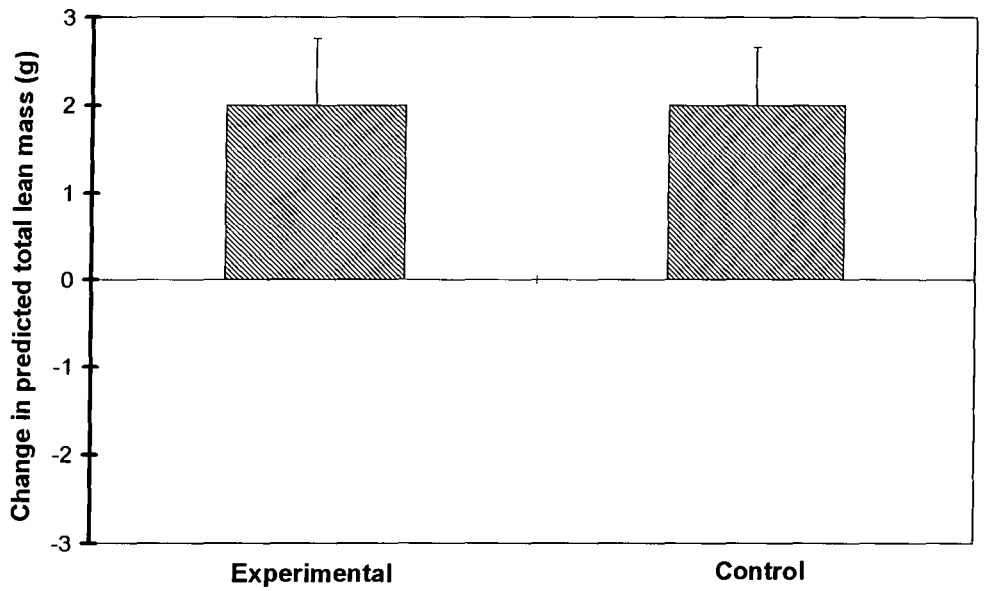
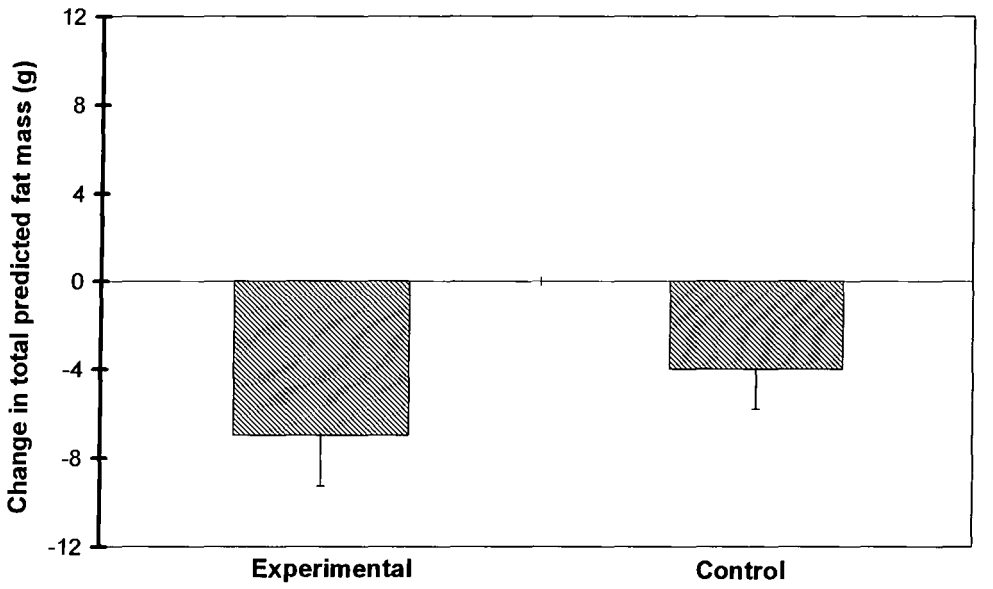


Fig. A3: Predicted total fat mass



carrying only string harnesses, showed reductions in BM during this time. The decrease seen in BM in these controls was due primarily to decreases seen in PFAT. Indeed, the control birds JLL and JWY exhibited considerable decreases in PFAT, by 9g and 11g respectively, between the start and end of the experiment 1. The reductions in BM seen in the control birds may have possibly arisen due to handling stress occurring during the experiment (see Discussion Section).

Table A2: Difference in total body mass (BM), predicted total lean mass (PTLM) and predicted fat mass (PFAT) in grams of both experimental and control birds during experiment 1.

Difference = Finishing mass (removal of weight/harness) minus starting mass at day zero (attachment of weight/harness)

ID		BM (g)	PTLM (g)	PFAT (g)	Mass of weight (g)
JYY	Experimental	-17	-1	-16	12.0
JWW	Experimental	-10	-1	-9	13.0
JWG	Experimental	-17	1	-18	12.2
JLW	Experimental	2	5	-3	11.8
RGG	Control	-3	-1	-2	-
JYG	Control	-3	2	-5	-
JLL	Control	-5	5	-9	-
JWY	Control	-8	3	-11	-

The graphs B9 to B16 and Table A3 show the that reductions in BM in the experimental birds during experiment 2 were considerable less than those seen in experiment 1. Small reductions in BM were seen in the experimental birds RGG(-2g), JYG (-1g) and in JLL (-1g) during experiment 2 but individual Knot JWY actually increased in BM during this time, despite carrying a 12g lead weight. As

mentioned earlier (see Table A1), at the start of experiment 2 the birds were considerably lighter than they were at the start of experiment 1 Knot JYG actually decreased in BM rapidly during the first 4 days after the attachment of the lead weight, but by the time the lead weight was removed 6 days later BM had increased again to a level similar to that seen at the start of experiment 2. Two control birds in experiment 2 (Knot JWG and Knot JYY) actually decreased in BM, primarily due to a decrease in PFAT, within 4 days of applying a string harness but BM then increased again to a level similar to the starting mass measured. On removal of the weights there was little evidence of BM returning to the starting levels in experiment 1, but BM in experiment 2 tended to increase again to the levels measured at the start of experiment 2.

Table A3: Shows difference in total body mass (BM), predicted total lean mass (PTLM) and predicted fat mass (PFAT) in grams of both experimental and control birds during experiment 2.

Difference = Finishing mass (removal of weight/harness) minus starting mass at day zero (attachment of weight/harness)

ID		BM (g)	PTLM (g)	PFAT (g)	Mass of weight (g)
JYY	Control	-1	3	-4	-
JWW	Control	6	2	4	-
JWG	Control	-4	4	-8	-
JLW	Control	3	0	3	-
RGG	Experimental	-2	4	-6	13.7
JYG	Experimental	-1	2	-3	12.7
JLL	Experimental	-1	3	-4	11.9
JWY	Experimental	6	4	2	12.1

Figures B1 to B8: Changes seen in predicted total lean mass and in predicted total fat mass in experimental and control birds, during experiment 1.

Day zero is start of experiment, i.e. BMR measurement and then application of weight + harness (experimental birds) or application of harness (control birds).

Figure B1. Experimental bird-Knot JWG (12.2 gram weight applied)

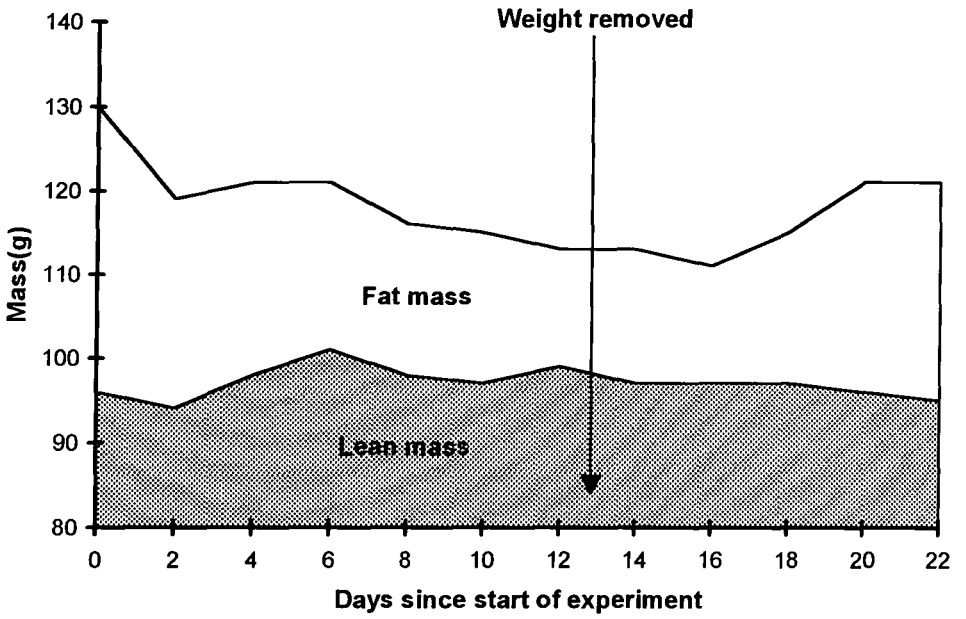


Figure B2. Control bird-Knot JLL

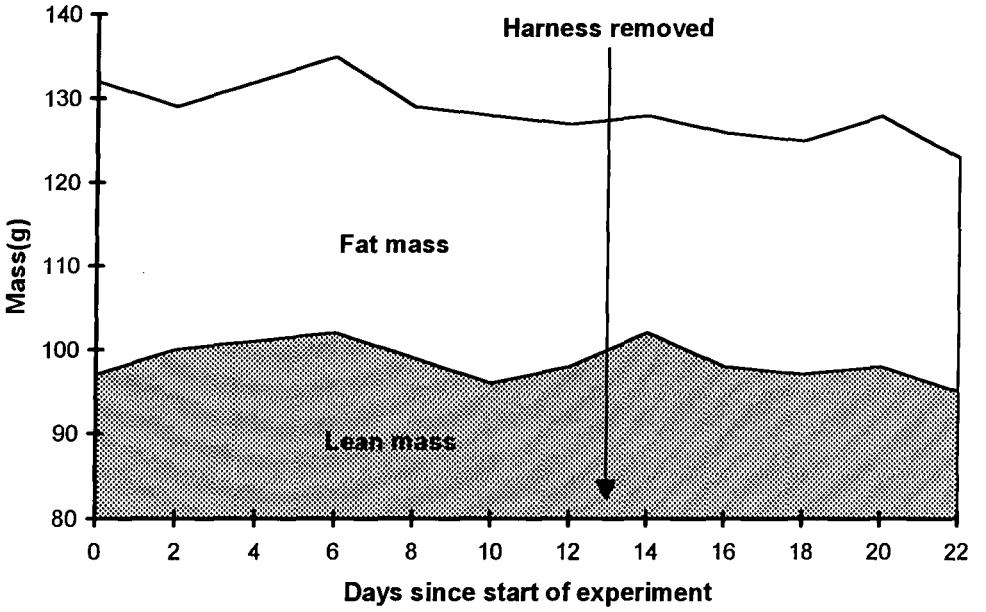


Figure B3. Experimental bird-Knot JLW (11.8g weight applied)

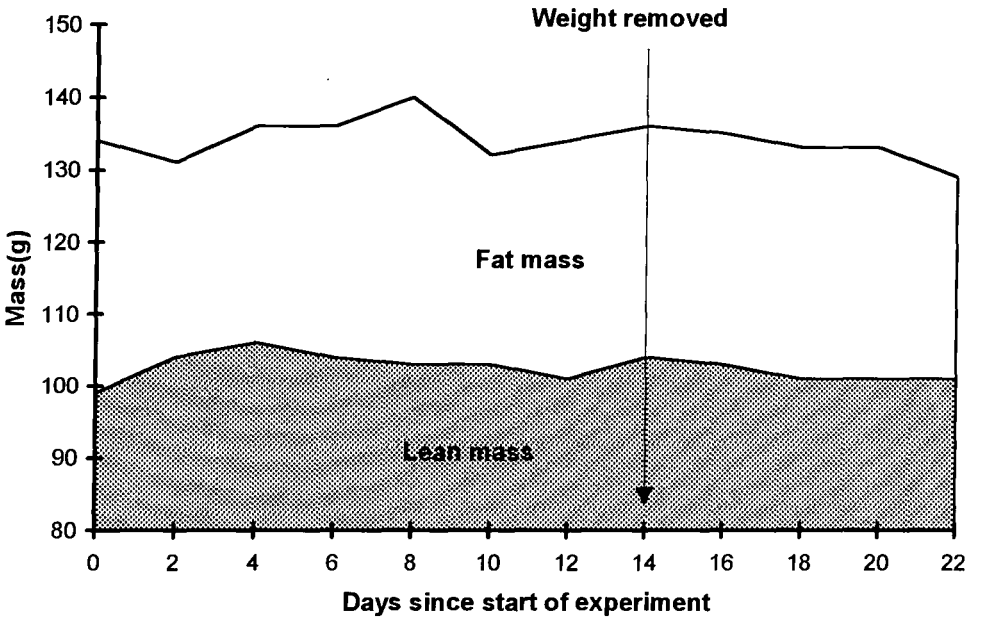


Figure B4. Control bird-Knot JWY

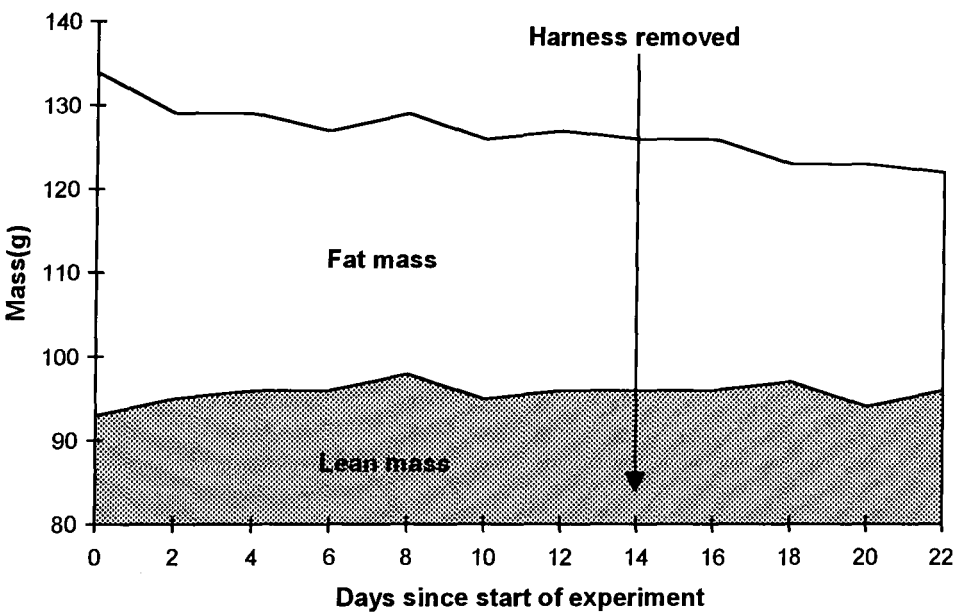


Figure B5. Experimental bird-Knot JYY (12.0g weight applied)

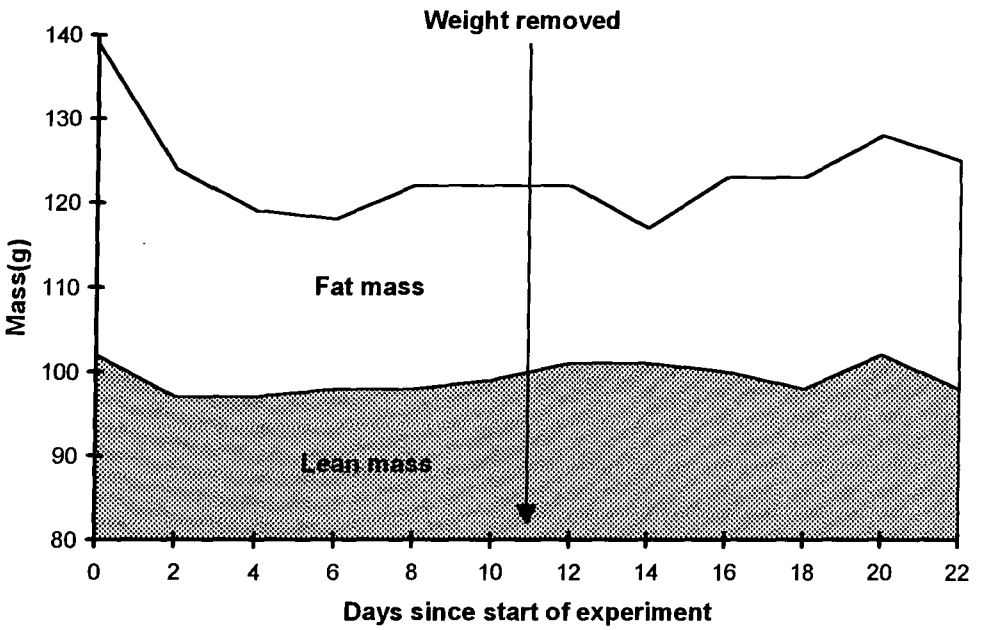


Figure B6. Control bird-Knot RGG

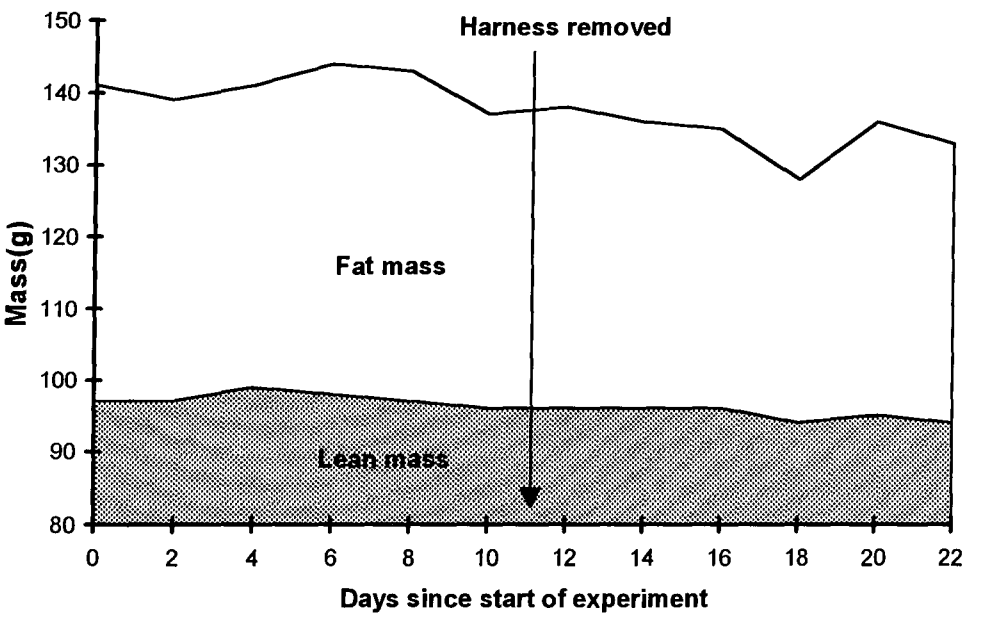


Figure B7. Experimental bird-Knot JWW (13.0g weight applied)

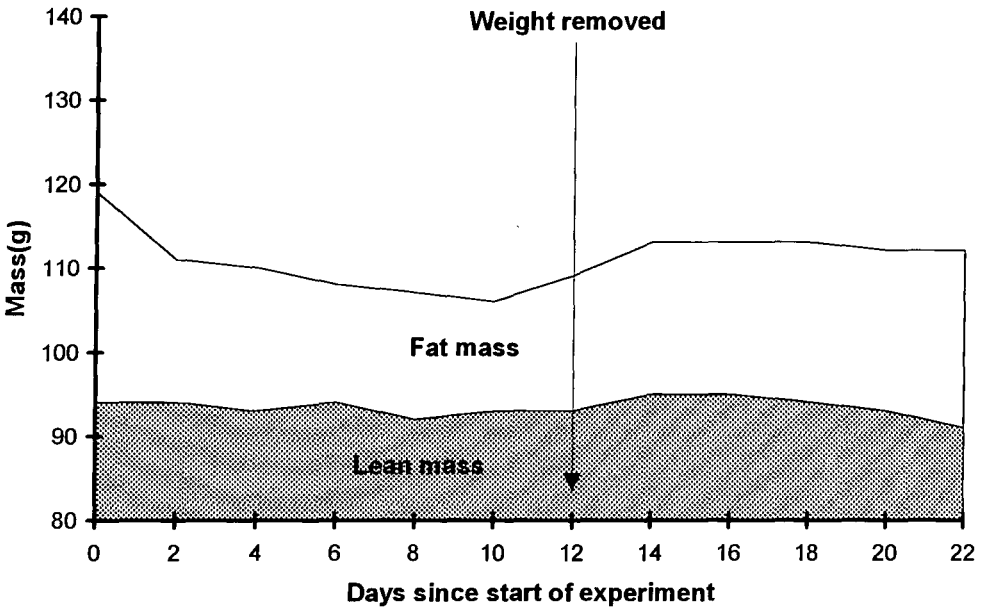
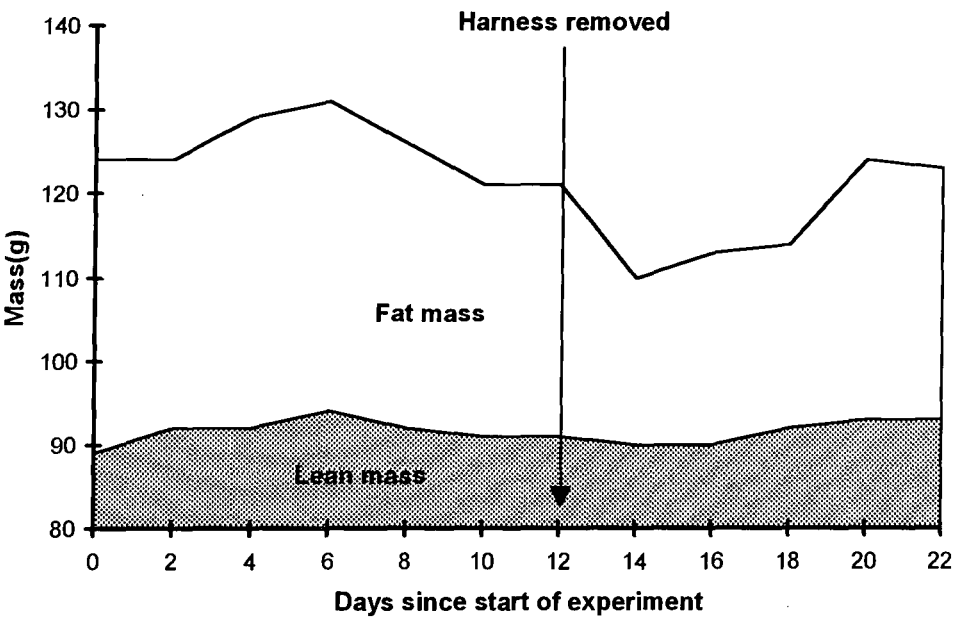


Figure B8. Control bird-Knot JYG



Figures B9 to B16: The changes seen in predicted total lean mass and in predicted total fat mass in pairs of experimental and control birds, during experiment 2.

Figure B9. Experimental bird-Knot JLL (11.9g weight applied)

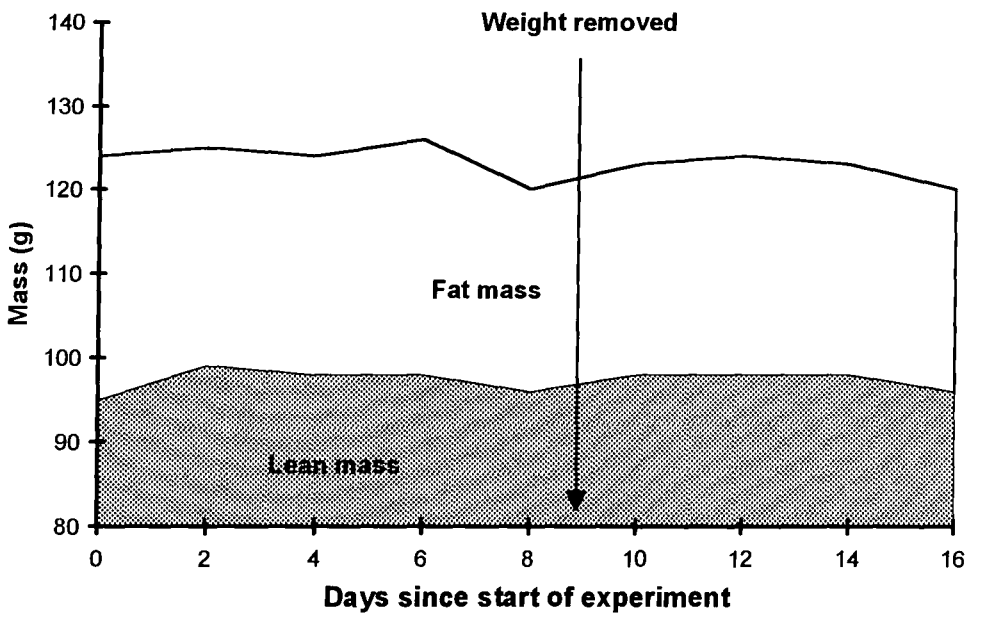


Figure B10. Control bird-Knot JWG

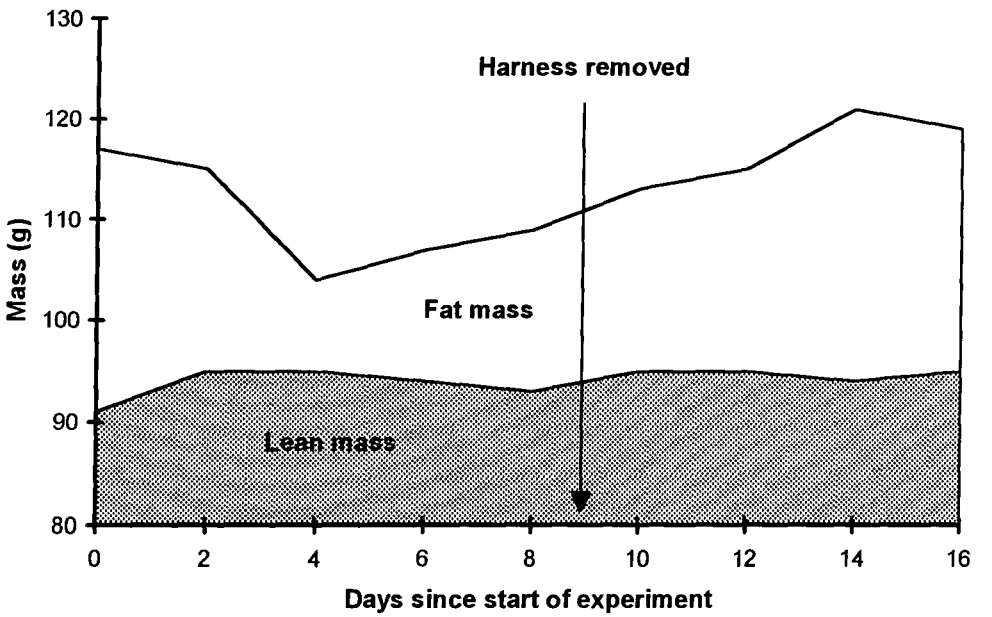


Figure B11. Experimental bird-Knot JWY (12.1g weight applied)

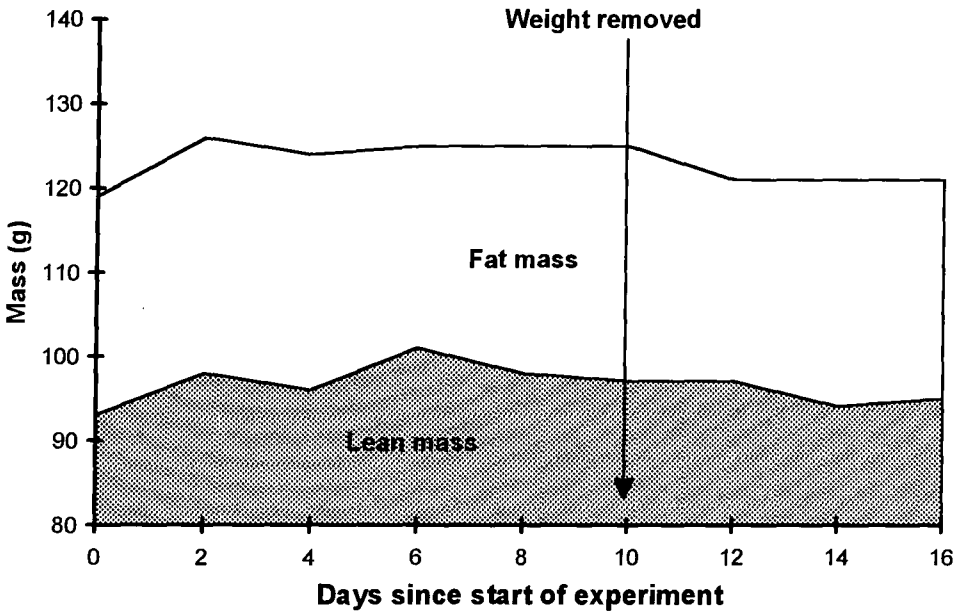


Figure B12. Control bird-Knot JLW

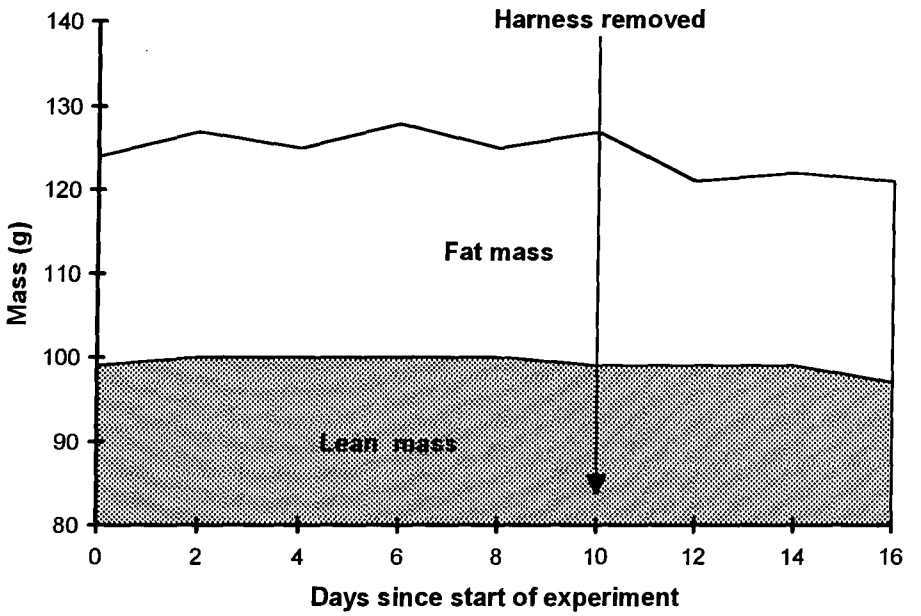


Figure B13. Experimental bird-Knot RGG (13.7g weight applied)

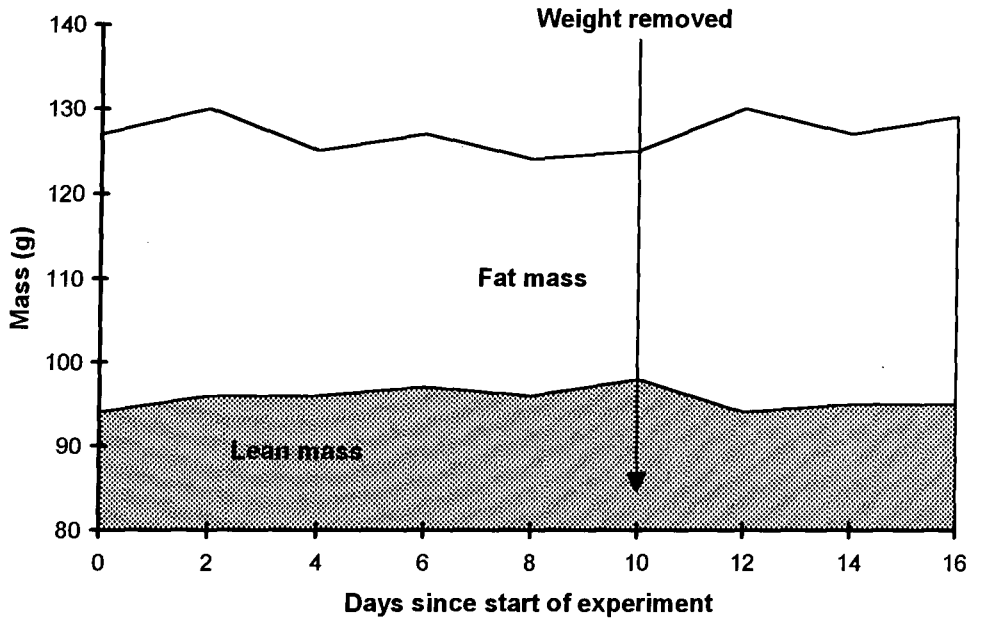


Figure B14. Control bird-Knot JYY

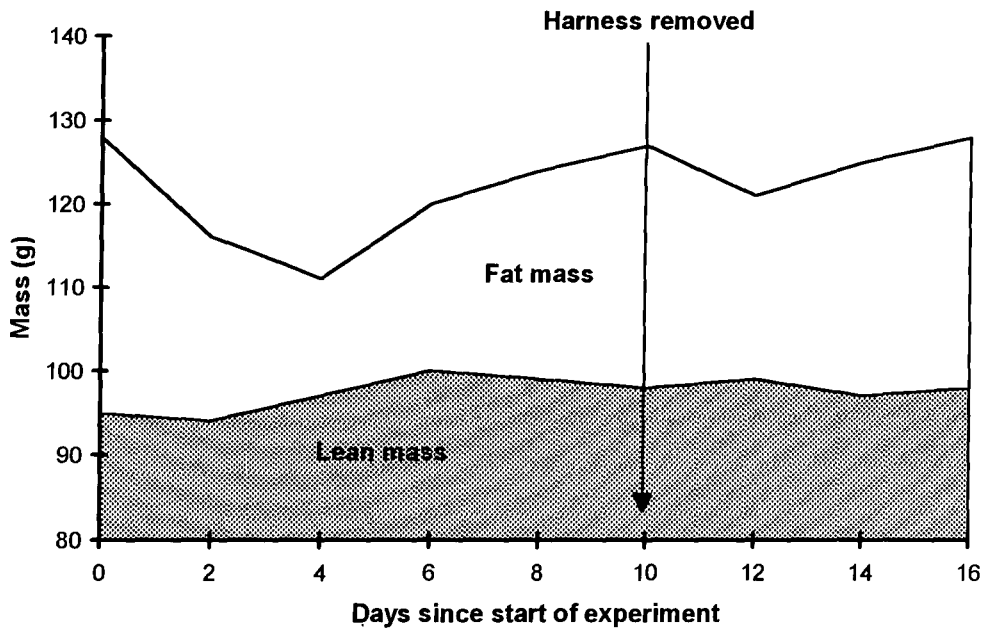


Figure B15. Experimental bird-Knot JYG (12.7g weight applied)

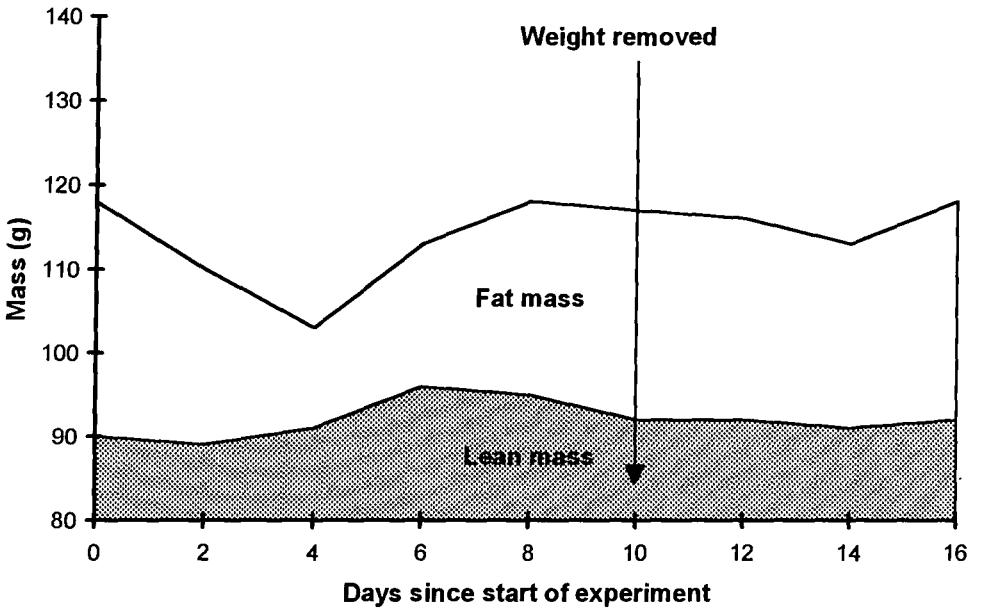
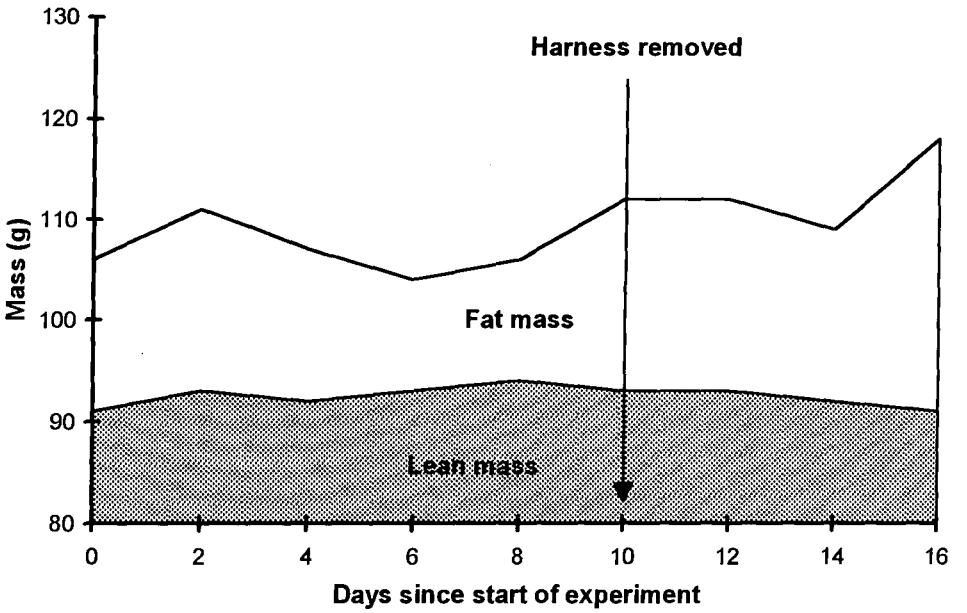


Figure B16. Control bird-Knot JWW



To investigate whether the changes seen in BM and body composition that occurred within individuals during the weight manipulation experiment, differed between experimental and control birds within a designated pair, analysis using paired T-tests was employed. An assumption when using paired t-tests is that there is a natural pairing of observations in the samples. The experimental and control birds in this experiment were paired at the start of the experiment according to body mass, body composition and skeletal measurements. The difference in BM and body composition that occurred between the start and finish of the experiment was calculated for each individual in each pair (experimental and control) and then compared. The change in BM seen during the weight manipulation experiment was not significantly different in the experimental bird in each pair when compared to the control birds (Paired T-test $T_8 = 0.80$, $P>0.05$). There was also no significant difference between individuals in a pair when the changes in PTLM (Paired T-test $T_8 = 0.15$, $P>0.05$) or in PFAT (Paired T-test $T_8 = 1.25$, $P>0.05$) that occurred during the weight manipulation experiment were analysed. Therefore it can clearly be seen from these results, when comparing between individuals in a pair, that the changes that occurred in both BM and body composition were not significantly greater within the experimental birds during the weight manipulation experiment when compared to the control birds.

While the birds in each pair were similar in BM, body composition and size, they were obviously not genetically identical. Therefore, I also investigated the differences that occurred in BM and body composition during this experiment in the group containing experimental birds and separately for the group containing the control birds, i.e. did BM within experimental birds significantly decline during the weight manipulation experiment. No significant change in BM was seen to occur within experimental birds during the weight manipulation experiment (Paired T-test $T_8 = 1.63$, $P>0.05$), although experimental birds did significantly increase in PTLM over this time (Paired T-test $T_8 = 2.62$, $P<0.05$). PFAT measured within experimental birds did however decrease significantly within experimental birds by the time the lead weights were removed (Paired T-test $T_8 =$

0.294, $P<0.05$). Therefore it can be seen from the above results that significant reductions did occur in the PFAT of experimental birds between the attachment and then removal of the artificial weights, with a concomitant significant increase in lean mass. BM did not significantly alter within the control birds between the start and finish of the weight manipulation experiment were not significant (Paired T-test $T_8 = 1.13$, $P>0.05$), although, as with experimental birds, the PTLM did increase significantly within individuals during this time (Paired T-test $T_8 = 3.21$, $P<0.05$). Unlike the experimental birds however, PFAT did not significantly decrease within the control birds during the running of this experiment (Paired T-test $T_8 = 2.08$, $P=0.08$).

The second aim of the weight addition was to investigate the effect that fat mass has on an individual's BMR. However, as seen from the above results, not all experimental birds lost fat mass during the weight manipulation experiment. The BMR measured did not significantly differ between the experimental and control groups at the start (T-Test, $T_{14} =0.48$, $P>0.05$) or at the end of the weight manipulation experiment (T-Test, $T_{14} =0.01$, $P>0.05$). It is clear from earlier results that the mean BM, PTLM and PFAT of the experimental and control birds were very similar at the start of the weighing experiment, but did differ, though not significantly so, at the end of the experiment, i.e. the experimental birds tending on average to lose more fat. The BMR (see Table A4) of the experimental birds did not alter, within an individual, significantly between the start and finish of the weight manipulation experiment (Paired T-Test, $T_8 =0.60$, $P>0.05$), despite PTLM tending to increase. The lean-mass-specific BMR (see Section 2.3 and Chapter 3) also did not significantly change either, within these experimental birds during this time (Paired T-Test, $T_8 =0.30$, $P>0.05$). A similar finding was also seen to occur within the control birds when comparing the BMR and lean mass specific BMR measured at the start and then at the finish of the experiment (Paired T-Test, $T_8 =0.71$, $P>0.05$ and Paired T-Test, $T_8 =1.31$, $P>0.05$, respectively). Within a pair of birds any change in BMR and lean-mass-specific BMR was not significantly different in the experimental birds when

compared with the control individuals (Paired T-Test, T_8 =0.14, P>0.05 and T_8 =0.38, P>0.05, respectively).

Table A4: BMR measurements in captive Knot prior to the attachment of lead weights (start) and prior to the removal of the same weights (finish) in experiment 1 and 2. All BMR measurements in Watts

Exp.1= Experiment 1 Exp. 2= Experiment 2

E= Experimental bird C= Control bird

Diff= Difference

ID	Exp.	Experiment 1			Exp.	Experiment 2		
	1	Start	Diff	Finish	2	Start	Diff	Finish
JYY	**E**	1.29	+0.08	1.37	**C**	0.95	+0.42	1.37
JWW	**E**	1.27	-0.11	1.16	**C**	1.07	+0.33	1.40
JWG	**E**	1.18	-0.14	1.04	**C**	0.94	0	0.94
JLW	**E**	1.07	+0.22	1.29	**C**	1.37	-0.23	1.14
RGG	**C**	1.42	-0.06	1.36	**E**	1.54	-0.18	1.36
JYG	**C**	0.96	+0.09	1.05	**E**	0.80	+0.55	1.35
JLL	**C**	1.22	-0.16	1.06	**E**	1.41	-0.12	1.29
JWY	**C**	0.93	+0.15	1.08	**E**	1.36	+0.12	1.48

Discussion

The primary objective of the work reported in this appendix was to test the hypothesis that captive Knot regulate both their total body mass (BM) and their total lean mass (TLM) directly so that fat mass they carry is regulated only indirectly as a consequence. From the results section, it was shown that 3 of the 4 experimental birds in experiment 1 did decrease in BM during the running of the weight manipulation experiment, primarily due to a decrease in the fat component

of body mass. These reductions in BM and predicted fat mass (PFAT) actually exceeded the mass of the artificial weights carried by the 3 individual Knot. The control birds in experiment 1 also all decreased in BM and fat mass between the attachment and removal of the string harnesses, although these harnesses only weighed approximately 0.2 grams. However, what is also evident from the results section is that the experimental birds during the running of experiment 2 did not decrease in BM or in PFAT to any great degree after the attachment of the lead weights. From the results section it is also clearly evident that during experiment 2 no clear trend in BM or PFAT was seen in the control birds either, with two individuals increasing in these BM parameters and two decreasing. Further evidence that BM regulation did not tend to occur was shown when the changes in BM and body composition that took place during the weight manipulation experiment were compared between the experimental and control bird in a designated pair. One would have expected that the experimental birds in each pair would have decreased in BM, primarily due to a decrease in PFAT, during the weight manipulation experiment to a greater degree than that seen in the control bird. However, the changes that occurred in BM and body composition in the experimental bird of each pair were not significantly greater than those measured in the control individual.

While the results presented in this appendix are fairly inconclusive, they did show that the mean BM and mean PFAT of the experimental group of birds did decrease to a greater extent, by the time the weights and harnesses were removed, than the decrease seen in the mean BM and mean PFAT of the control birds. During the running of experiments 1 and 2, individual birds in both the control and experimental groups tended to increase in the lean component of BM, which may imply that some seasonal changes in body composition were taking place. The apparent lack of good evidence to suggest that captive Knot can internally regulate their BM through a process of internal weighing, is given further credence with what happens to an individual's BM after the removal of the lead weights. The prediction, following on from the original hypothesis, would have

been that on removal of the artificial weights an individual's BM would increase back to a level similar to that seen at the start of the experiment. This was clearly not the case in experiment 1, where on removal of the weight BM tended to remain lower that at the start. Mean BM, PTLM and PFAT of the 8 birds used in this experiment were all significantly lower at the start of experiment 2, than at the start of experiment 1.

The second objective in this weight manipulation experiment was to investigate the effect that fat mass has on an individual's basal metabolic rate (BMR). The prediction in this experiment being that if fat mass decreased, due to the carrying of an inert lead weight, the indirect costs of carrying this weight would still be present, therefore the BMR measured in that individual should not differ from that measured prior to the weight being applied. In those 3 individual Knot that lost a considerable amount of fat, no discernible trend was seen the BMR measured before and approx. 12 days after the attachment of the lead weights. The Knot JWG lost the greatest mass of fat during the weight manipulation experiment at 18g, but the actual direct O_2 consumption (see Chapter 4) of 18g of fat, using the value measured by Scott & Evans (1992), is only 0.07 Watts, or less than 5% of the total BMR measured. Therefore to determine the effects that fat mass has on an individual's BMR, it may be necessary to use individuals that carry considerably larger fat masses. Although PTLM tended to increase within both experimental and control birds during the course of the weight manipulation experiment by an average of 2g, there was no discernible trend seen in BMR during this time, i.e. some individuals in both experimental and control groups increased in BMR during the experiment and some decreased in BMR, irrespective of the increase or decrease in PTLM measured in that individual.

The possible reason or reasons why there appeared to be little evidence of BM regulation due to internal weighing in these captive Knot, particularly during experiment 2, will now be discussed. The first obvious reason why the application

of weights on to the back of individual Knot did not lead to a decrease in BM and fat mass may simply be because the mechanism involved in BM regulation in these birds is not affected by mechanical loading. It is possible that some biochemical cue, possibly released by the fat mass itself, regulates the BM of individual birds. It has been hypothesised that leptin, a 16-kDa protein, is a mammalian humerol signal from adipose tissue that acts on the central nervous system, reducing food intake and increasing energy expenditure in a negative feedback manner (Ahren *et al*, 1997). It is feasible therefore that some biochemical substance is released directly from the fat mass, probably due to some diurnal stimulus, and this regulates BM in birds, although leptin has not as yet been discovered in birds (J. Speakman, *pers. com.*). This however does not explain why captive waders tend to maintain seasonally similar body masses to wild conspecifics (Scott *et al*, 1994). It has been well recorded that when waders are brought into captivity, they decrease in the lean component of BM (primarily in the 'digestive organs') and increase in the fat mass carried, thereby maintaining BM at levels similar to those seen in the wild conspecifics (Piersma, 1994; Piersma *et al*, 1996; Mitchell, 1996; Appendix III). Regulation of BM, primarily to variation in the fat mass, may be due to 'stretch receptors' in the skin that are analogous to the baroceptors found in vertebrate arteries. A decrease in overall BM may lead to a decrease in the tensile stress acting on these putative receptors, possibly leading to an increase in hyperphagia. It may be that some stimulus, such as photoperiod, acts indirectly on these stretch receptors, leading to the seasonally shifting optimal BM seen in wild waders (Dugan *et al*, 1981).

An assumption of this experiment was that no seasonal variation occurred within-individuals in BM or in body composition during time-scale of the experiment. From Chapter 3 and Appendix I it can clearly be seen that captive Knot exhibit fluctuations in BM and particularly in the fat component of BM during the annual cycle. Therefore, although the experiment was run during a period (March-early April) of fairly stable BM and body composition, it may be that the lead weights were just too light to counter any seasonal effect seen within individual birds. The

birds at the start of experiment 2 were significantly lighter in BM, PTLM and PFAT when compared to the start of experiment 2. The lack of any evidence of BM regulation in experiment 2 may simply because these individuals were not at their optimal BM, but were underweight. It may therefore be that BM regulation is over-ridden if BM is low, i.e. these experimental birds could not 'afford ' to decrease in BM or in fat mass during experiment 2 anymore, even though the lead weight were attached to their backs. The mean BM of individuals at the start of experiment 2 is particularly low for adult Knot during March-April in captivity (see Appendix III). It must however also be mentioned that one of the experimental birds in experiment 1 that did decrease in BM (Knot JWW), had a starting mass of only 119g.

The lack of any clear evidence of BM regulation in experiment 2 would not appear to be due to the complicating effect of pre-migratory fat deposition taking place, as experiment 2 finished in late April and the Knot used in this experiment generally started to exhibit pre-migratory fat deposition in early June. That these birds tended to deposit fat somewhat late, when compared to other captive Knot that were not subjected to the carrying of artificial weights (see Chapter 3 and Appendix I), may indicate that the regulatory processes involved in pre-migratory fattening were upset by the application of the artificial weight. It may also simply be that the Knot used in the weighing experiment were in poor physical condition after the experiment, hence the fact that they did not tend to deposit fat until somewhat later in the year. There was also no connection between an individual's apparent ability to regulate BM and that individual exhibiting pre-migratory fattening. Indeed, the 3 individuals in experiment 1 that did appear to regulate BM (JYY, JWW, and JWG) did not exhibit a spring increase in BM typically associated with other captive adult Knot in this study. Of the other 5 individuals used in this study, only individual JWY did not increase in mass in spring.

Another possible reason for the lack of evidence that Knot regulate their BM internally through a process of internal weighing may be due to the attached weights being too light, or being positioned in the wrong area. Witter *et al* (1994) attached weights of up to 8g on to the backs of Starling *Sturnus vulgaris*, which were then still able to fly with these weights attached. If the mean mass of Starling are taken to be approx. 74g (Witter & Goldsmith, 1997), then these 8g weights accounted for around 11% of total body mass. In my study, the mass of artificial weights applied was approx. 12g and the mean BM measured was 126g. Therefore the artificial weights in my study accounted for around 9.5% of starting BM. Although the relative mass of the lead weights in my study were less than those in Witter *et al's* (1994) study, captive Knot had great difficulty walking if weights any greater than around 14g (11% of starting BM) were attached to their backs. This inability to cope with weights greater than 14g may have been, in part, due to the fact that the weights used in my study were occasionally prone to slipping to the side of an individual's back. This will have undoubtedly have altered that individual's centre of gravity and affected its gait biomechanically. Witter *et al* (1994) suggested that modelling clay of equal mass positioned on each leg of captive Starling did not affect manoeuvrability during flight as much as when the weights were attached to the tail or back. However the modelling clay in their study was removed at the end of every day and the conditions the experiments were run in was dry, unlike in my experiment. Modelling clay, is likely to go soggy in damp conditions and this could well affect the accuracy of TOBEC measurements, therefore this clay was not deemed suitable for a study such as mine. The attachment of lead weights to the legs would also have affected the TOBEC output, unless they were easily removed prior to a TOBEC measurement.

An increase of stress in individual birds during the running of the experiment may have affected that individual's ability to regulate total body mass. All individuals used in this experiment however had been in captivity for over 3 months and all had previous experience of being handled during TOBEC and BMR

measurements. Skin abrasions were present in experimental birds during both experiment 1 and experiment 2, but were slightly more severe during experiment 2. It may be possible that these slight skin abrasions that occurred in some experimental birds, allied to an increase in handling, may also have affected an individual's ability to regulate BM, possibly through the corticosterone stress response. This stress response is known to occur in wild birds during their capture (Harvey *et al*, 1984; Wingfield *et al*, 1995). Corticosterone levels are known to be associated with both food-intake rate and metabolic expenditure and there is experimental evidence that corticosteroids regulate fattening in some birds (Dolnik & Blyumental, 1967; Wingfield *et al*, 1990) and therefore any alteration in the level of corticosteroids within an individual may possibly affect that individual's ability to regulate its total body mass. However, if the birds were physiologically stressed one would think that this may affect an individual's BMR and one would also not expect that BM in some of the experimental birds would increase, although some individual's may have coped with the stress better than others.

From the results section it can be seen that the BMR of individuals birds and their lean mass-specific BMR did not increase in all birds between the application and removal of the lead weights. Therefore, it can be seen that there only very tentative evidence from this work that captive Knot regulate their BM through a process of internal weighing, although experimental birds did tend to decrease in BM, primarily due to a decrease in the fat component of BM. That is not to say that a mechanism of internal weighing can be discounted. A longer-term study, with an improved experimental design (particularly in weight attachment) may provide a better insight into what factors are involved in BM regulation in waders.