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by

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This thesis is presented in candidature for the degree of doctor of philosophy in the University of Durham, August 1991

To my parents Bert and Veronica and to all those
people both past and present with whom
I have had the pleasure of climbing
both crag and mountain with.

ABSTRACT

Studies on seasonal variation in metabolic rate related to changes in body composition, with particular reference to shorebirds (*Charadrii*).

The basal metabolic rate of three species of shorebird was measured throughout the non-breeding season. These measurements were related to change in body mass and body composition. No seasonal pattern in BMR was apparent after variation related to changes in body mass and body composition had been accounted for.

Seasonal variation in body mass of captive Grey Plover and Redshank was found to resemble that of the same species in the wild. This was not so for Sanderling.

Body composition changes were either inferred from destructive analysis, or measured using a technique known as total body electrical conductance (TOBEC). The intraspecific relationship between TLM (Total lean mass) and TOBEC index was found to be best described by a linear equation.

Separate intraspecific allometric equations were derived relating BMR to body mass for two shorebird and one wildfowl species. The mass exponents in these equations were found to be 1.03, 0.62 and 0.61 for Redshank, Grey Plover and Wigeon respectively. The results were related to the current interpretations of the BMR/body mass exponent.

The within-individual BMR/body mass relationship was investigated for Redshank and Grey Plover. The mean mass exponent was found to be 1.23 and 0.92 respectively. No significant relationship was found for any individual Sanderling.

Variation in BMR within an individual was related to variation in body composition. In most cases variation in body fat was found to be the most important predictor of within-individual variation in metabolic rate.

In Vitro determinations of the oxygen uptake of avian fat, liver and muscle tissues indicated that the energy consumption of fat was less than one tenth that of liver and muscle. This indicates that within-individual increases in BMR with increased levels of fat are probably associated with increased metabolic output of the lean tissues.

CONTENTS

Acknowledgements

This study was funded by a Natural Environment Research Council Studentship. I am grateful to the Department of biological sciences of the University of Durham for the use of research facilities.

Throughout this study I have received much help from many people in the often frustrating task of catching birds. I would like to thank in particular John Richardson, Stewart Lowther, Sarah Wilson and Neil Anderson. I am grateful to the Tees and South Cleveland ringing groups for providing me with their data. I would like to thank Murray Grant for his help with the work presented in Chapter 2.

During the course of my work I have benefited from discussions with and information provided by friends and colleagues at the University of Durham. I would like to take this opportunity to thank Digger Jackson, Dr. Brian Huntley (for statistical advice), Roland Ascroft and Prof K. Bowler. In this context I would also like to thank Jerremy Hills for the many enlightening conversations that were had whilst sitting around the much used tea pot.

Thanks must also go to Dr. and Mrs Grant for the use of their garden and to Michael Bone for his technical support and endless enthusiasm.

Finally, I wish to thank and acknowledge Prof. P.R. Evans for his help and advice throughout this project.

INTRODUCTION

The metabolic rate is the overall rate of energy utilization by an organism. It is the summation of all anabolic and catabolic energy transformations. Such energy transformations are the basis for all biological activity. The metabolic rate of an animal dictates its food requirements, is intricately linked to food availability and is thus an important influence on its ecology. It is increased by life processes such as food processing, growth, locomotion and most significantly (for endothermic animals) temperature regulation. The minimal level of energy utilization that an individual animal requires to maintain life is called its basal metabolic rate (BMR) (sometimes referred to as standard or maintenance metabolism). For an endotherm this is usually taken to be the rate of energy utilization by an individual at complete rest unstimulated by the digestion and assimilation of food, or by low temperatures. My study was concerned with the basal metabolic rates of endotherms and principally with those of birds. I have focussed on inter-individual and intraspecific difference in BMR in a few species of birds examined over the non-breeding season and on seasonal changes within individual birds.

Interspecific variation in BMR

Interspecifically, basal metabolic rate has been shown to be highly correlated with mean body mass and size of each species (Kleiber, 1947, 1961; Hemmingsen, 1960; Lasiewski and Dawson,

1967, and Kendeigh *et al*, 1977). This relationship accounts for about 80% of the observed variation in metabolic rate of endotherms (McNab, 1988a). The rate of metabolism is thought by some authors to be central to the scaling of other life parameters such as "physiological" time (Lindstedt and Calder, 1981), reproduction and growth (Reiss, 1989) and brain size (Harvey and Bennet, 1983).

The form of the relationship between metabolic rate and mass can be expressed according to the allometric equation $MR = aM^b$ where MR = Basal metabolic rate M = Mass a = mass coefficient and b = mass exponent. When expressed logarithmically such a relationship between metabolic rate and mass is linear, (LogMR = Log a + b Log M). The intercept term is referred to as the proportionality coefficient and the slope term the mass exponent. Historically most attention has focused on the importance and relevance of the mass exponent. This is probably because the mass exponent has been seen as being highly conserved across animal classes and has been considered by many to represent a fundamental facet of similarity between organisms (Hemmingsen, 1960). The mass coefficient, conversely, has been seen as being a measure of the intensity of metabolic activity of a particular class or group or organisms and consistent within that class.

In actual practice the definition of basal metabolic level for ectothermic classes of animals is extremely difficult, as differing results are obtained depending on temperature, presence of food, light, season and thermal history of the animal under study (Schmidt-Nielson, 1984). Closer examination of Hemmingsen's

(1960) data shows that between ectothermic classes there is little consistency in the mass exponent. Values have been produced of 0.83-0.86 for reptiles (Bennett and Dawson, 1976; Wheeler, 1984), 0.66 for amphibians and of 0.75-0.94 for fish (Schmidt-Nielson, 1984). For invertebrates, slopes listed by Altman and Dittmer (1968) varied from less than 0.67 to over 1.0. Having noted the large degree of heterogeneity with respect to the mass exponent of BMR, it is true to say that the overall mass exponent for ectothermic, endothermic and unicellular animals is 0.75 (Hemmingsen, 1960). Nevertheless, during the pursuit of the functional interpretation of the mass exponent, most attention has focussed on mammals and birds, due largely to the difficulty in defining basal conditions for ectotherms.

For Mammals the mass exponent has been found to be between 0.73 and 0.75 (Brody *et al*, 1926; Kleiber, 1932, 1961; and Hemmingsen, 1960). The value is usually quoted as 0.75. This value is satisfactory, as Klieber (1961) has reasoned that, given all the potential available information, it is not possible to distinguish between a slope of 0.73 and that of 0.75.

For birds the mass exponent has been found to be between 0.72 and 0.74 for non-passerines and 0.65-0.72, depending on season, for passerines (Lasiewski and Dawson, 1967 and Kendeigh *et al*, 1977). When both passerines and non-passerines are taken together the value of the exponent is found to be between 0.66 and 0.68 (Kendeigh *et al*, 1977; Elgar and Harvey, 1987 and Daan *et al*, 1989). This arises because, the proportionality coefficient (the

intercept of the log form of the allometric equation) is higher for passerines than for non-passerines.

The reason for separate analysis of passerines and non-passerines has been argued by some authors (see Schmidt-Nielsen 1985) as follows: characteristically, passerines tend to have higher body temperatures than do non-passerines. Since it is known from work on ectothermic animals that a rise in body temperature causes an increase in the metabolic rate, and it is observed that passerines tend to have higher basal metabolic rates than non-passerines of the same body size, separation of the passerines from non-passerines eliminates, or helps to eliminate, variation in body temperature as a variable in the equation. As passerines also tend to be small birds (6g-1000g), when the metabolic rate/mass relationship is plotted passerines dominate the lower end of the graph. This tends to pull the slope of the graph upwards at its lower end, thus making the overall slope shallower. This effect on the slope is enhanced if least-squares regression analysis is used (as is common) due to the disproportionate effect of out-lying points.

This argument has been countered by Daan *et al*, (1989). Based on the work of Bennett and Harvey (1988), they argue that within the non-passerines there is as much variation between orders as there is deviation from the Passeriformes.

Causes of interspecific variation in BMR

The functional significance of the mass exponent has given rise to much debate and speculation (Kleiber, 1961; McMahon, 1973; Blum, 1977; Kendeigh *et al*, 1977; Boddington, 1978; Economos, 1979a; Feldman and McMahon, 1983; Heusner, 1983, 1984, 1987; Wieser, 1984; and McNab, 1988a&b). A satisfactory explanation based on physical principles has yet to be found. Many of the suggested arguments are examined in reviews by Schmidt-Nielsen (1984) and Blaxter (1989).

Traditionally the so called "surface law " has been invoked to explain the disproportionate relationship between mass and basal metabolic rate increase. It is assumed here that all of the cost of basal metabolism can be explained by the cost of maintaining constant deep-body (core) temperature. This argument was first developed by Sarrus (1839) and experimentally examined by Rubner (1883). The implication is that an increase in metabolic rate will be proportional to an increase in surface area, as it is from this that an animal loses heat. The increase in surface area of a constant density isometric object, with mass, will have an exponent of 0.67 and so,it is thus argued, should metabolic rate. Whilst this value does agree with the experimentally determined value obtained by Kendeigh (1977) for all birds, it does not agree with that for mammals or that for birds when passerines and non-passerines are treated separately. Schmidt-Nielsen (1984) remarks that, as metabolic rate varies with $body\ mass^{0.75}$ even for ectothermic animals, the surface area argument is spurious. I believe that this conclusion is incorrect, and that we should not

be surprised if animals whose body temperature depends mainly on behaviourally controlled exchanges of heat with the environment, via their external surfaces, expend energy in a manner which scales with surface area.

The "surface law" theory could still remain acceptable if surface area varies with mass with an exponent which differs from 0.67 eg. 0.75. In practice however, although surface area has proven a very difficult parameter to measure with sufficient accuracy, the available evidence suggests that it does vary with body mass with an exponent of 0.67 (Schmidt-Nielsen, 1984). Other authors whilst accepting the fundamental importance of the "surface law" hypotheses, have attempted to incorporate other physical parameters to explain the discrepancy between the observed and the expected exponent. On the basis of Blum's (1977) argument that $volume^{0.75}$ would be the exponent of the surface area of a hypothetical four dimensional sphere, Boddington (1978) suggested that the fourth dimension required to raise the 0.67 mass exponent to 0.75 may be time. On the other hand, Economos (1979a,b) put forward the theory that the increase in gravitational force as mass increases would account for the discrepancy between observed and expected value of the mass exponent. Economos calculated that the metabolic cost of gravity should scale with $mass^{0.89}$ and postulates that basal metabolism under conditions of terrestrial gravity is the sum of gravity's metabolic cost and a surface related metabolic expenditure which is independent of gravity. It is difficult to see how this argument would apply to those animals which live in water and are neutrally buoyant, as they expend little or no energy in

supporting themselves. Wieser (1984) suggested that the discrepancy is a result of the evolutionary trend to greater size. He postulates that the increase in metabolic intensity (mass coefficient) with size detected by Heusner (see below) represents a qualification of "Cope's law" (Cope, 1885; Newell, 1949), that in the course of evolution the representative of each taxon grew bigger.

McMahon (1973) developed an elaborate theory based on the principle that animals are not isometrically similar but show the property of elastic similarity. This argument has been summarized by Schmidt-Nielsen (1984) in the following way: animals cannot remain geometrically similar (isometric) as their sizes increase, because the cross-sectional area of their limbs would increase only as the square of the characteristic linear dimension "l", although they would need to support an increase in mass proportional to l^3. MacMahon argues further that the limbs of an animal are exposed to similar forces of buckling and bending. Thus, all proportions of an animal should change with size in the same way. From this argument it is possible to derive the result that metabolic rate scales interspecifically with $mass^{0.75}$ without having to contend with the "surface law".

Heusner in several papers (1982, 1984 and 1987), suggested that the 0.75 mass exponent has no physical basis and is the result of the inappropriate application of regression analysis, *ie.* it is only a description of the data. Heusner suggested that the mass exponent for any individual species should be 0.67 and demonstrated that the proportionality coefficient "a" varies with

mass, ie. it is not a constant. When physical interpretations are placed on the value obtained for the exponent "b" from the interspecific regression of mass and metabolic rate, it has been assumed that it is a constant over all mass ranges. Whilst this argument has been countered in part (Feldman and MacMahon, 1983; and Iberall, 1984), it is compelling, as the surface law argument then becomes satisfactory.

Ecological and evolutionary implications of the BMR/mass relationship

Several authors have sought to examine the deviation that occurs from the expected BMR/mass slope in terms of the feeding habits and habitats of differing taxonomic groups. McNab (1988a,b), concludes that much of the remaining variation in basal rate among birds and mammals is associated with feeding habits; but Elgar and Harvey (1987) report that among mammals the only consistently significant association that occurs is in the higher relative basal metabolic rate of vertebrate eaters. Similarly Bennet and Harvey report (1987) that among birds they could detect no significant associations between variation in resting metabolic rate and differences in diet. Bennet and Harvey (1987) contend that phylogenetic considerations are of more importance than feeding habits or habitat preference. All of the above mentioned authors warn of the potential theoretical problems that can arise from a failure to take into account the sample size mass range and phylogenetic make up. This warning could be interpreted in the form that animals of different species do not necessarily possess a high degree of functional similarity and so

support the contention that there is no functional significance behind the overall BMR/mass exponent.

It can be argued that descriptions of deviations from an expected value or base line equation can only remain descriptions of the data, and that identification of reasons for such deviations will only be facilitated when the physical mechanism for the basic equation is understood. To some extent defining the theoretical physical basis (if there is one) for the observed relationship between BMR and body mass may be of little consequence to the applied biologist as its ecological implications still remain regardless of functional understanding and the ecologist will still require to be able to make predictions of energy consumption rates in the field. Many authors have published work pertaining to the evolutionary and ecological implications of this relationship and other similar size-related relationships (for example, Bonner, 1965; Calder, 1974; Peters, 1983; Schmidt-Nielsen, 1984; Reiss, 1989).

One of the most widely quoted implication of the BMR/mass relationship is that as mass increases so mass specific demands decrease. A consequence of this is that smaller species will have a higher rate of energy turnover per unit mass of tissue than do larger animals. As described by Peters (1983), a 500 Kg. Moose *Alies americanes* had an energy expenditure at basal conditions of 436 Watts whilst 500 Kg of mice would expend 5430 Watts. Measured in simple terms of energetics, for a given biomass, smaller animals have a larger impact on the ecosystem than do larger ones.

The fasting endurance time of an animal depends on the size of its energy reserves and the rate of energy consumption. Given a mean energy content for fresh tissue of $7x10^6 JKg^{-1}$ (Cummins and Wuychek, 1971) and constant body temperature, at basal levels of energy expenditure, a 20g homeotherm will metabolize 100% of its body tissues in about 7.3 days, whilst a 500 Kg homeotherm would take 91 days. Survival time for birds has been found to be (in hours), 24.3 $Mass^{0.39}$ for temperatures between +2°C and +6°C but only 5.4 $Mass^{0.58}$ at temperatures between -1°C and -9°C (Kendeigh *et al*, 1977). The disproportionate decrease in survival time as ambient temperature decreases in smaller birds compared to larger birds is brought about by the relationship between heat conductance and body mass. This can be expressed as a power function of mass with an exponent of between 0.45 and 0.65. As this slope is shallower than that for BMR/body mass, the increase in metabolic rate required to counter heat loss to a given ambient temperature is proportionately less for larger birds than for smaller birds. This argument can be evoked to explain why some small homeotherms enter into torpor in response to cold and lack of food.

It can thus be expected that smaller animals are much more dependent on a stable food supply than are larger ones and will generally spend more time foraging than larger ones (Peters, 1983). Gibb (1954) reported that the time spent feeding was inversely related to body size in a series of species of tits (Paridae). The mass exponent of this relationship was found to be

$M^{-0.28}$, which is close to the inverse of the BMR/mass curve for passerines.

A consideration of the BMR/mass relationship at the cellular level suggests that in smaller animals the cells use energy at a higher rate than do the same cell type of a larger animal. Whilst there have been several studies relating tissue respiration to overall rates of oxygen consumption (Grafe *et al*, 1925; Krebs, 1950; Martin and Fuhrman, 1955), these have proved difficult to interpret (Schmidt-Nielson, 1984). As most energy generated by homeotherms is derived via oxidative-phosphorylation it would be expected that mitochondrial density, or more exactly, cristae surface area, should scale with body size. Smith (1956) reported results that indicate that the total number of liver mitochondria per gram body mass decreases with increasing body size with the mass exponent of -0.28 (for rat rabbit sheep and cattle), which is similar to the mass specific exponent for whole animal metabolism (-0.25). This is supported by the findings of Mathiew *et al* (1981). Such data suggest that the allometric dependence of metabolic rate on mass may reside in the structural and functional organisation of the cells themselves. In addition, the difference in metabolic intensity between mammals and reptiles has been accounted for by the variation in cristae surface area and cytochrome c activity found in liver and muscle tissues taken from these animals (Else and Hulbert, 1981).

The importance of cellular considerations is that metabolic scope, ie. the ability to increase metabolic rate above basal, may be limited by the cellular capacity to generate energy at a

sufficient rate. The relationship between maximum metabolic rate and body mass has a mass exponent between 0.82 and 0.84 (Taylor *et al* 1980 and Koteja, 1987). Thus, in general larger animals have a greater metabolic scope, measured in absolute terms, than smaller ones.

It would appear at first that there are advantages to being larger rather than smaller. Indeed, through the course of evolutionary history it would seem that there has been a trend for an increase in size (Bonner, 1965). Whilst there have been some decreases in size in particular evolutionary lineages, the capacity for increased size and mean size has almost certainly increased. Being larger does carry costs, however. In absolute terms larger animals require more food than do smaller ones. Thus, larger animals will have less ability to exploit marginal habitats than smaller ones. In addition, other parameters such as home range may increase disproportionately with size. For birds, home range increases according to $Mass^{1.16}$ (Schoener, 1968) so although being larger may mean having to spend less time foraging, it may mean having to spend more time defending territory.

Intraspecific variation in BMR

One would expect that some of the evolutionary drive towards an increase in size should manifest itself at the species level. Nevertheless one of the most overlooked levels through which an insight may be gained is at the intraspecific level. For mammals, Heusner, (1983) could find only 7 species (all domestic) in the

literature for which he could examine the intraspecific relationship and only a few more data are available to date (Wheeler, 1984; Schofield, 1985 and Blaxter, 1989) For birds, although values can be found for nine species for the mass exponent of Average Daily Metabolic Rate (ADMR), or Daily energy expenditure (DEE), only one value is available for BMR (a recently published study by Daan *et al.* (1989)). This study found that the intraspecific exponent for Kestrels *Falco tinnunculus* was 0.78 (SE=0.226) which is not statistically distinguishable from the mass exponent for all birds (whether the passeriforms are included or not). Nevertheless it raises the possibility that the intra-specific mass exponent may differ from that produced from interspecific data. If either Heusner's or Feldman and MacMahon's arguments are correct then it would be predicted that intraspecifically the mass exponent should be 0.67, whilst if the theories of Blum and Boddington or Economos are correct the value of the exponent should be 0.75, ie the same as the value for the interspecific relationship.

Seasonal variation in BMR

Several authors have indicated that BMR varies with season (Pohl, 1971; West, 1972; Kendeigh *et al.* 1977 and Saarela, 1980), although few of these studies have involved continuous monitoring throughout the season. It is well known that many species show large seasonal variation in the body mass (King, 1972; and Blem, 1973), chiefly attributable to changes in the levels of fat being carried (Helms, 1968; Blem, 1973 and Dugan *et al.* 1981), although it may also result from considerable build up of flight muscle

before migration in some species (Davidson and Evans 1990). This raises the question: as to what extent can seasonal variation in BMR be explained simply by change in body composition and to what degree do factors such as cold acclimatization, changing hormone levels and migratory preparation alter the BMR throughout the season?

Fat or lipid is the principal form of energy store used by birds. The ability to store energy increases a bird's chance of surviving periods when energy demands are great or when energy supplies are limiting. Fat storage may be sub-divided into three closely related groups: 1) in preparation for migration; 2) for non-migratory seasonal and daily cycles; 3) in preparation for breeding.

There is sufficient evidence to assume that birds actively control levels of fat deposition within certain limits, with environmental stimuli affecting the set points at which fat levels are regulated via physiological mechanisms. The environmental conditions can restrict the degree to which physiological responses can alter the fat reserves carried by a bird. The physiological control mechanisms of avian fat homeostasis are poorly understood (Blem, 1973; Leclerq, 1984).

Severe weather conditions, such as those associated with persistently low ambient temperatures strong winds or both cause increased energy expenditure largely associated with thermoregulation. In addition food can become less available. During strong winds for instance, shorebirds which hunt by visual

cues may be unable to feed because they cannot localize food items (principally invertebrate prey). The ability of an animal to survive periods without food is positively correlated with its fat reserve (LeMaho *et al*, 1981; Dugan *et al*, 1981 and Cherel *et al*, 1988). With respect to the northern hemisphere, harsh weather conditions are more frequent with increasing latitude especially during the winter. It has been predicted that within a species, fatter and thus heavier birds should occur during the winter months and further north. This pattern has been reported by several authors (Blem, 1973, 1981; Pienkowski *et al*, 1979; Davidson, 1982; Nolan and Ketterson, 1983).

With respect to the fat deposits of Charadrii wintering in northern regions the most common interspecific pattern observed is that of an increase in body fat between arrival on the wintering grounds and late December, preceding a relatively rapid decline until the end of February, and followed by a pre-migratory increase in March, and April or May depending on species and timing of migration. This pattern has been observed for Bar-tailed Godwit, *Limosa lapponica* (Evans and Smith, 1975), Redshank *Tringa totanus* (Davidson, 1981a), Dunlin *Calidris alpina* (Davidson, 1981a&b and Geode *et al*, 1990) and Knot *Calidris canutus* (Davidson, 1981a&b) and is also inferred from body mass measurements for Sanderling *Calidris alba* (Davidson, 1981a and Wood, 1987), Ringed Plover *Charadrius hiaticula* Turnstone *Arenaria interpres* and Grey Plover *Pluvalis squatarola* (Davidson, 1981a). Fat levels in mid-winter can rise to 20% of body mass and at pre-migratory periods to as much as 50% of total body mass. It is considered by some authors that the observed decline

in reserves following mid-winter is the result of a change in the trade-off between the risk of starvation and the risk of predation. As winter progresses, the chance of encountering severe weather and thus temporary starvation, due to inability to feed or inability to obtain sufficient food, becomes less (Lima, 1986; Rogers, 1987). It is assumed that carrying more fat increases the chance of an individual being killed because it becomes less adept at escaping from predators. In addition, whilst feeding to obtain and maintain high levels of fat reserves, the bird may become less vigilant and thus more exposed to predation.

Given the large variation which can occur in fat reserves and the high percentage of body mass that fat can form, Tuite (1984) warned that this could have important implications when using allometric equations to determine energy output. That is, when the body mass of an individual bird has a seasonal variation resulting from fat deposition and utilization, which value of should be choosen as a predictor of metabolic rate? This is equivilant to asking whether adipose tissue is a significant contributor to the metabolic rate of the whole organism and what bearing does this have on predictive allometric equations. It also raises the question of the relationship between BMR and body mass at the level of the individual.

Changes in BMR during the 24-hour day

Most birds exhibit pronounced daily variation in BMR, Measurements of BMR recorded at night are 30-40% lower than those recorded during the day for small birds (5-50g) and 10-25% lower for large birds >500g (Kendeigh *et al*, 1977). This daily rhythm in BMR is probably due to increased muscle tone during the day-time or active phase (the pattern is reversed in nocturnal birds) and may serve to conserve energy during the night. Daily cycles of fat deposition are commonly seen in passerines, which do not feed at night. In such birds, day-time energy intake must exceed or be greater than the day and night energy expenditure. Winter fat storage in birds is frequently thought to be too small to allow survival without food for a period of time greater than that of the overnight period plus a few hours of the following day (Newton, 1972; Blem, 1976 and Blem and Pagels, 1984). Stube and Kettersen (1982), found however that some passerines could survive without food periods in excess of eighteen hours. This may indicate an ability to regulate body temperature below normal during the resting phase thus enhancing energy conservation. Evans (1969) suggested that small birds maintain enough fat reserves to survive the coldest night they normally encounter at a particular time of year. For comparative purposes BMR is normally recorded during the resting phase; metabolic rate measurements recorded under basal conditions but in the active phase, are termed fasting metabolic rate (FMR).

Summary of the aims of this thesis

This thesis aims to examine the intraspecific relationship between body mass and BMR in birds, particularly shorebirds

(Charadrii), allowing further examination of the functional relationship between BMR and mass. It also aims to examine the role that seasonal change in body composition may have in altering an individual's BMR. This involved measurement not only of BMR but of the metabolic output of adipose and lean tissues in vitro. The study of seasonal intra-individual and intraspecific variation in BMR is based on determinations of BMR from 3 species of shorebird held in long term captivity during the non-breeding season. The species studied were Redshank, Sanderling and Grey Plover. Intraspecific variation in BMR was also investigated in one wildfowl species, Wigeon *Anas penelope*, over a short time period. Body mass changes were monitored in the captive birds under study. Comparison with seasonal mass changes of birds in the wild was made which facilitated further examination of the hypothesis that the seasonal changes in fat deposition seen in many species of shorebird are internally pre-programmed.

During my work on this thesis two techniques became available for the evaluation of body composition non-destructively, These were Total Body Electrical Conductance (TOBEC) and ultrasonic probing. Evaluations of these two techniques are presented in Chapter 2. In particular, the changes in body composition of those birds held in captivity were monitored using TOBEC during the final year of this study.

CHAPTER 1

SEASONAL VARIATION IN BODY MASS AND BODY COMPOSITION OF WILD AND CAPTIVE POPULATIONS OF REDSHANK, GREY PLOVER AND SANDERLING.

1.1: INTRODUCTION

This chapter describes the seasonal patterns of variation in body mass that occur in three species of shorebird wintering on the Tees estuary (54°8'N 1°10'W). The patterns of mass change in the wild are related to changes in body composition evaluated from dead birds and compared to those of birds of the same species held in captivity at Durham University over the non-breeding season. This comparison facilitates the testing of the hypothesis developed by Pienkowski *et al,* (1979) and Lima (1986), that fat reserves during the wintering period are regulated internally to match the variation in the probability of occurrence of inclement weather (which prevents feeding), rather than being controlled externally by prey availability and abundance. If the pattern of body mass change in the captive population of shorebirds (supplied with food *ad libitum*) matches that of birds in the wild population and if changes in mass of fat comprise most of the changes in body mass, then the external regulation hypothesis is nullified and internal regulation is the most plausible hypothesis. The three species studied were the Redshank, the Grey Plover and the Sanderling.

Based on the reasoning outlined in the General Introduction, during periods of food shortage the smallest individuals should

deplete their energy reserves fastest on a percentage basis. Thus, in order to withstand a similar period of time without food, smaller individuals need to carry proportionately more fat. For Mammals, interspecific fasting endurance should vary with a mass exponent of 0.44 (Millar & Hickling, 1990), because fat content varies as $mass^{1.19}$ (Lindstedt & Boyce, 1985) and BMR as $mass^{0.75}$ the difference between these exponents is 0.44. From the limited data available, the intraspecific mass exponent of fasting endurance has been estimated to be 0.40 (Millar & Hickling, 1990). These intraspecific data are based on samples of small mammals and thus do not cover a wide size range. For flying birds the overall energetic cost of carrying additional fat may be greater than for mammals. Interspecifically the theoretical power requirement for flight increases with a mass exponent of 1.16 (Phillips *et al*, 1985), whilst for an individual the exponent is has been calculated to be 1.6 (Rayner, 1990). This reinforces the hypothesis that at both an inter and intraspecific level, all other factors being equal, larger individuals should carry proportionately less fat than smaller con-specifics (given that all individuals within the population are anticipating the need to survive a similar time period without food). At the least it would be anticipated that the mass of fat carried by birds should increase with total body mass with a lower exponent than for mammals. This hypothesis is tested below for both a single species using data derived from body composition analysis of Sanderling carcasses and interspecifically for shorebirds using data derived from the literature.

1.2: METHODS

1.2a Ringing data

Information has been collected from three bird ringing groups, operating around the Tees estuary. Durham University, South Cleveland, and Tees ringing group. Birds were caught using either mist nets or cannon-nets.

1.2a (i) Sanderling

A total of 1811 Sanderling was caught and weighed between 1979 and 1989, of which 369 birds were caught more than once. For analysis, birds were sexed using the discriminant function, based on wing and bill measurements, described by Wood (1987). Using this function the probability of mis-classification as to sex is 14%. Those birds caught by South Cleveland ringing group were caught using mist nets at nocturnal roosting sites whilst those caught by Durham University were caught using cannon nets. The majority of these were caught while they were feeding at tide-edge or at day-time roosts.

1.2a (ii) Redshank

A total of 984 Redshank was caught and weighed between 1983 and 1989 of which 96 birds were caught more than once. For analysis, birds were raced either as being British breeders (*T.t-britannica*) or Icelandic breeders (*T.t robusta*) using the predictive discriminant function, based on wing, bill, and tarsus

plus toe measurements described, by Summers *et al* (1988). Birds were caught by Durham University with cannon nets at high water roost sites and by the Tees ringing group with mist nets placed between feeding and roosting sites at night.

1.2a (iii) Grey Plover

A total of 487 Grey Plover was caught and weighed between 1975 and 1989 of which 43 birds were caught more than once. All the birds providing data for analysis were caught by Durham University using cannon nets at high water roost sites.

Individuals from all three species used in the analysis were aged either as juveniles or adult based on plumage characteristics (Prater *et al.* 1977) and weighed to the nearest gram using either Salter or Pesola spring balances. Where data from large catches have been used, correction factors were applied to allow for loss in body mass between time of capture and time of weighing. These were 1.0, 1.4, and 1.7 % of body mass per hour for Sanderling, Redshank and Grey Plover respectively, (figures are based on the work of Davidson (1981a)). Mass values were corrected to the nearest gram. Birds caught by mist nets were all weighed soon after capture and therefore no correction for mass loss was considered necessary.

In order that it can be asserted that changes in body mass (all species caught) and body composition (Sanderling) can be said to be representative of those of over-wintering individuals, it is important to be able to determine the population structure and

turnover. For the three species studied here the population turn over has been assessed using biometrics, ringing recoveries and past literature. Because a large number of people were involved in the collection of these data, no check has been made for systematic sampling bias caused by any one individual. It is anticipated that the overall summation of errors has produced results that are without systematic error.

1.2b Body composition analysis

The samples used in this study were collected from the Tees estuary between 1972 and 1989. Processing of the carcasses was carried out by N.C.Davidson, P.R.Evans, K.C.Kwon and the author of the present study (see Appendix 1. Table 1). Samples were obtained as cannon netting casualties or taken under licence (from the N.C.C.). Sacrificed samples were weighed to the nearest gram on either a Pesola or Salter spring balance and each bird measured before being killed by cervical dislocation or thoracic compression. Samples obtained as cannon netting casualties were weighed and measured as soon as practically possible after death.

Body condition analyses followed the methods of Evans and Smith (1975) and Davidson (1981a). Prior to fat extraction the pectoral muscles of the right side were dissected out. The abdomen was opened and carcases were sexed by gonadal examination. In some cases the liver and kidneys were also removed and defatted separately to facilitate determinations of heavy metal concentrations in these tissues as part of a separate study.

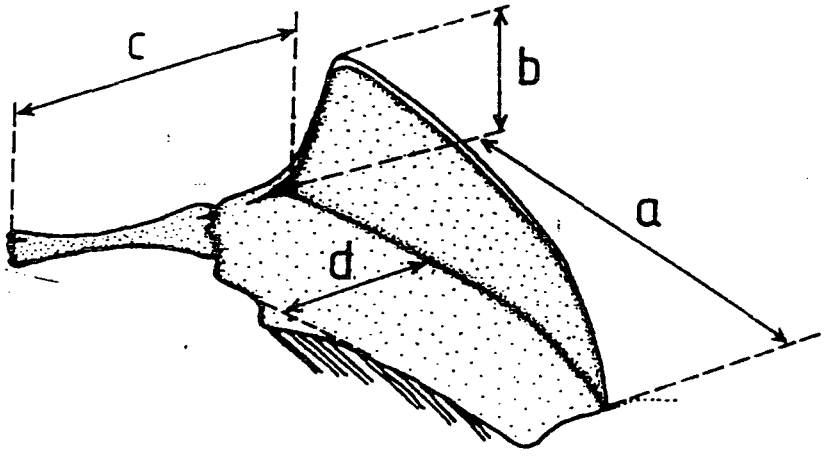


Fig 1.1 Skeletal measurements of the sternum and coracoid bone used to calculate the area of attachment of the pectoral muscles (SMV) and standard muscle index, (See section 1.1e). Diagram is taken from Davidson (1981a)

The muscle (from which the subcutaneous fat had been removed), kidney, liver and remaining carcass were dried to constant mass in vacuum ovens at 40-50°C. Following drying, the samples were weighed and the fat was extracted in a Soxhlet apparatus using 60-80°C bpt petroleum ether. The samples were then re-dried to constant mass. The fat content was calculated by subtraction of the mass after extraction from that before. Samples from which the fat had been extracted are described hereafter as "lean".

1.2c Condition indices

An index of the protein reserve was obtained by comparing the lean dry mass of one pectoral muscle to a standard volume (SMV) which was calculated from four skeletal measurements determining the skeletal attachment area, according to the equation given below.

$$SMV = b(ad + 0.433c^2)$$

Where SMV=Standard Muscle Volume, *a*=length of sternum, *b*=height of the keel of the sternum, *c*=distance from the keel to the end of the coracoid and *d*=is the width of the bony raft of the sternum (Evans and Smith 1975, See Fig 1.1).

The Standard Muscle Index (SMI) is the mass of lean dry pectoral muscle divided by the SMV. The SMI is thus a measure of available protein reserve which takes animal size into account and is independent of seasonal variation in mass.

The lipid index (LI) is defined as the fat content (g) divided by the total fresh mass of the sample and is expressed as a percentage.

The Total Lean Mass (TLM) is the total fresh mass(g) of the sample minus the fat content.

The Total Lean Dry Mass (TLDM) is the total fresh mass(g) of the sample minus both the fat and water content.

The water content (WC) is expressed as a percentage of the total lean mass(g).

For statistical analysis of body composition Sanderling were assigned to groups of limited physiological heterogeneity. These groups were: 1) birds caught in August and not yet in moult; 2) birds in early moult (moult score<15); 3) birds in late moult (moult score>15); 4) birds caught in November not in moult; 5) birds caught in December; 6) birds caught in early February; 7) birds caught in mid-February; 8) birds caught in early March;, 9) birds caught in late March; 10) Birds caught in April; 11) Birds caught in early May; 12) Birds caught in mid-May; 13) Birds caught in late May. Moult scores were calculated by the method of Evans (1966). Sampling biases are associated with both cannon and mist netting techniques. (Pienkowski & Dick 1976 and Furness & Galbraith 1980). Although biases in age structure and population composition may have occurred in this study due to sampling technique, no differences in body composition or mass assignable to method of collection could be detected.

1.2d Maintenance of captive birds

During the first season of study (1988-89) birds were maintained in outdoor aviaries on grass. The aviaries measured (1.8m x 0.5m(h) x 1.5m) and (2m x 0.5m(h) x 2m) for Sanderling and Grey Plover respectively. Their diet consisted of blow fly larvae occasionally supplemented with ragworm and meal worms. Water was provided in large trays to facilitate both bathing and drinking. Both food and water were available *ad libitum*.

During the second season of study (1989-90) birds were kept in outdoor aviaries measuring 1.2m x 0.5m(h) x 2.5m on grass. Their diet consisted of blow fly larvae with lean minced beef supplemented with Sluis, (a commercially available bird diet) and occasionally, meal worms.

In total, 10 Redshank, 5 Grey plover and 6 Sanderling were kept during the non-breeding season. Unfortunately in both years deaths occurred in the captive populations due to outbreaks of *Coccidiosis emeiria*. In addition on the 1 March 1990 violent storms blew over some cages and allowed 4 Redshank and a Grey Plover to escape. Details of birds held and their fates are given in Appendix 1, Table 2.

1.2e Statistical analysis

Throughout this thesis data were analysed using the spss^{x} statistical computer package. Details of the numerous statistical

tests employed are given in the result sections. Except where stated, 'statistically significant' implies $P<0.05$.

1.3: RESULTS

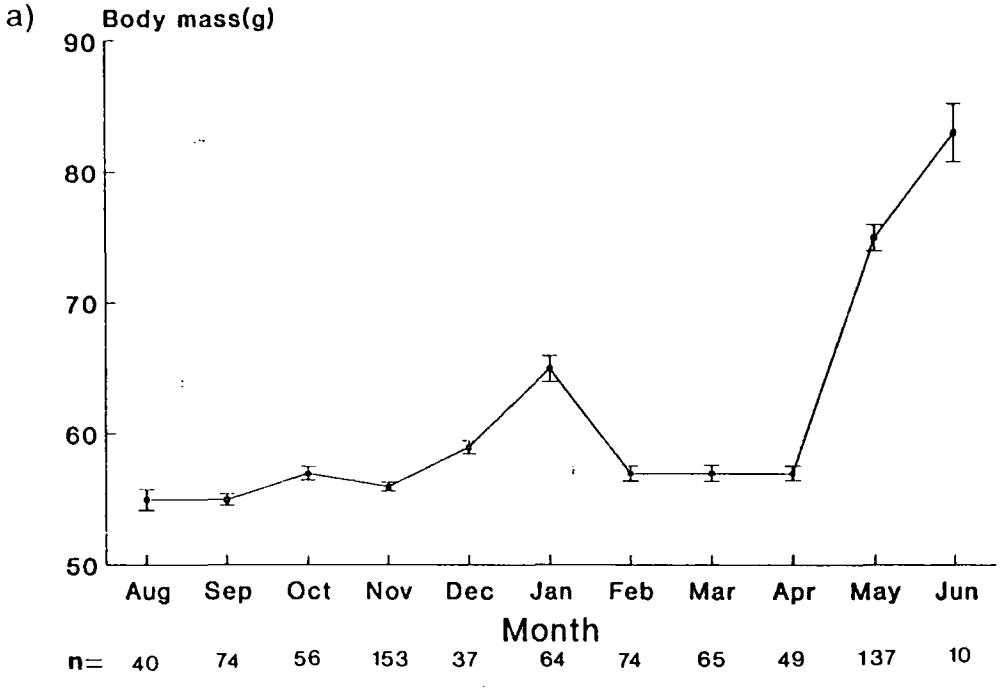
1.3a Body masses of Sanderling in the field

Figures 1.2a & b show the mean monthly mass of adult Sanderling caught during the non-breeding season with males (a) and females (b) birds shown separately (identified by the method of Wood (1987)). Figure 1.3a and 1.3b are similar plots for those Sanderling caught as juveniles. The masses of males are consistently lower than those of females, but the overall pattern of seasonal variation in body mass is similar for both sexes. This result is consistent with that of Wood (1987).

As it is widely believed that Sanderling do not show appreciable levels of Sub-speciation it is not anticipated that the interpretation of the above result will be affected by influxes of individuals of populations with differing size/body mass structure.

Analysis of variance has been used to examine the data set. Potential sources of variation were considered to be month, year, sex, age, capture site and ringing group. Before analysis, data were log-transformed to normalize variances. Results of the ANOVA (Tables 1a, b & c) indicated that site ringing group and age do not contribute significantly to the variation in body mass but

Fig 1.2a&b Seasonal variation in mean body mass of adult Sanderling caught during the non-breeding season at Teesmouth between 1979 and 1989 for a) Males and b) Females. Horizontal bars represent 2x the standard error of the sample mean. n= the sample size.



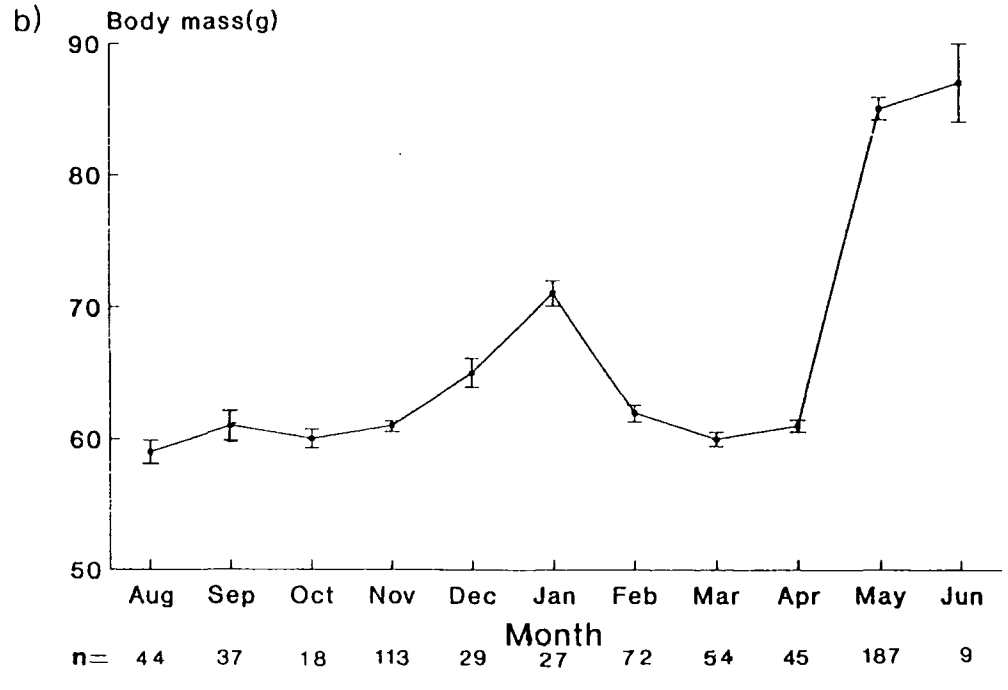
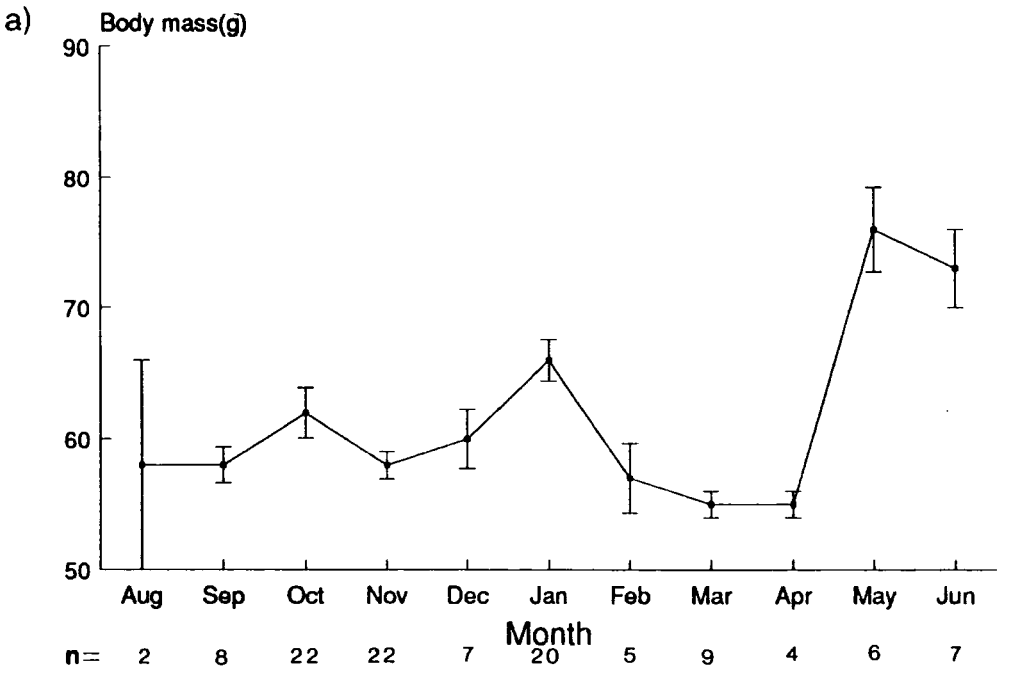
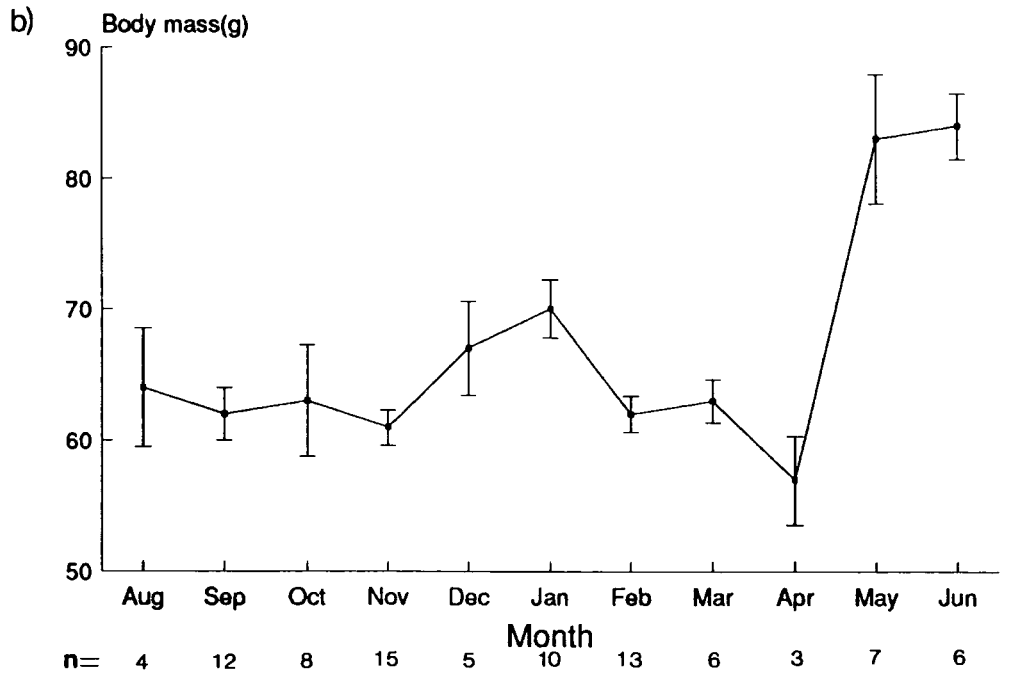


Fig 1.3a&b Seasonal variation in mean body mass of juvenile Sanderling caught during the non-breeding season at Teesmouth between 1979 and 1989 for a) Males and b) Females. Horizontal bars represent 2x the standard error of the sample mean. n= the sample size





significant variation occurs on a year-to-year basis. Appendix 1 Table 3 shows monthly mean masses for individual years; in most years data were available from only a limited selection of months.

TABLE 1.1a. ANOVA of log10 body mass of Sanderling with Month, Year, Sex and Age as independent variables.

Source of variation	Sums of squares	DF	Mean squares	F	Signif. of F
Main effects	6.22	25	0.249	125	P<0.0001
Month	3.51	10	0.351	177	P<0.0001
Year	0.31	13	0.024	11	P<0.0001
Sex	0.58	1	0.584	293	P<0.0001
Age	0.002	1	0.002	1.08	P>0.05
Explained	6.220	25	0.249	125	P<0.0001
Residual	3.106	1569	0.002		
Total	9.326	1594	0.006		

TABLE 1.1b. ANOVA of log10 body mass of Sanderling with month, year, sex and ringing group as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif. of F
Main effects	6.22	25	0.249	125	P<0.0001
Month	3.45	10	0.345	174	P<0.0001
Year	0.31	13	0.024	12	P<0.0001
Sex	0.58	1	0.585	295	P<0.0001
Ringing group	0.0001	1	0.0001	0.24	P>0.05
Explained	6.22	25	0.25	125	P<0.0001
Residual	3.12	1569	0.002		
Total	9.31	1594	0.006		

Table 1.1c ANOVA of Log10 body mass of Sanderling with Month Year sex and site as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif. of F
Main effects	6.22	15	0.249	125	P<0.0001
Month	3.4	10	3.45	174	P<0.0001
Year	0.29	13	0.2	173	P<0.0001
Sex	0.58	1	0.58	295	P<0.0001
Site	0005	1	0.005	2.4	P>0.05
Explained	6.22	25	0.25	125	P<0.0001
Residual	3.10	1569	0.002		
Total	9.3	1594	0.006		

The sources of variation among both months and years have been investigated using the honestly significant difference test (HSD) for post hoc comparisons (Hays 1988). Tests were based on ANOVA with month and year as independent variables after controlling for sex (Table 1.2 a & b). Significant variation in body mass occurred between January and all other months, and between June and May and all other months. This pattern is consistent for both males and females. The higher body masses recorded in May are a result of pre-migratory fattening. The mean increase in mass between December and January was 6g, that between April and May was 33g an increase of 31.5% in the mean for males and 39% for females. This is consistent with the results of Wood (1987) who suggested that the greater increases seen in females may be due to a requirement for extra reserves to lay a full clutch of eggs soon after arrival on the breeding grounds (see also Davidson and Evans 1990). This was as found in high-arctic breeding geese (Ankney And Macinnes, 1978).

Table 1.2a ANOVA of Log10 Body mass of Male Sanderling with Month and Year as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif. of F
Main effects	2.301	23	0.100	54.66	P<0.0001
Month	1.423	10	0.142	77.76	P<0.0001
Year	0.219	13	0.017	9.194	P<0.0001
2-Way interactions	0.241	37	0.007	3.564	P<0.0001
	0.241	37	0.007	3.564	P<0.0001
Explained	2.543	60	0.042	23.15	P<0.0001
Residual	1.486	812	0.002		
Total	4.026	872	0.005		

Table 1.2b ANOVA of Log10 Body mass of female Sanderling with month and year as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif. of F
Main effects	2.979	23	0.130	73.45	P<0.0001
Month	2.062	10	0.206	116.97	P<0.0001
2-Way interactions	0.137	29	0.005	2.685	P<0.0001
	0.137	29	0.005	2.685	P<0.0001
Explained	3.116	52	0.060	33.98	P<0.0001
Residual	1.183	671	0.002		
Total	4.299	723	0.006		

The rate of increase in body mass between April and May did not differ significantly between the two sexes ($t=1.58$ $df=195,241$ $P>0.05$).

Interpretation of these results could be affected by population turnover. Birds caught in May probably contain both wintering and passage individuals. An estimation of the population stability has been made by examining the relative numbers of unringed and previously ringed birds caught in each month throughout the non-breeding season (Table 1.3). A large proportion of the birds which over winter on the Tees estuary are ringed; thus a high through-put of birds is indicated by a dilution in the of previously marked birds in catches. Thus it can be seen that the highest rates of turnover are seen in September, May and June. This result is consistent with that of Goodyer and Evans (1980) who show that at least three populations of Sanderling use the Tees estuary during the non breeding season. These are: 1) moulting birds; 2) over wintering birds and; 3) spring passage birds. Evans and Pienkowski (1984) showed that many of the

colour-marked wintering population remain at Teesmouth until mid-May.

TABLE 1.3 Ratio of previously marked to unmarked Sanderling caught on the Tees estuary during the non-breeding season.

Month	Number of birds Marked	Total caught	Pre. Marked/ total x 100
Jan	48	123	39
Feb	71	183	39
Mar	39	165	23
April	14	101	14
May	17	368	4
June	-	32	-
July	-	-	-
Aug	16	90	18
Sept	18	244	7
Oct	30	119	25
Nov	92	308	29
Dec	24	78	30

The overall inter-year variation in body mass cannot be attributed to any one year or group of years in particular. Given that the adaptive significance of winter fat deposits are thought to be related to temperature (King, 1974 and Blem, 1976) and or to wind chill factor (Dugan *et al*, 1981). I investigated the possibility that these environmental parameters can account for the between-years variation in body mass using correlation analysis of annual monthly mean temperatures and wind speeds with mean monthly body mass of each year. However no significant correlations were obtained.

As indicated by ANOVA, significant variation can also be attributed to interaction between months and years. This variation probably arises from the patchiness of the data collected Appendix 1 Table 3. To establish if the seasonal

pattern described above is real, I have controlled for year by subtracting from the mean mass of each monthly sample the value of the relevant annual mean. I then divided the resultant by the standard deviation of the appropriate annual mean. Thus the body mass of each monthly sample is thus expressed in units of standard deviation from the overall monthly mean with variation due to year controlled for. Figure 1.4 summarizes the results for males and females combined. As can be seen, the pattern obtained by this analysis resembles those derived from untransformed body mass values (Figs 1.2 & 1.3). These results are consistent with the seasonal pattern of mass change observed in other shorebird species wintering on coastlines of more northern temperate latitudes (Davidson 1981a).

The results presented here disagree with those of Wood (1987) who found that mid-winter body mass of Sanderling wintering on the Tees estuary peaked in February. My study indicates a mid-winter peak in January, as also found for Sanderling wintering on the Wash (Johnson 1985). The discrepancy probably arises from the low sample sizes used by Wood (1987). Figure 1.5 shows the pattern of change in body mass shown by individual birds caught twice or more within any one non-breeding season. The pattern revealed parallels that seen when the data are viewed as a whole.

1.3b Body masses of Grey Plover in the field

Figures 1.6 show the mean monthly body masses of adult and juvenile Grey Plover caught during the non-breeding season on the Tees estuary. The patterns shown are consistent with those

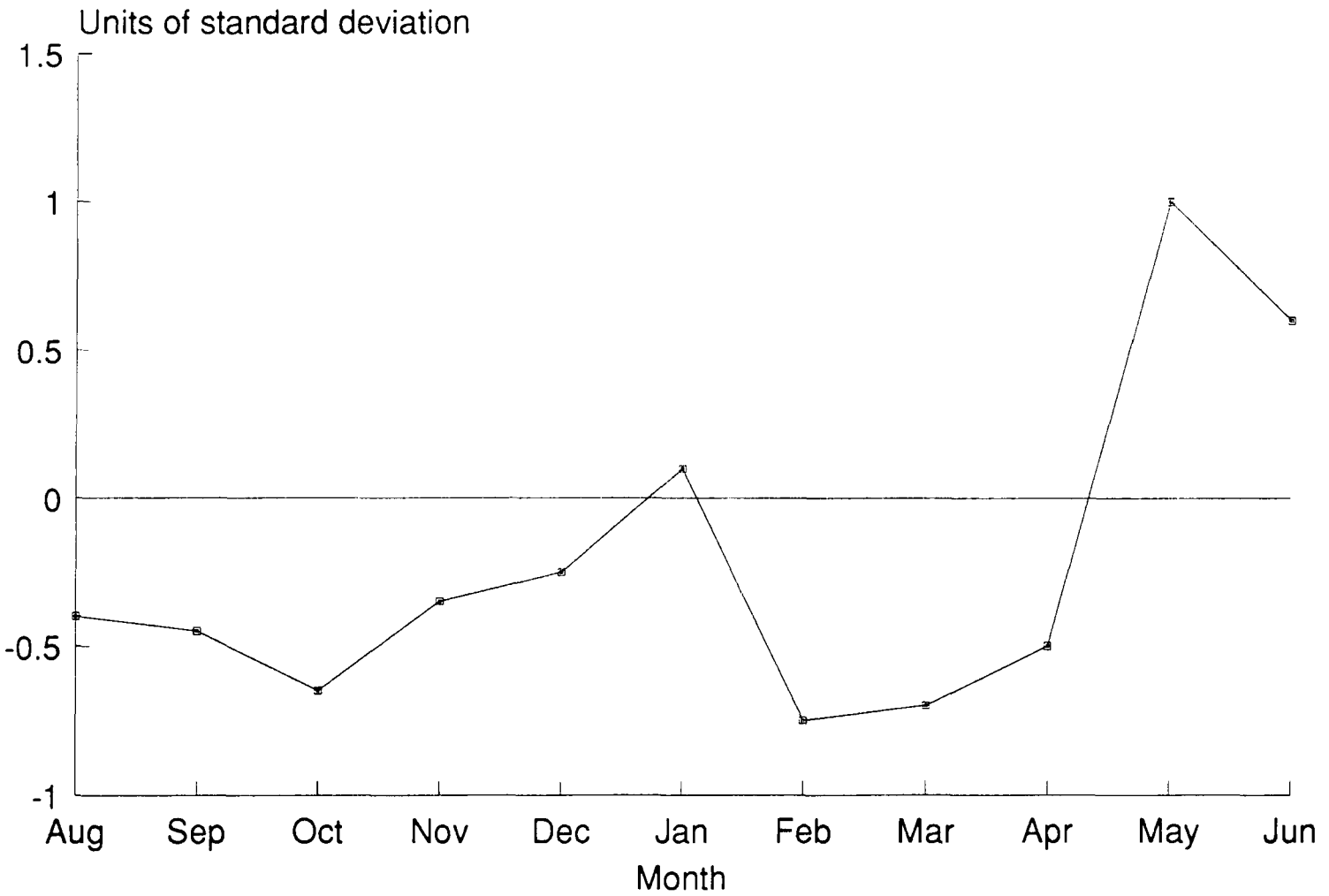


Fig 1.4 Seasonal variation in body mass of adult Sanderling caught at Teesmouth between 1979 and 1989 body mass is expressed in units of standard deviation from the overall monthly mean with variation due to year controlled for, (See section 1.2a for details).

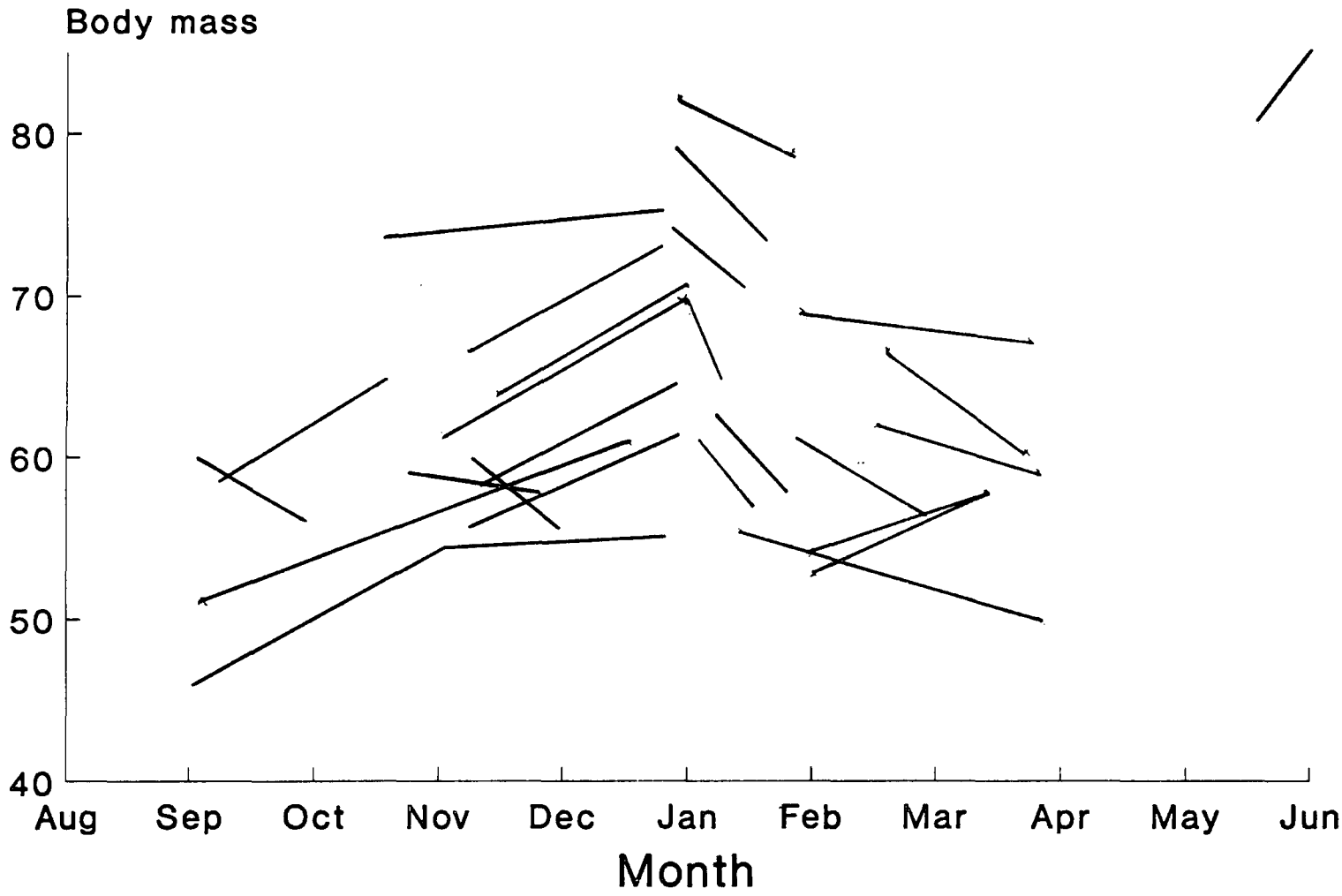


Fig 1.5 Within-year body mass change of Sanderling at Teesmouth. Each line joins the value obtained for one individual. Only birds caught twice or more during early or late winter are shown.

reported by Davidson (1981a,b) and Dugan *et al* (1981). A three way ANOVA (using log-transformed data to standardize the variance), with month, year and age as independent variables, indicates that age does not contribute significantly to the variation in body mass (Table 1.4).

Table 1.4 ANOVA of Log10 body mass of Grey plover caught on the Tees estuary with month, year and age as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	0.41	22	0.199	8.4	P<0.0001
Month	0.17	9	0.019	8.7	P<0.0001
Year	0.21	12	0.017	7.8	P<0.0001
Age	0.002	1	0.002	0.86	P>0.05
Explained	0.413	22	0.019	8.6	P<0.0001
Residual	1.03	464	0.002		
Total	1.44	486	0.003		

The sources of variation within months and years has been investigated using the HSD test. Birds caught in December have a body mass which is significantly higher than in all other months except November and January. The mean mass in May is significantly lower than that for all other months and refers to first year birds only; and the mean for March is significantly lower than that for February. Figure 1.7 shows the pattern of mass change in individual birds which have been caught twice or more within the same non-breeding season. Birds marked 1 & 2 on Figure 1.7 show a pattern which is contrary to that shown in Figure 1.6 whilst that marked 3 shows a much steeper decline than would be expected if the general trend were being followed. Other reweighed individuals provide data consistent with the general pattern.

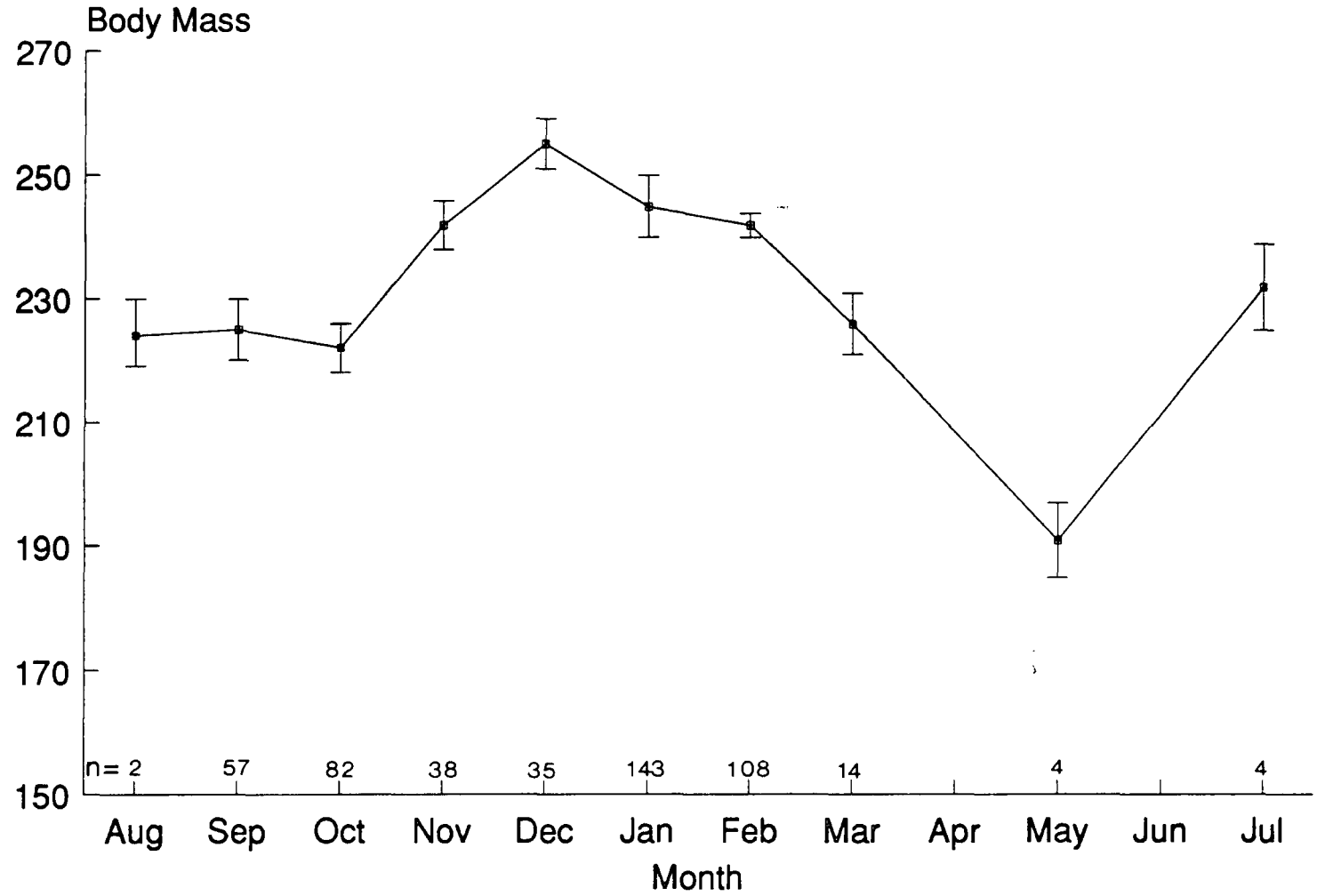


Fig 1.6 Seasonal variation in mean body mass of Grey Plover caught during the non-breeding season at Teesmouth between 1975 and 1989. Horizontal bars represent 2x the standard error of the sample mean. n= the sample size.

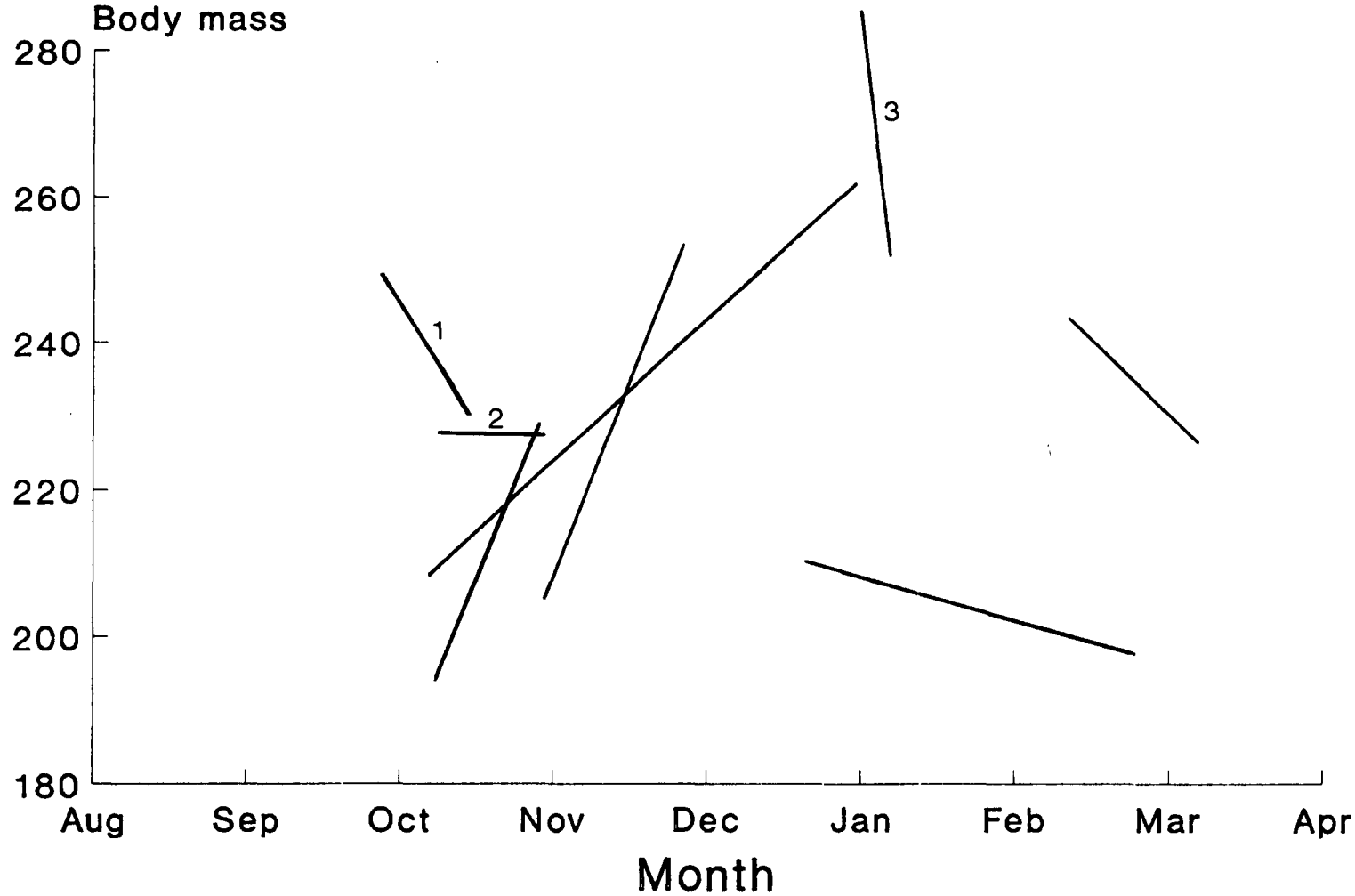


Fig 1.7 Within-year body mass change of Grey Plover at Teesmouth. Each line joins the value obtained for one individual.

In general there is relatively little early autumn passage of Grey Plover through the Tees estuary. Grey Plover wintering on British estuaries tend to move only a short distance to the Waddensea in March before migrating to the Siberian breeding grounds (Pienkowski and Evans, 1984). This accounts for the lack of a premigratory increase in body mass which is seen in most other shorebirds. Many Grey Plover are resident throughout the wintering period on the Tees estuary as shown by individual-marking studies although some individuals use the estuary only in cold winters (Townshend, 1985 and Evans & Townshend, 1988).

Year-to-year variation arose chiefly from the relatively high mean masses recorded in 1987 and 1977 in conjunction with low values in 1979 and 1988. Mean monthly body mass for each year are shown in Appendix 1. Table 1.4. As with Sanderling there were no significant correlations betwen annual monthly mean temperatures and wind speeds with mean monthly body mass of each year.

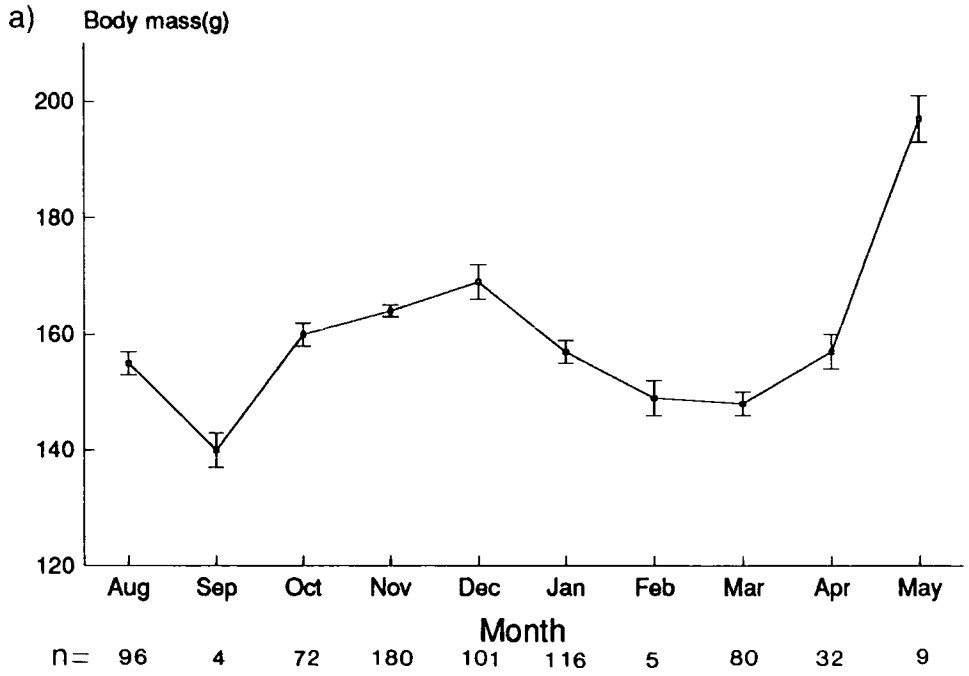
1.3c Body masses of Redshank in the field

Figures 1.8a & b show mean body masses in different months for adult (a) and juvenile (b) Redshank wintering on the Tees estuary. It has not proved possible to sex Redshank satisfactorily using biometrics. Whilst females are on average larger than males there is much size overlap between the sexes and considerable geographical variation. Where possible, adult Redshank have been assigned to races using the discriminant

function developed by Summers *et al*, (1988), based on measurements of tarsus plus toe, bill length and wing length. In Figure 1.9 mean monthly body masses of the two races are shown separately. Only those birds whose race could be predicted to within 70% certainty were included in this plot or in subsequent analyses where race is included as a variable. Unfortunately this excluded many birds, as tarsus plus toe measurements often were not taken. Fig 1.9 shows the proportions of *T.t britannica* and *T.t robusta* caught throughout the non-breeding season. Only in November did the Icelandic to British ratio differ significantly from 1. The notion that British Redshank may move away from the Tees estuary as winter progresses is supported by Hale (1973), who suggested that, in the north of England, both young and adults leave the breeding grounds and go first to the nearest suitable coastal feeding area, but as winter progresses and the weather becomes harsher some move away from these sites in a southward direction. It is not possible to make firm statements concerning the overall level of population turnover on the Tees estuary. Davidson (1981a) indicates that there is indeed a progressive decline in numbers of Redshank from October until late November and an increase in late February.

Results of ANOVA (using log-transformed data to normalize the variance) with month, year, ringing group and age as independent variables indicate (Table 1.5) that the identity of the ringing group does not contribute significantly to the overall variation in body mass but that month year and age do.

Fig 1.8a&b Seasonal variation in mean body mass of Redshank caught during the non-breeding season at Teesmouth between 1983 and 1989 for a) Adults and b) Juveniles. Horizontal bars represent 2x the standard error of the sample mean. n= the sample size.



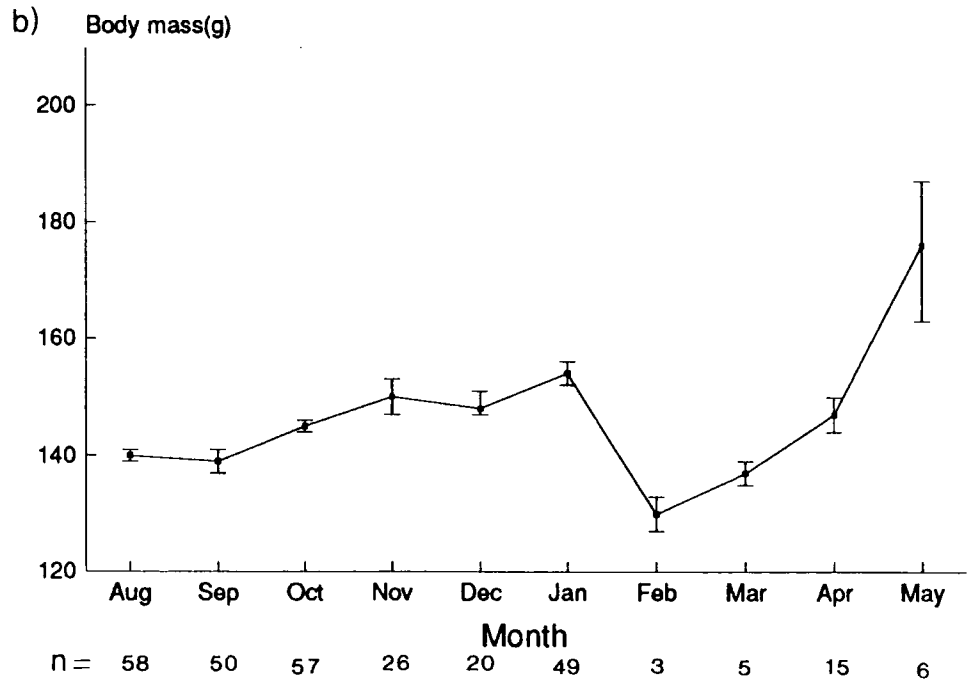


Table 1.5 ANOVA of Log10 body mass of Redshank caught on the Tees estuary with month, year,age and ringing group as independent variables.

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	0.877	18	0.049	31.98	P<0.0001
Month	0.380	10	0.038	24.95	P<0.0001
Year	0.056	6	0.009	6.161	P<0.0001
Ringing group	0.0001	1	0.0001	0.142	P>0.05
Age	0.223	1	0.223	146.4	P<0.0001
Explained	0.877	18	0.049	31.98	P<0.0001
Residual	1.470	965	0.002		
Total	2.347	983	0.002		

Juveniles were consistently lighter than adults throughout the non-breeding season, as found also by Buxton (1988) for Redshank wintering in the Western Isles of Scotland. When race is included in the analysis, variation in body mass related to month is no longer significant (Table 1.6), although this is probably due to the greatly reduced sample size.

Table 1.6 ANOVA of Log10 body mass of Redshank caught on the Tees estuary with month, year, and race. (Redshank included are those whose race can be determined with 70% confidence or more)

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	0.104	10	0.010	12.82	P<0.0001
Month	0.010	6	0.002	2.163	P<0.01
Year	0.010	3	0.003	4.127	P<0.0001
Race	0.026	1	0.026	31.79	P>0.05
Explained	0.104	10	0.010	12.82	P<0.0001
Residual	0.102	126	0.001		
Total	0.205	136	0.002		

Thus without an accurate picture of the change in *T.t.-britannica* to *T.t. robusta* ratio throughout the non-breeding season interpretation of seasonal trends in body mass becomes very difficult. The overall pattern of seasonal change in body

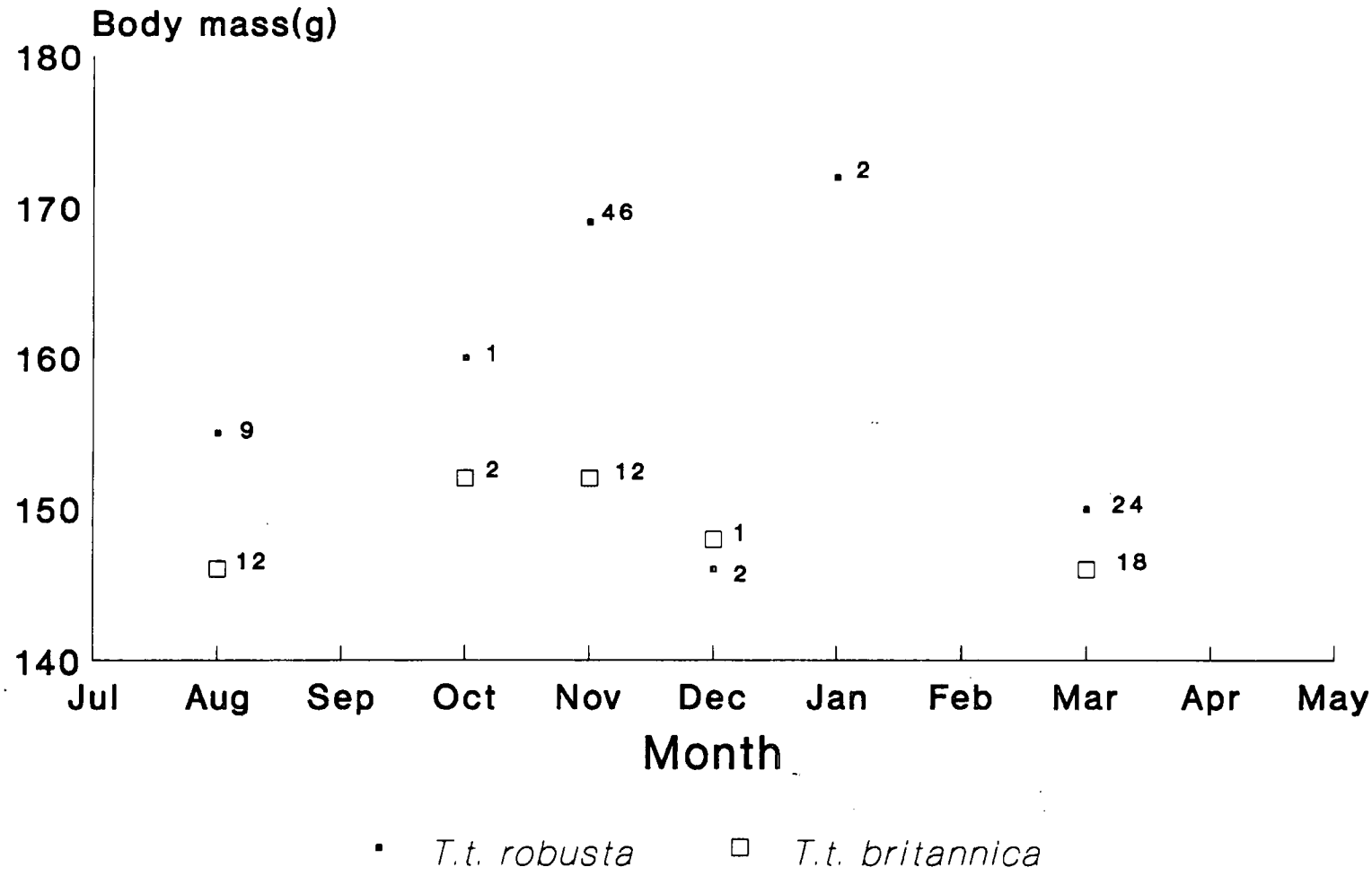


Fig 1.9 Seasonal variation in mean body mass of Redshank caught during the non-breeding season at Teesmouth. Birds have been raced either as *T.t robusta* or *T.t. britannica*. Only those birds whose body mass could be predicted to within 70% certainty are shown. Numbers adjacent to points indicate sample sizes.

mass is consistent with that reported by Davidson (1981a&b), who also found no marked mid-winter peak in body mass. The apparent increase until Dec/Jan could be due to changing ratio of the two races, or to increase of mass in one race alone (*T.t.robusta* see Fig 1.9). After December/January body mass declined rapidly through to February but then increased slowly through March, April and May. It is noteworthy that the general trend reported by Davidson (1981a&b) was derived from birds that had not been separated by race and that figure 1.9, in which the races are shown separately does not reflect the overall general pattern.

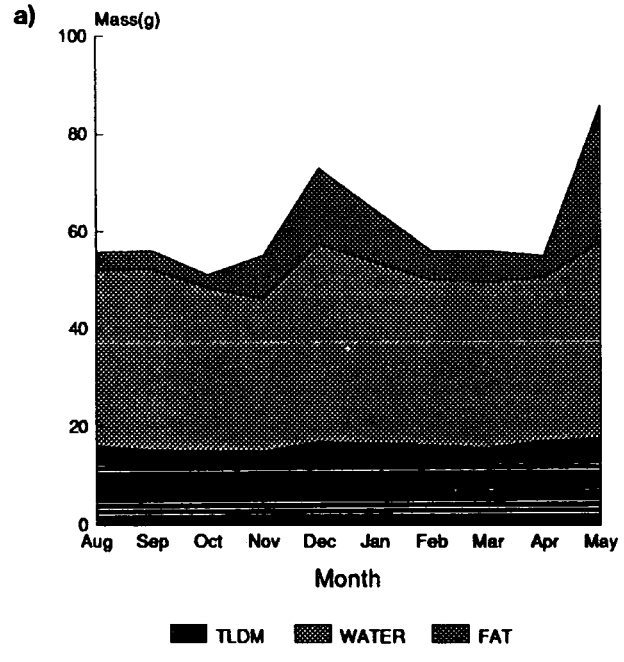
1.3d Sanderling body composition analysis

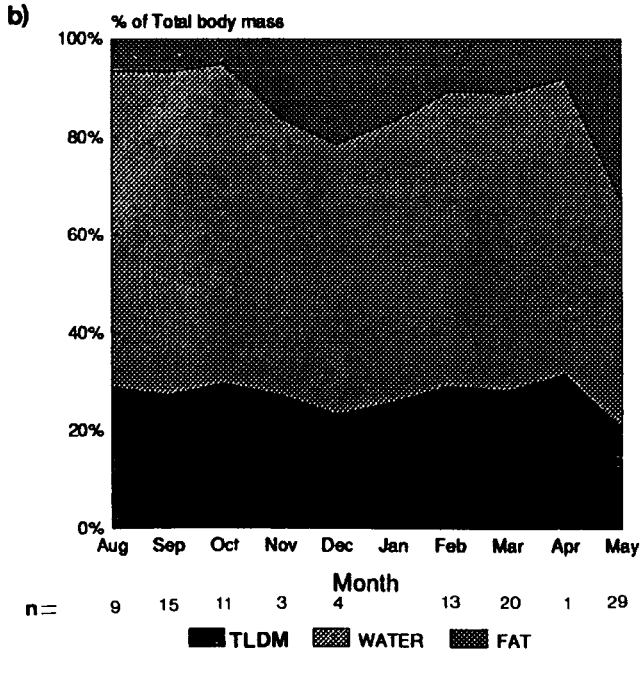
104 Sanderling, analyzed for body composition, had been obtained between 1972 and 1989. Details of date of capture are given in Table 1 (Appendix 1). All birds were caught whilst they were feeding or at high water roost.

Figure 1.10a summarizes seasonal variation in body mass, fat, lean dry mass (LDM) and water with month in absolute terms and Figure 1.10b in relative terms. Variation in fat accounts for most of the variation in total body mass; the coefficient of variation is 1.00 for fat, but only 0.15 for water and 0.11 for LDM.

The mean water content (WC) of birds in wing moult is slightly but significantly higher (0.3%) than of birds not in moult ($t=2.15$ $\underline{P}=0.013$).

Fig 1.10 Seasonal variation in body mass, fat, lean dry mass, and water of Sanderling caught at Teesmouth as revealed by destructive analysis. a) in absolute terms b) in relative terms. n= the sample size.





No convincing evidence was found for premigratory dehydration, although WC for birds caught during May was 0.5% lower than for birds sampled between November and April, this difference was not statistically significant (t=0.72 df=79 P=0.479).

The TLDM of birds caught during wing moult was significantly lower (by 0.8g) than for birds sampled during the winter months (t=2.41 P=0.019). Many species also show a decrease in total mass during wing moult (Chilgren 1977, Murphy and King, 1984). An HSD test based on ANOVA (Table 1.7) with physiological group (see methods section) as an independent variable, shows that, after controlling for sex, the total mass of those birds analysed for body composition during moult was not significantly different from that of birds analysed during any winter month except December. However for the birds caught for ringing, discussed in Section 1.4a, mean body mass during winter was 1.4g lower than for those birds not in moult and caught before December (t=3.07 df=626 P=0.002).

Table 1.7 ANOVA Log10 body mass of Sanderling (analysed for body composition) with assigned physiological group for body composition analysis as an independent variable.

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Physiological status	0.850	12	0.071	37.98	P<0.0001
Residual	0.173	92	0.002		
Total	1.127	104			

Figure 1.11 shows the monthly variation in lipid index. ANOVA of LI with sex as the independent variable shows that males and female Sanderling do not differ in lipid index (F=1.53 P=0.218)

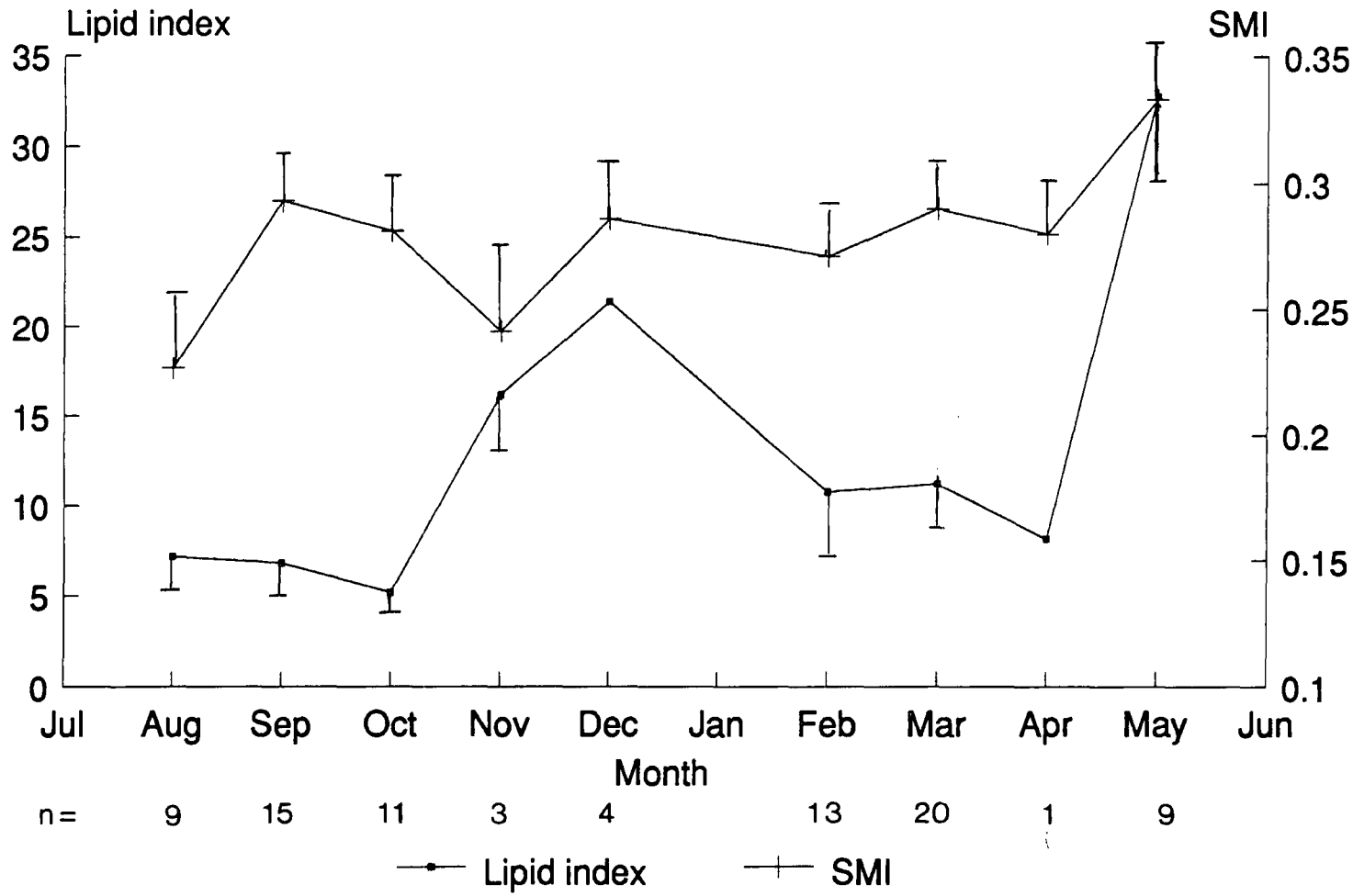


Fig 1.11 Seasonal variation in Lipid index (LI) and Standard muscle index (SMI) of Sanderling caught at Teesmouth during the non-breeding season. Horizontal bars represent 1x the standard error of the sample mean. n= the sample size.

This is also true of SMI (F=0.634 P=0.43) and WC (F=0.18 P=0.670).

As can be seen, the lipid index remained comparatively steady during the months of August, September and October (during moult). This period of stability is followed by a rise to a peak LI of 17.33±0.55% in December and then a decrease until April. LI increased rapidly prior to migration. The maximum LI recorded in the pre-migratory period was 47%, whilst the mean LI for birds caught during the last third of May was 33.7±1.9.

HSD tests based on ANOVA (Table 1.8) with month as an independent variable of LI (arc sine transformed) indicate that the mean LI for May was significantly higher than all other months, whilst that for December did not differ significantly from those for November, March or February. The index for November, was significantly higher than that for October. Moult status had no significant effect on the lipid index.

Table 1.8 ANOVA of arc sine transformed lipid index of Sanderling with month

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Month	1.23	8	0.15	41.58	P<0.0001
Residual	0.15	96	0.004		
Total	1.58	104			

This pattern of LI change is similar to that reported for other shorebird species wintering in N.E. England (Evans and Smith 1975 Davidson 1981a&b and Pienkowski *et al*, 1979). The mid winter peak LI of 17.3% is similar to that of 20% predicted by Davidson

(1981a). Figure 1.11 also shows the variation in SMI. No definite mid winter peak is apparent. Thus high fat reserves are not necessarily correlated with high levels of SMI (protein reserve). This is confirmed by a partial correlation of SMI against LI controlling for body mass which does not yield a significant correlation ($P>0.05$ $r= 0.11$). Relatively low values of SMI were found in both August and November. Low August values may reflect recent arrivals of birds from the breeding grounds, with low levels of protein reserves following migration. This phenomenon has been reported for Bar-tailed Godwits (Evans and Smith 1975) Dunlin (Davidson 1981a) and for Pacific Golden Plover *Pluvalis fulva* (Johnson *et al* 1989). The muscle indices of birds in moult and those not in moult during the same period did not differ significantly. Of only three Sanderling sampled during November one bird had an exceptionally low SMI (below 0.195). It may have been starving or possibly newly arrived to the estuary. The high SMI recorded in May is a reflection of pre-migratory hypertrophy (Johnson and Macfarland, 1967; McNeil, 1970 and Evans and Smith, 1975).

An HSD test based on ANOVA with month as the independent variable indicates that the mean SMI for May was significantly higher than for all other months and was lower in August than in all other months except November. SMI for September was significantly higher than August but not October. The overall pattern is thus one of relative stability throughout the wintering period with a steep rise between April and May prior to spring migration. This is consistent with results published for adult Bar-tailed Godwits (Evans and Smith, 1975) and adult

Dunlin and Knot (Davidson 1981a) wintering on estuaries in N.E. England.

An ANOVA with year month and data set was used to test the extent of the variation between birds used for body composition analysis and birds caught for ringing (Section 1.4). Data were log-transformed before analysis, to standardize the variance. Significant variation between the two groups was apparent for male birds only (Table 1.9). This difference disappeared when those birds caught during October are excluded from the analysis. Birds killed in October had significantly lower body mass than those caught and released. Of the former all were in moult, whilst for the ringed group only 36.5% were in moult; this may account for the discrepancy. Pearson's correlation has also been used to check for the extent of correlation between the monthly mean body masses of the two sample groups. The values obtained for the correlation coefficient were 0.88 when the birds caught in October are included and 0.89 when they are excluded.

Table 1.9 ANOVA of Log10 body mass of Sanderling with month, year and Sample group (Body composition sample or Wild sample) as independent variables.

a) Males

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	2.07	24	0.086	41.67	P<0.0001
Month	1.45	8	0.182	87.83	P<0.0001
Year	0.16	15	0.11	5.15	P<0.0001
Sample group	0.008	1	0.008	4.04	P<0.05
Explained	2.07	24	0.086	41.67	P<0.0001
Residual	1.63	790	0.002		
Total	3.705	814	0.005		

b) Females

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	3.29	24	0.137	68.48	P<0.0001
Month	2.41	8	0.301	150.38	P<0.0001
Year	0.110	15	0.007	3.680	P<0.0001
Sample group	0.0001	1	0.0001	0.068	P>0.005
Explained	3.29	24	0.137	68.48	P<0.0001
Residual	1.635	711	0.002		
Total	3.705	735	0.005		

1.3e Grey Plover body composition

Only 15 Grey Plover were analyzed for body composition. Capture details are given in Table 1, of appendix 1. Because of low sample size, these data have not been subjected to rigourous statistical analysis. Student's t-tests indicate that there were no significant differences between adults and juveniles with respect to total body mass, LI or SMI. The patterns of seasonal change in SMI and LI are shown in figure 1.12. The pattern revealed is consistent with the pattern of body mass flux obtained from the samples discussed in Section 1.4b, although the mean body mass of the sample analysed for body composition is somewhat higher than of the sample caught for ringing. The pattern of LI change is consistent with that described by Davidson (1981a), although the peak value shown in Figure 1.12 for November is higher than was found by Davidson (1981a). Mean mass of females was 273g whilst that of males was 227g. Although this difference is statistically significant ($t=2.65$ $P=0.02$ $df=13$) no confident biological interpretation can be given as birds were obtained in different months and sample sizes were low.

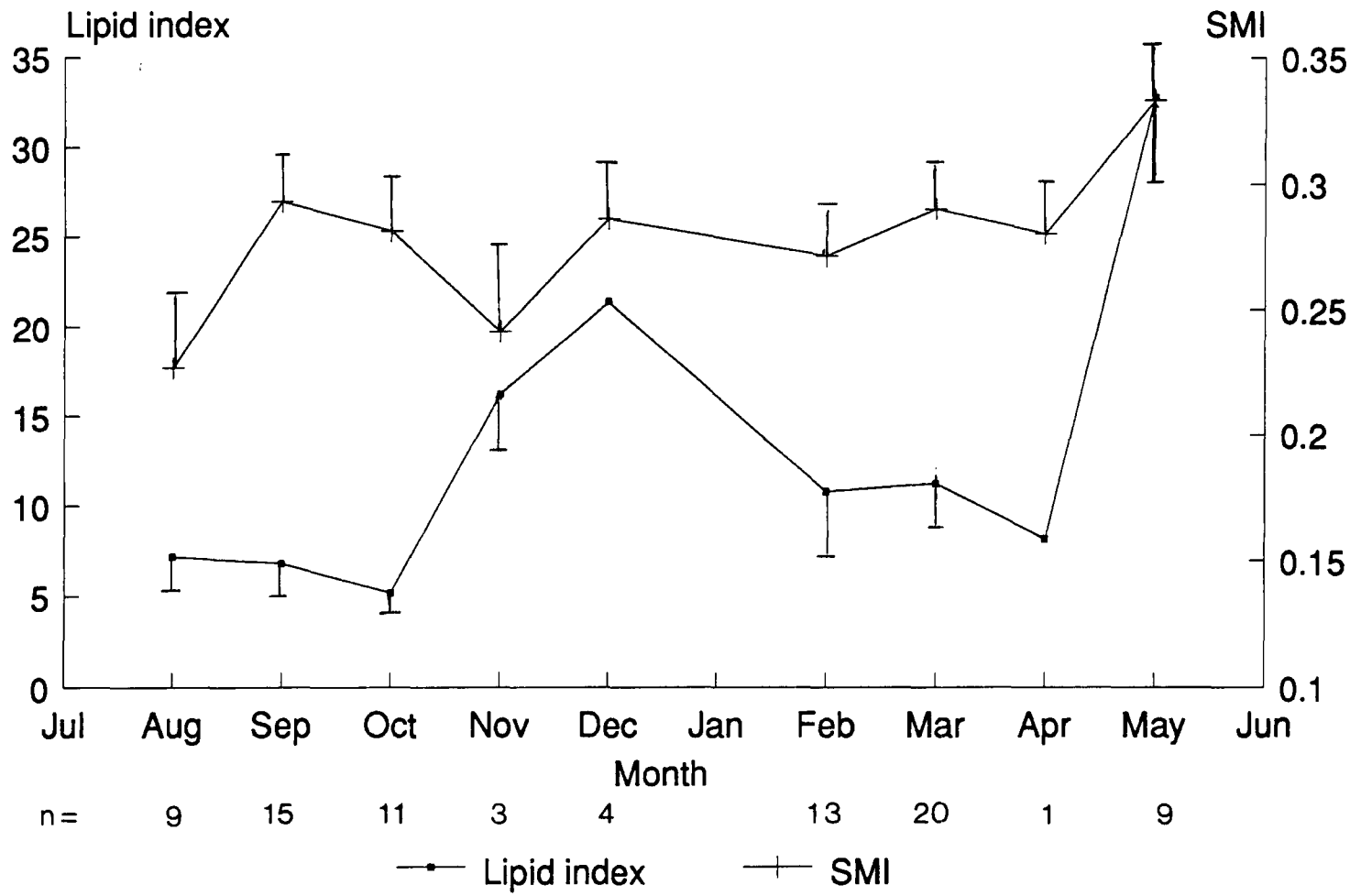


Fig 1.11 Seasonal variation in Lipid index (LI) and Standard muscle index (SMI) of Sanderling caught at Teesmouth during the non-breeding season. Horizontal bars represent 1x the standard error of the sample mean. n= the sample size.

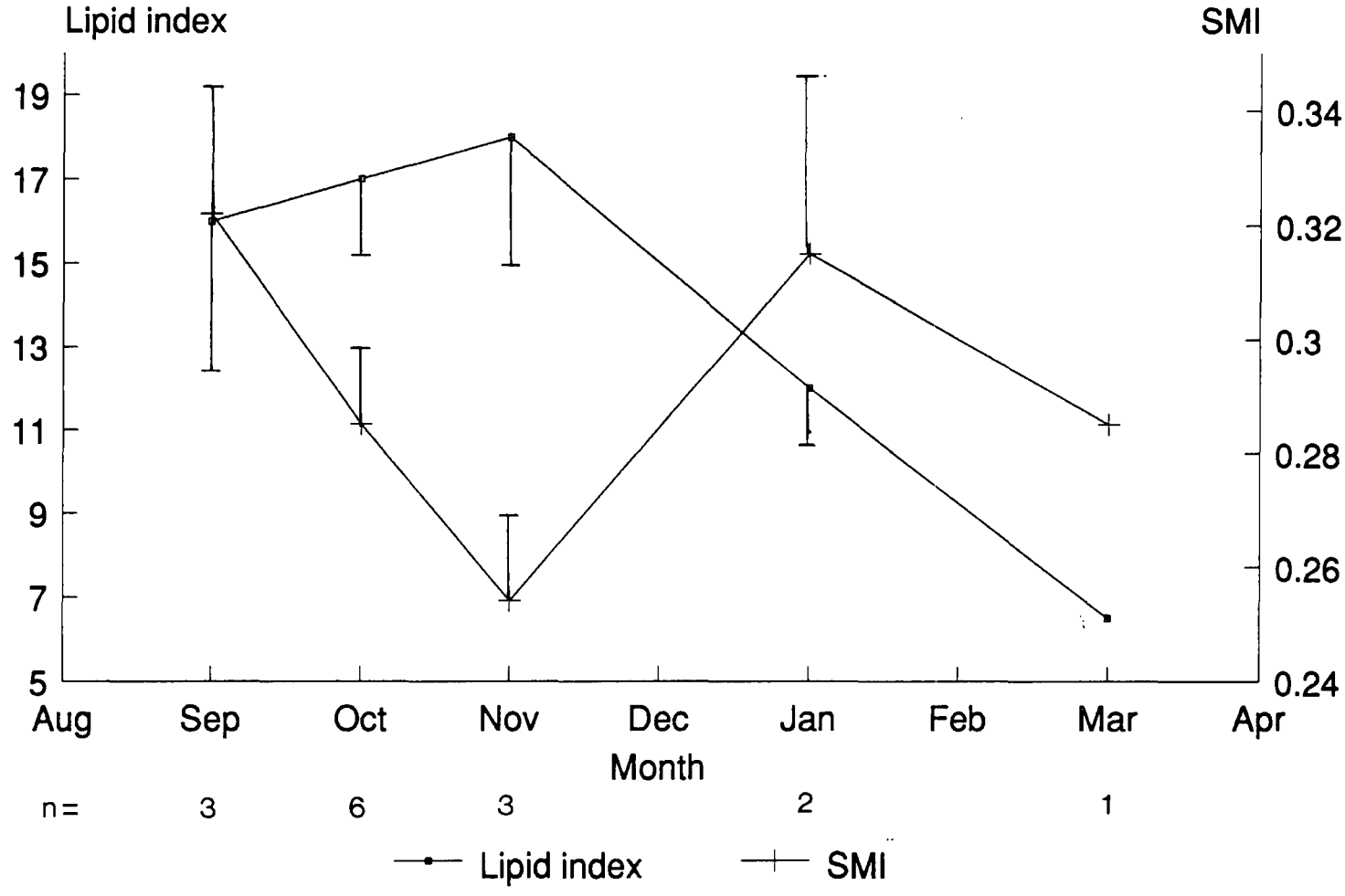


Fig 1.12 Seasonal variation in Lipid index (LI) and Standard muscle index (SMI) of Grey Plover caught at Teesmouth during the non-breeding season. Horizontal bars represent 1x the standard error of the sample mean. n= the sample size.

1.3f The relationship between fat content and body size.

In order to examine interspecific the relationship between fat content and body size between shorebird species, data were drawn from this study and from the literature on six: Grey Plover, Sanderling, Redshank, Bar-tailed Godwit, Knot and Dunlin. Only those species were used for which fat contents had been determined for the mid-winter period. Data were taken from Davidson (1981a) except for Sanderling (this study).

Least-squares regression analysis was used of Log10 of the mid-winter fat content(g) against Log10 TLM(g). This provided the relationship given below.

$$LogFat(g) = Log0.229(0.16)+0.95(0.183)\ LogTLM$$

$$(\underline{P}< 0.005\ r^2=0.84)$$

Numbers in brackets represent the standard error of the slope and intercept respectively. least-squares regression was also carried out with Log10 body mass as the independent variable of Log10 fat content as the dependant, even though this violates the assumption of non-independence of the x and y variables. The equation produced by this analysis is given below (see also Figure 1.13).

$$Log\ Fat(g) = 0.169(0.13)+0.98(0.17)LogBody\ mass$$

$$(\underline{P}<0.002\ r^2=0.89)$$

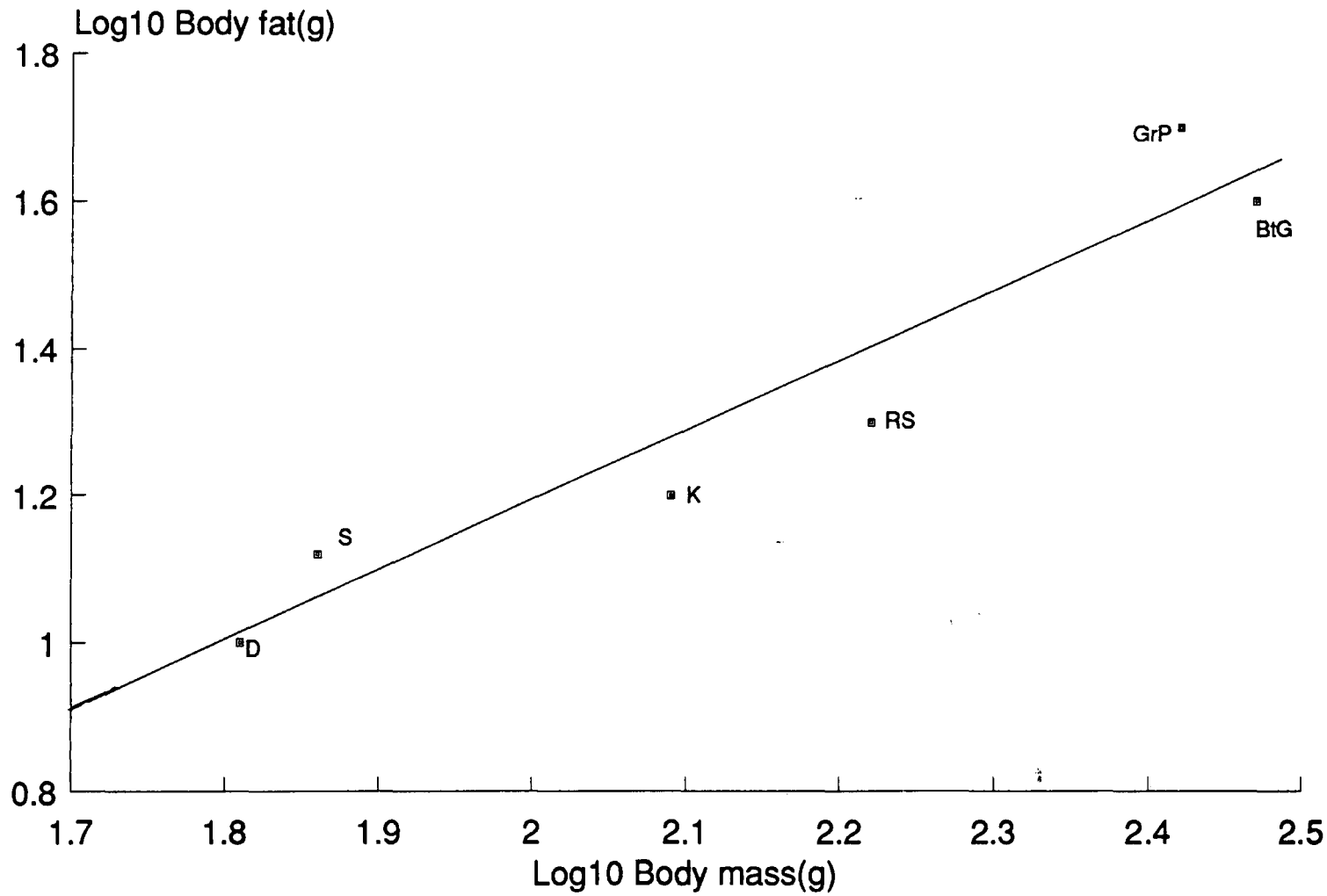


Fig 1.13 The relationship between body mass and peak mid-winter fat mass for 6 species of shorebird. Line was fitted using least squares regression analysis (equation given in text). D= Dunlin S= Sanderling K= Knot Rs= Redshank GrP= Grey Plover BtG= Bar-tailed Godwit.

The slope term can be converted to that value obtainable if reduced major axis regression (RMA) was used instead of least squares regression (LSR) using the equation given below.

$$b\text{RMA} = b\text{LSR}/r$$

Where b is the slope term and r is the correlation coefficient.

In the case above the value for the slope becomes 1.04 when Reduced major axis (RMA) is employed. RMA does not assume independence of the x and y axes. The mass exponent (ie. slope of the log/log plot) for shorebirds is thus lower than that found interspecifically for mammals (Pitts and Bullard, 1968). Assuming a mass exponent of metabolic rate of 0.75 for shorebirds, starvation endurance should vary as the 0.29 power of mass, interspecifically.

Intraspecifically, the relationship between size and fat content has been examined for Sanderling based on those data discussed in Section 1.5. In order to examine this relationship the Sanderling body composition data were divided into three groups which were considered to be physiologically homogeneous with respect to level of fat deposition. These groups are: 1) Birds caught between August and November; 2) Birds caught between November and April and; 3) Birds caught in May. Least squares regression analysis of Log10 Fat against Log10 TLM revealed no significant relationships between fat content and body size except for Sanderling caught during the winter period (Group 2). For these the relationship is given below.

$$Log\ Fat = 3.65\ LogTLM(1.02) - Log5.43(1.74)\quad r^2 = 0.25\quad \underline{P} < 0.02$$

If birds caught in December are removed the relationship between fat content and body size is no longer significant at the $P>0.05$ level. Month by month analysis of the above relationship does not reveal a significant relationship between Fat(g) and TLM(g) in any month except December. The relationship seen in December is due to the bimodality of the sampled birds with respect to body mass and lipid index The bimodality of the sample within this month is thus implicated as the source of the relationship between TLM and fat mass for group 2 discussed above.

The intraspecific relationship between fat carried(g) and body size found above for Sanderling contradicts those found interspecifically in mammals by Pitts and Bullard, (1968) and those found intraspecifically for small mammals by Millar and Hickling, (1990). They contrast with those presented here for shorebirds interspecifically. They are supported however by the findings both of this study and those of Johnson and McFarland (1967), Evans and Smith (1975), Mascher and Marcstrom (1976), Pienkowski *et al* (1979) and Davidson (1981a) that although females are larger in most species of shorebird they do not carry lower lipid indices.

1.3g Body mass changes in captive birds

Values discussed here exclude those recorded within 10 days of capture, those of birds considered to be ill and for a period of 10 days prior to death even if there were no visible symptoms of ill health.

1.3g (i) Sanderling

The pattern of body mass change in those Sanderling held in captivity, is shown in (Figure 1.14). Monthly variation in mass of captives was not shown to be significant when bird No.5 was excluded from the data set. This individual was the only Sanderling to show signs of pre-migratory mass increase in captivity. ANOVA indicates that the pattern of body mass change for those individuals held in captivity was significantly different from that of the wild population (Table 1.10).

Table 1.10 ANOVA of Log10 body mass of Sanderling with month, year and sample group (Captive or Wild) as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	4.977	18	0.277	100.24	P<0.0001
Month	2.533	6	0.422	153.07	P<0.0001
Year	0.330	11	0.030	10.88	P<0.0001
Sample group	0.123	1	0.123	44.47	P<0.0001
Explained	4.977	18	0.277	100.25	P<0.0001
Residual	2.882	1045	0.003		
Total	7.859	1063	0.007		

The general trend was for a slight progressive increase in mass throughout the season although the mass of Bird No.1 declined slightly from 54g to 52g, Whilst that of bird N0.3 declined rapidly during January and February (by 9g) but then increased for the remainder of the spring (Figure 1.15a-f).

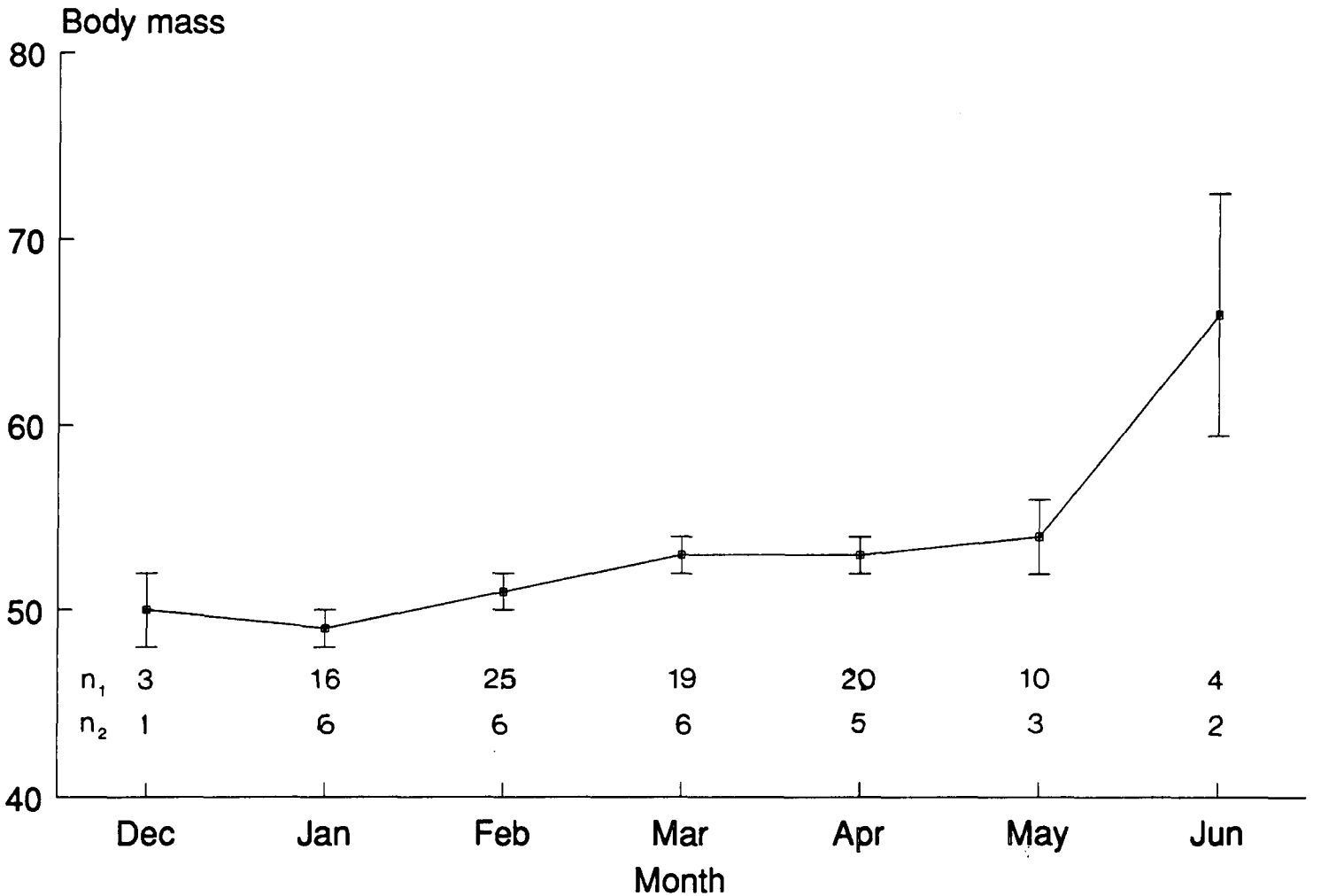
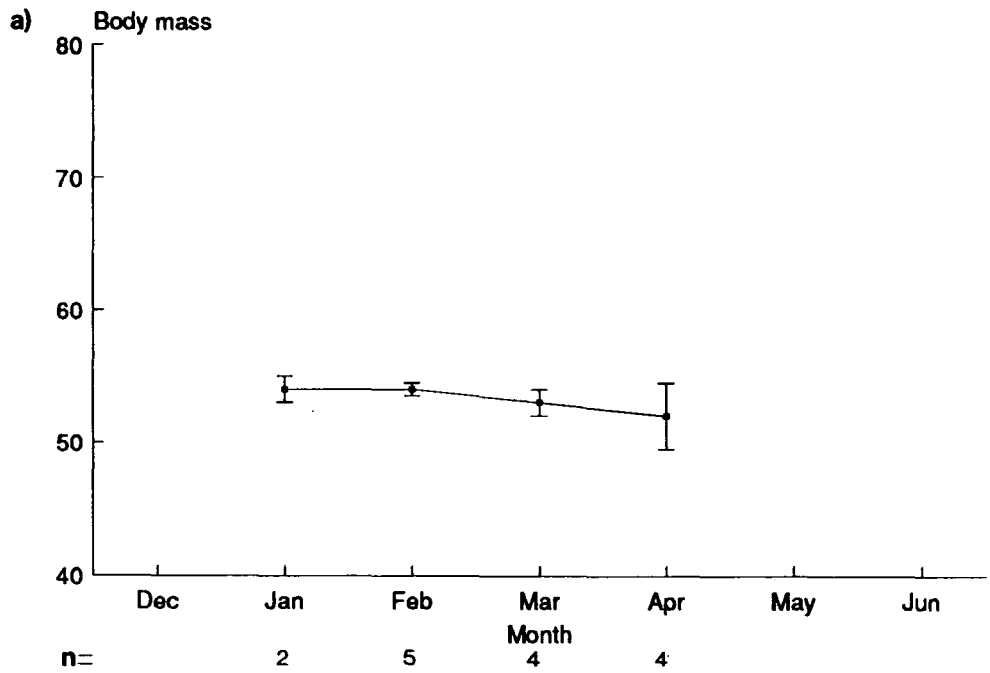


Fig 1.14 Seasonal variation in mean body mass of Sanderling held in captivity during the non-breeding season, n_1= sample size n_2= number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

Fig 1.15a-f Seasonal variation in body mass of individual Sanderling held in captivity during the non-breeding season, n= sample size. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.4 e)=bird No.5 f)=bird No.6



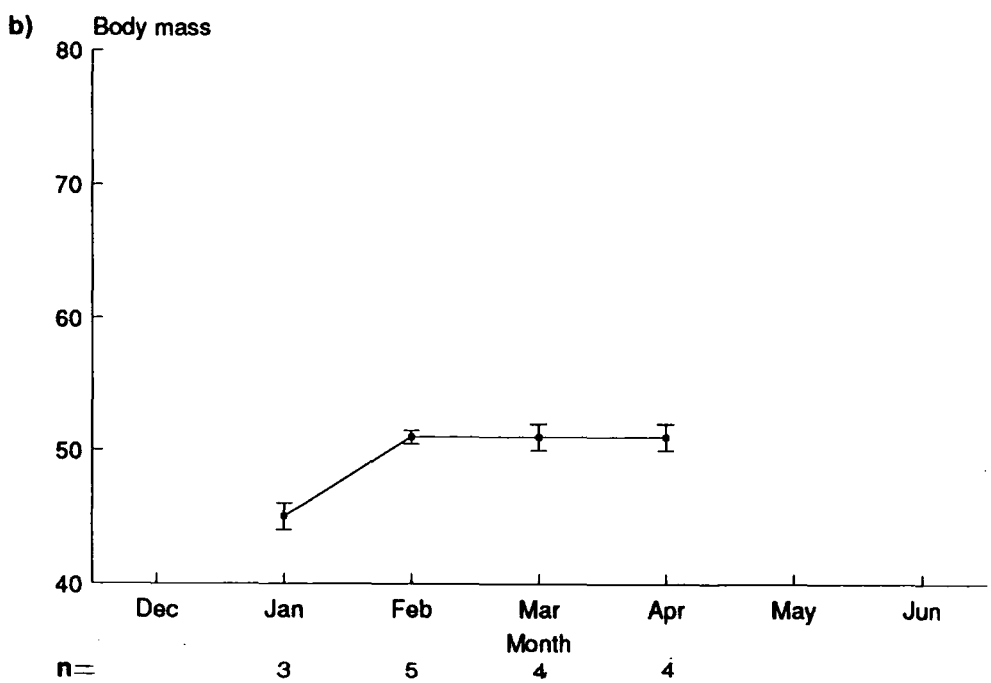
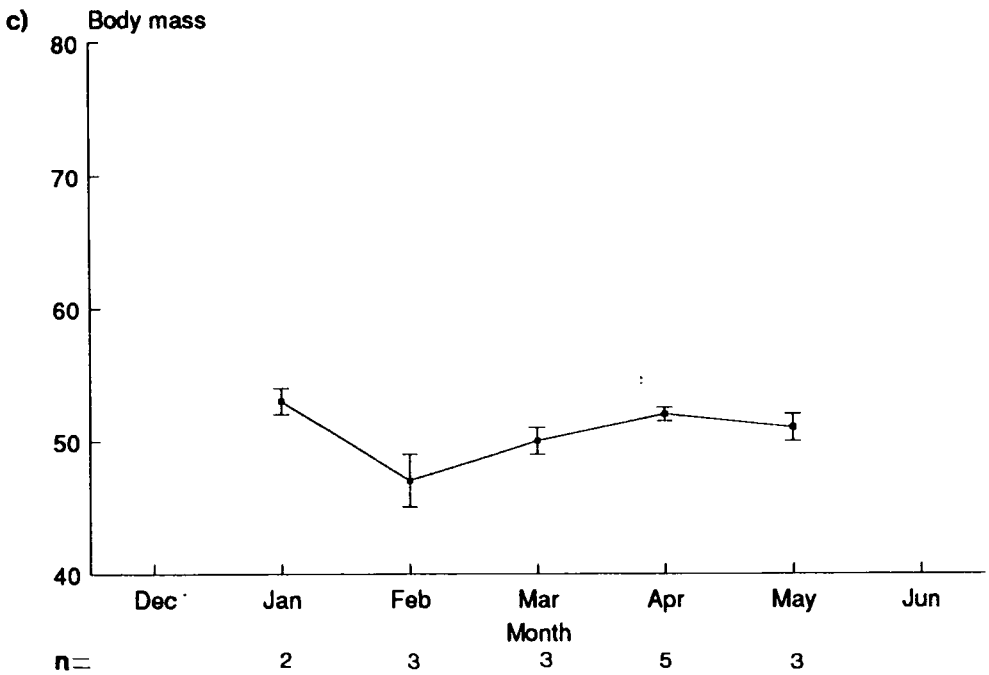


Fig 1.15 c & d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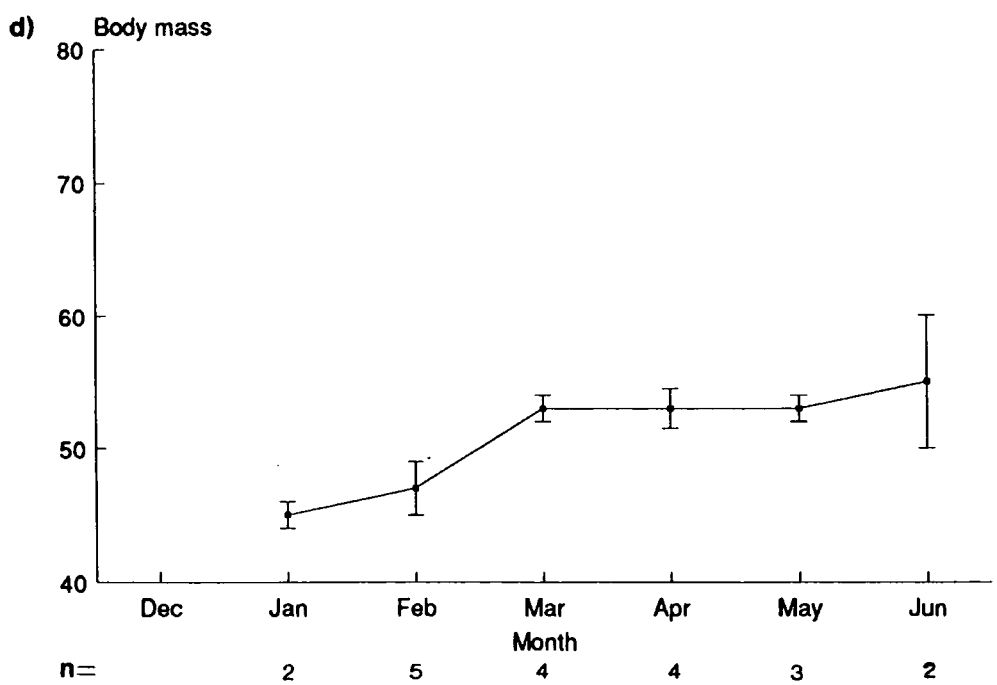
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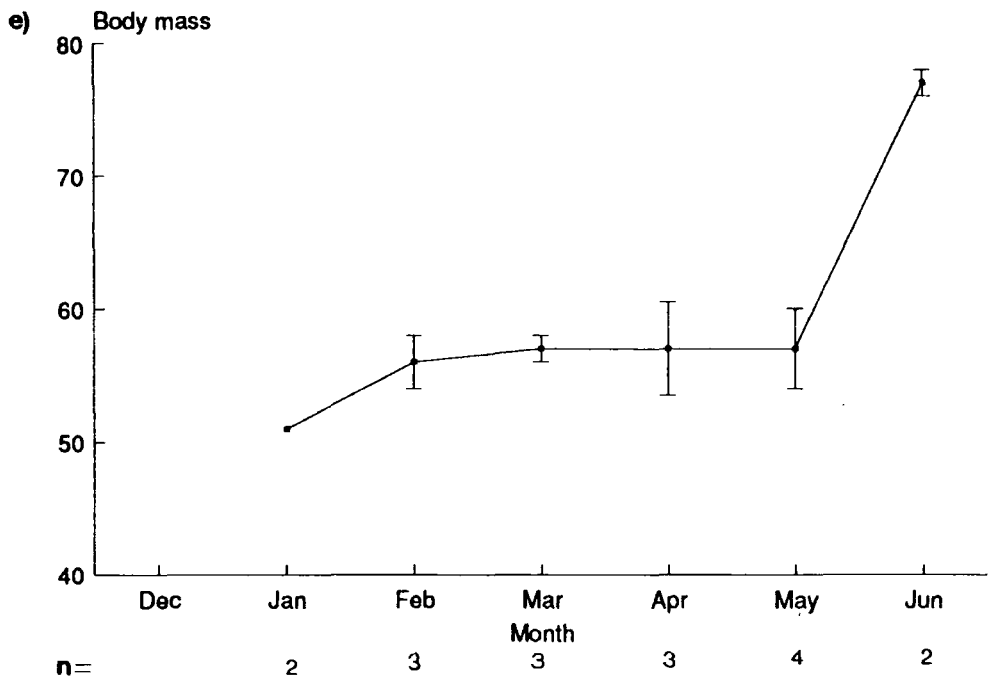
Fig 1.15 e & f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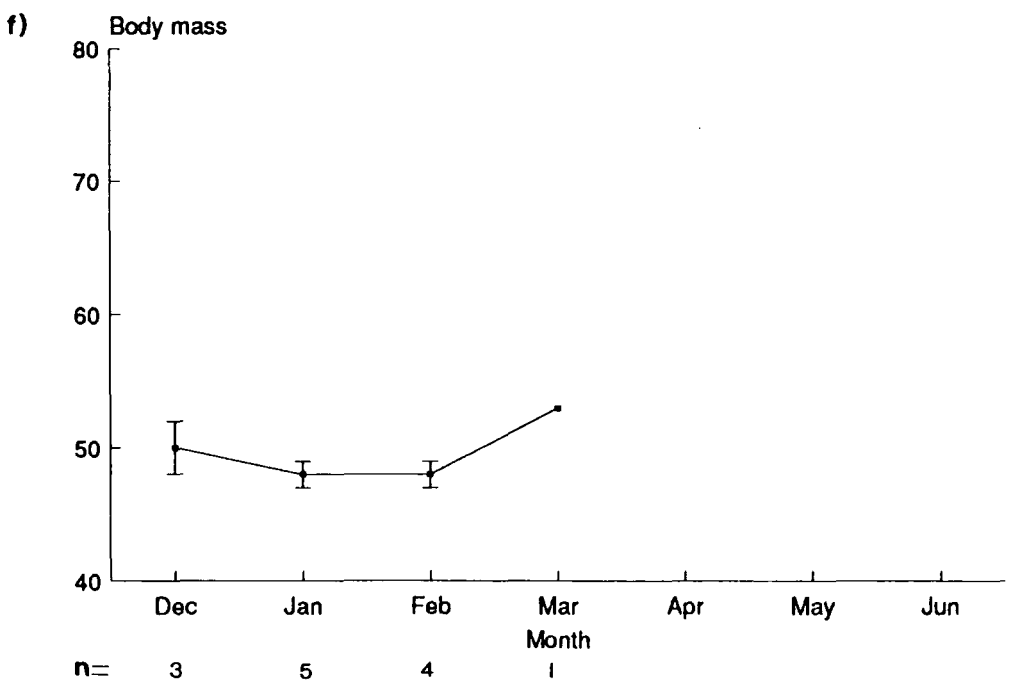
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1.3g (i) Redshank

The overall pattern of mass change of those Redshank held in captivity was not statistically distinguishable from that of wild birds (ANOVA Table 1.11). It should be stressed however that the seasonal pattern shown in figure 1.16 was obtained from that of two captive populations which only overlapped during February.

Table 1.11 ANOVA of Log10 body mass of Redshank with month, year and sample group (Captive or Wild) as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	0.386	15	0.026	13.31	P<0.0001
Month	0.110	7	0.016	7.91	P<0.0001
Year	0.021	7	0.003	1.75	P<0.05
Sample group	0.003	1	0.003	1.61	P>0.05
Explained	0.386	15	0.026	13.31	P<0.0001
Residual	1.232	669	0.002		
Total	1.600	684	0.002		

Those birds brought into captivity during February had an overall mass 18g lower than those birds already in captivity. From the discriminant function described in section 1.4c it is predicted that birds numbered 5, 3, 2 and 8 are *T.t.robusta* whilst birds 1, 4, 6, 7, 9 and 10 are *T.t britannica*. Of those birds held in captivity No.8 alone showed a pre-migratory increase in mass although the other animals held at this time were probably *T.t britannica* and thus were not expected to show large gains in mass at this time of year. Those birds caught in November showed a rapid increase in mass through this month followed by relative stability; all showed a slight drop during December (max 7%) except bird No.2 which showed a 1g rise. Body mass in January to February was relatively constant except that

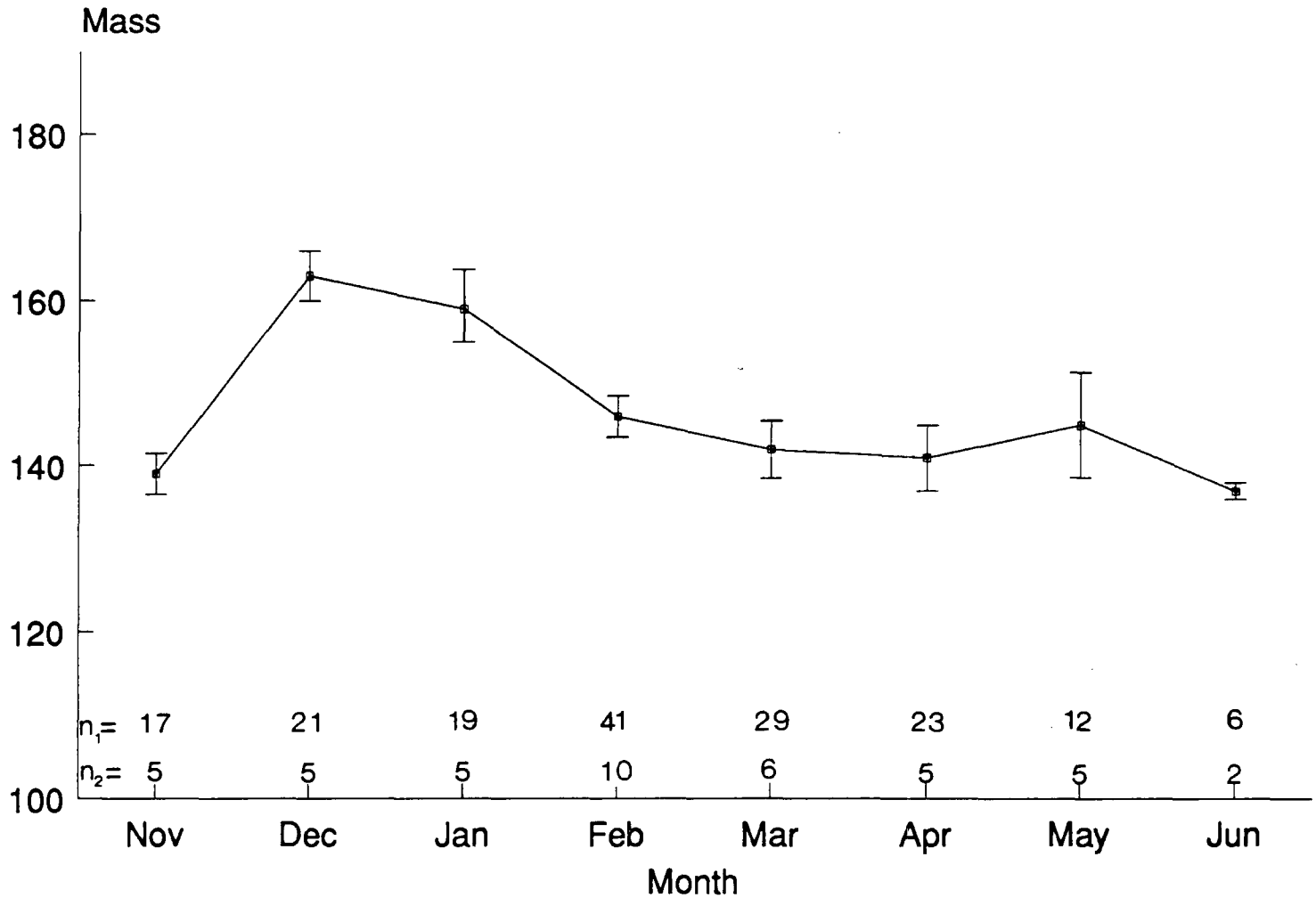
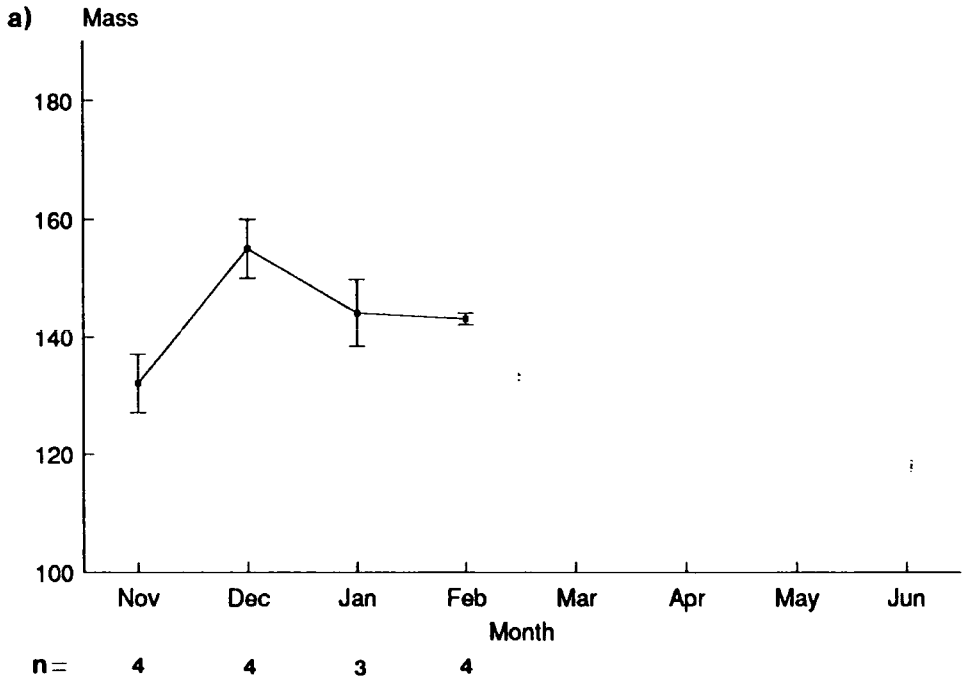


Fig 1.16 Seasonal variation in mean body mass of Redshank held in captivity during the non-breeding season, n_1= sample size n_2= number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

Fig 1.17 a-j Seasonal variation in body mass of individual Redshank held in captivity during the non-breeding season, n= sample size. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)= bird No.4 e)=bird No.5 f)=bird No.6 g)=bird No.7 h)=bird No.8 i)=bird No.9 j)=bird No.10



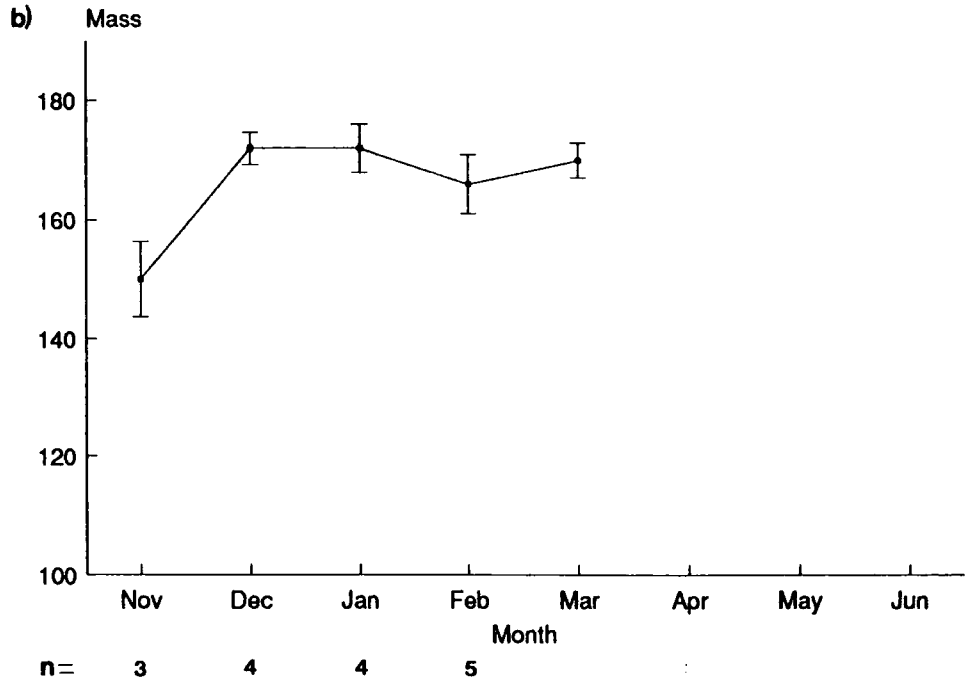
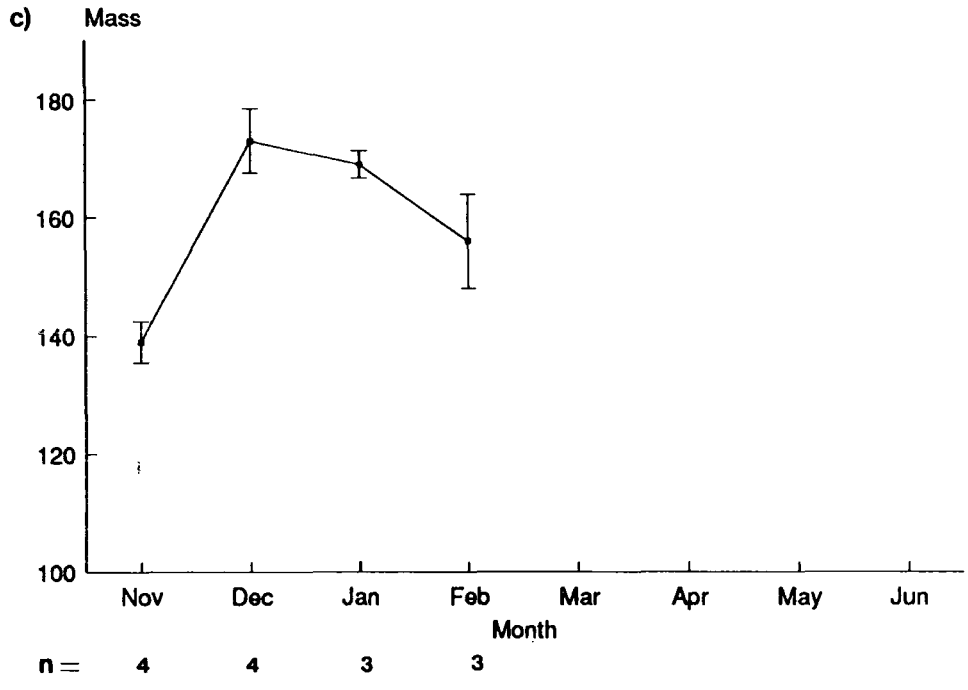


Fig 117 c & d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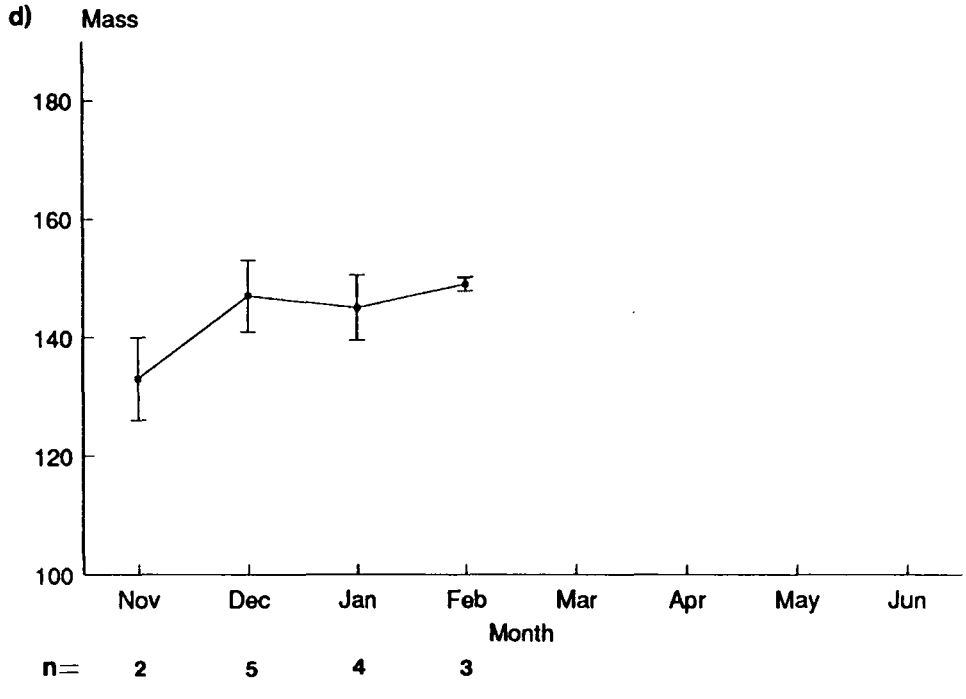
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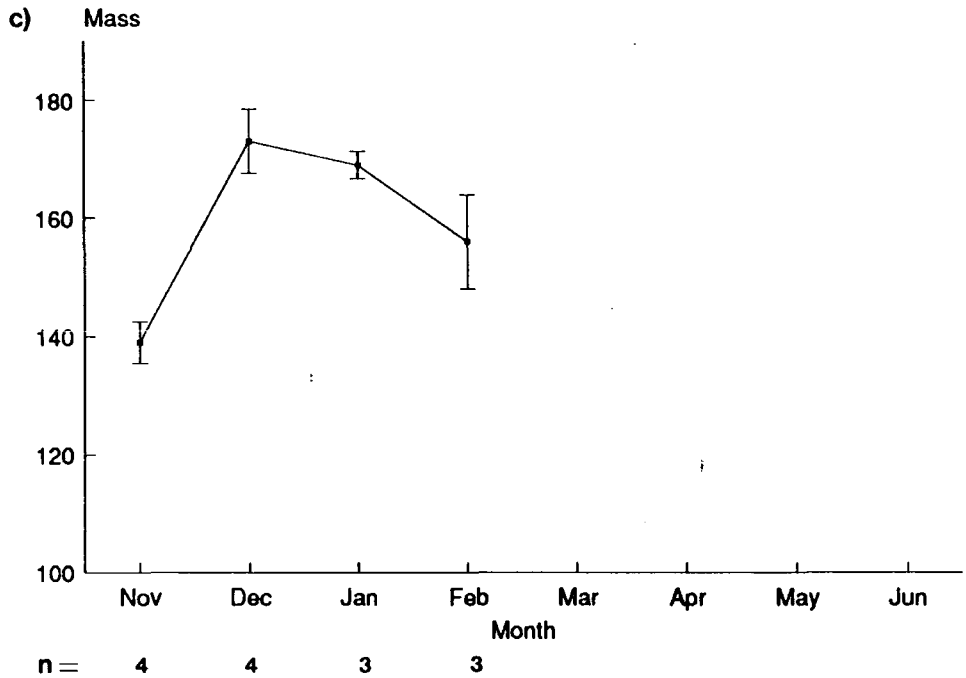
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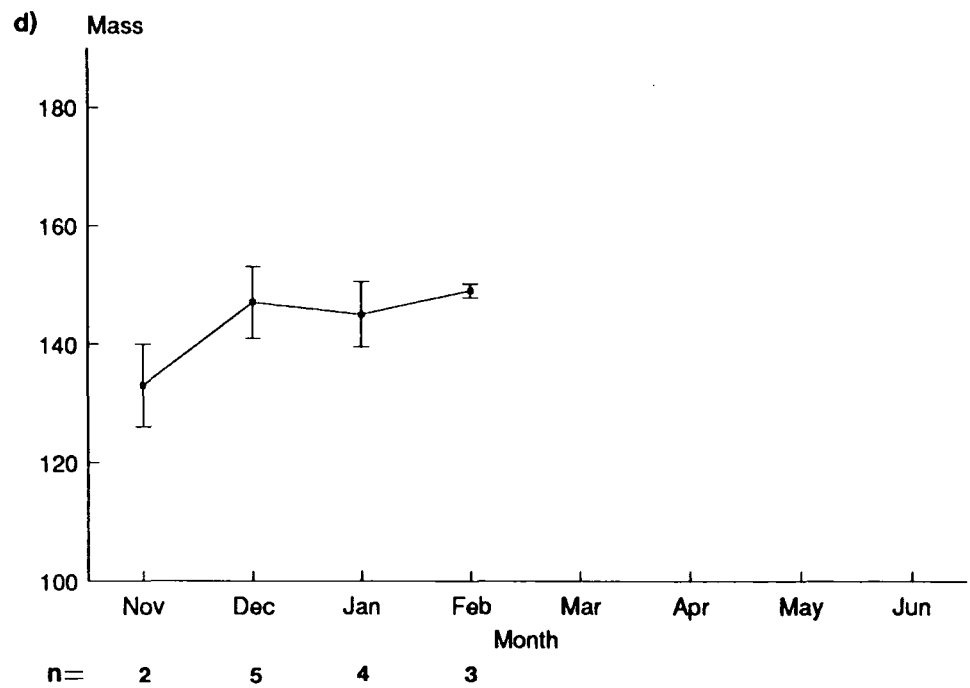
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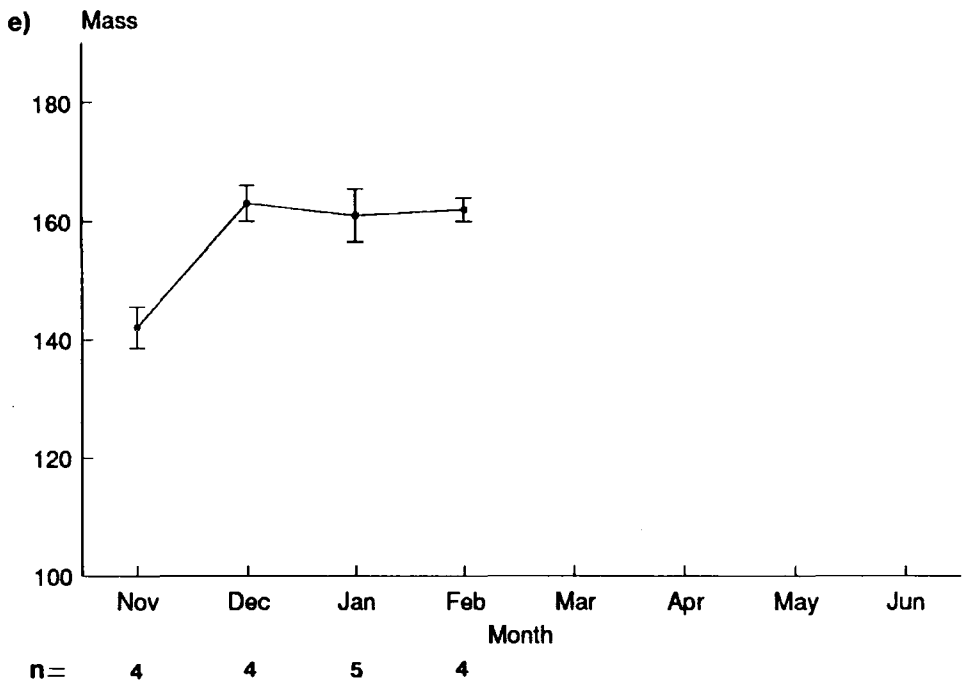
Fig 1.17 e & f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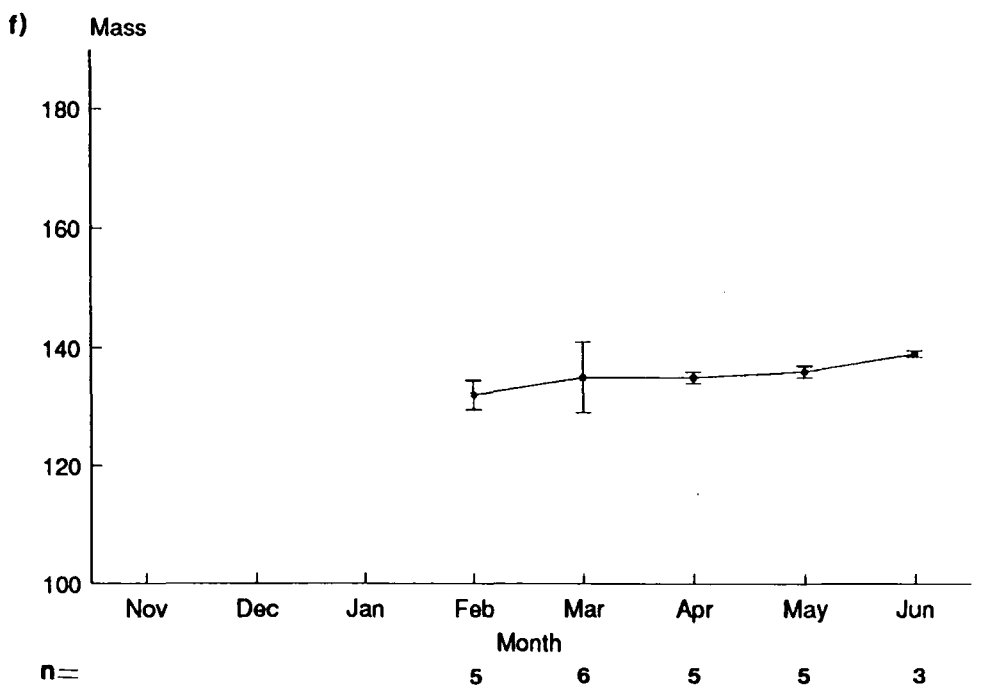
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Mass
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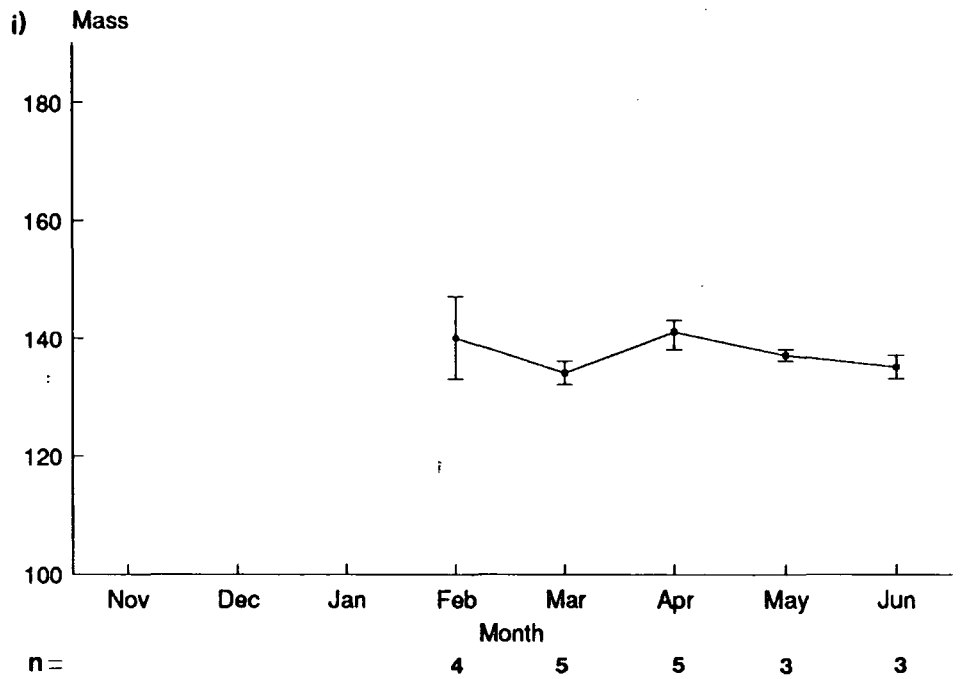
160
140
120
100
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Month
n=
5
6
5
5
3

Fig 1.17 i & j


i) Mass
180
160
140
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100
Nov
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n =
4
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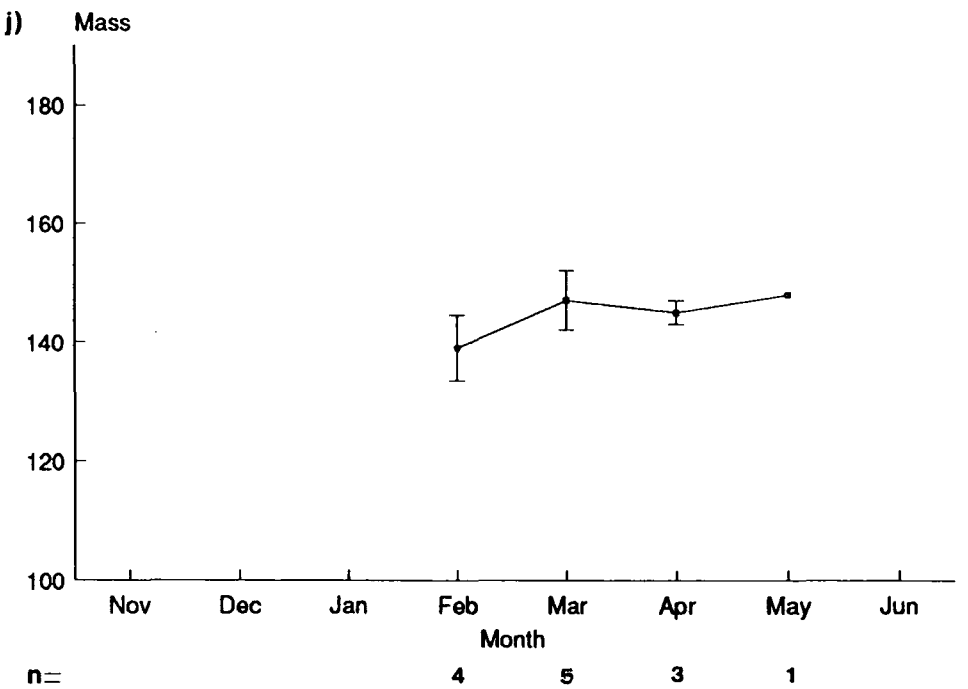
j) Mass
180
160
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Nov
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Month
n=
4
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1

of bird No.3 which fell by 14g (See Figs 1.17 a-j). From February throughout the remainder of the season most of those animals remaining in captivity showed a progressive increase in mass. Some animals showed a marked drop in mass following a temporary change in cage location for one week following storm damage. It is apparent therefore that under caged conditions individual Redshank may show body mass changes that differ from the general trend within the captive group of birds.

1.3g (iii) Grey Plover

Captive Grey Plover displayed an pattern of seasonal mass change which was not distinguishable from that of animals in the wild (ANOVA Table 1.12 Fig 1.18). The mid-winter peak masses of birds No.1 & No.2 were high, possibly reflecting obesity caused partially by inactivity, (See Figures 1.19 a-d).

Table 1.12 ANOVA of Log10 body mass of Grey Plover with month, year and sample group (Captive or Wild) as independent variables

Source of variation	Sums of squares	DF	Mean squares	F	Signif of F
Main effects	0.346	20	0.017	10.618	P<0.0001
Month	0.312	8	0.039	23.917	P<0.0001
Year	0.068	11	0.006	3.766	P<0.0001
Sample group	0.003	1	0.003	1.654	P>0.05
Explained	0.346	20	0.017	10.618	P<0.0001
Residual	0.403	247	0.002		
Total	0.749	267	0.003		

The highest mass value recorded for a bird caught in the wild in January was 305g The pattern of mass change for birds 1, 2 & 3 were very similar. All displayed an increase in mass until January followed by a decline through to April/May. The mass of

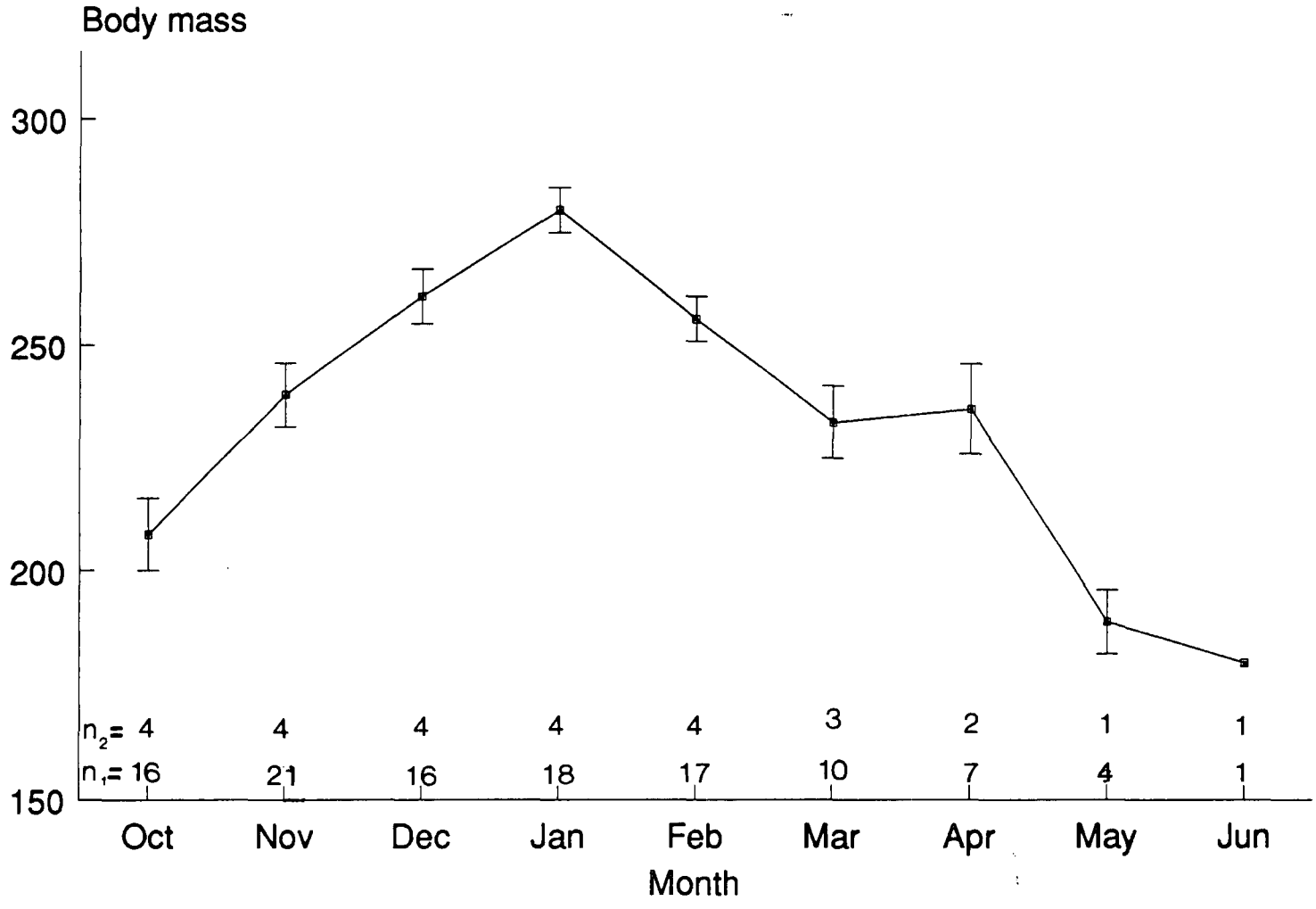
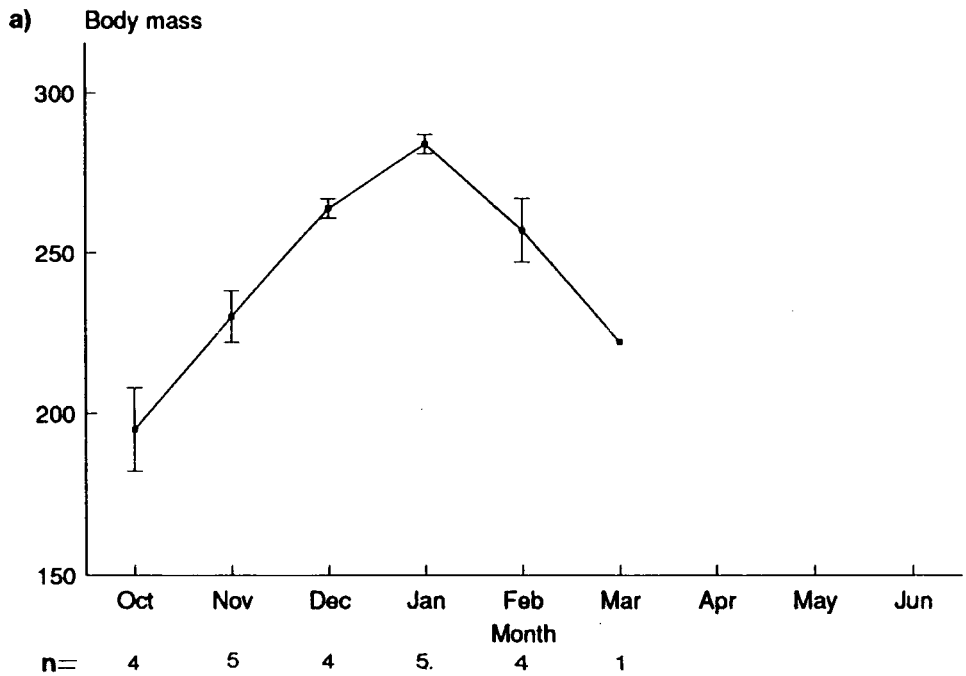


Fig1.18 Seasonal variation in mean body mass of Grey Plover held in captivity during the non-breeding season, n_1= sample size n_2= number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

Fig 1.19 **a-d** Seasonal variation in body mass of individual Grey Plover held in captivity during the non-breeding season, n= sample size. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.4



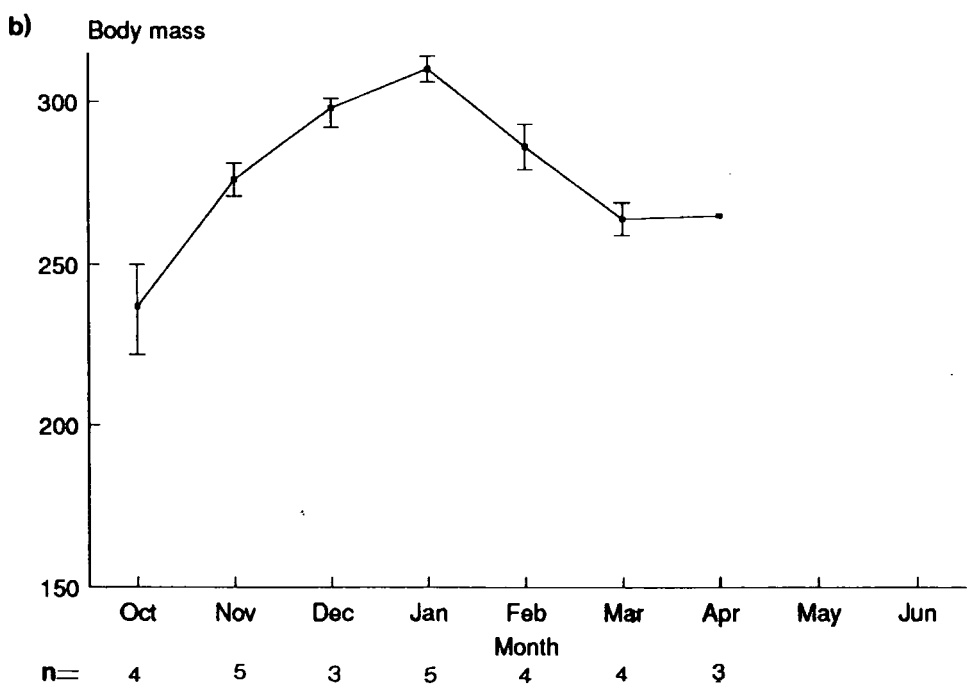
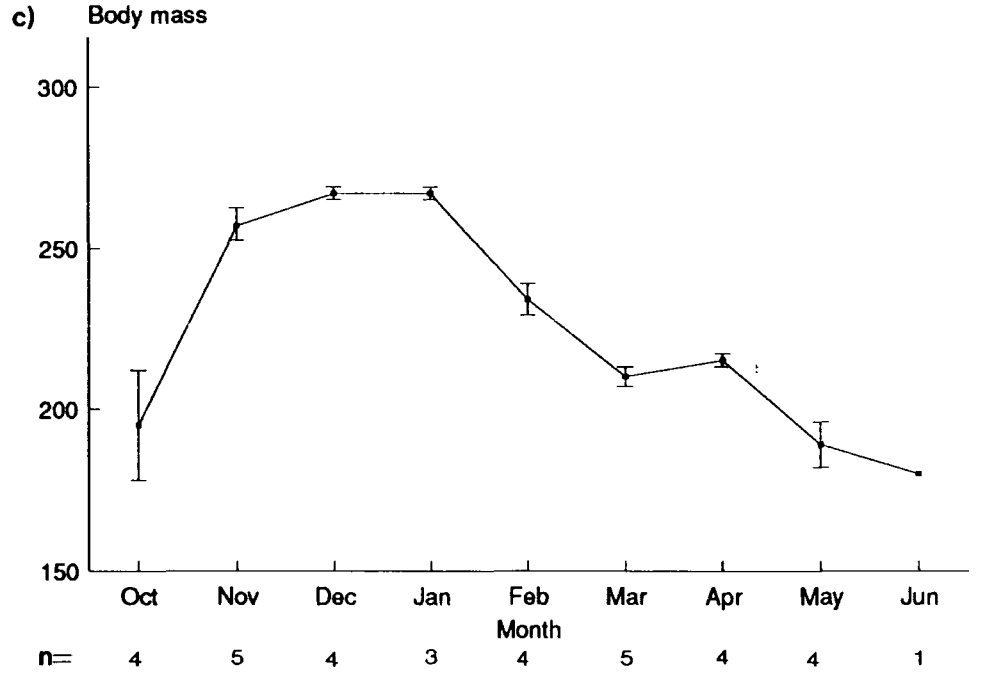
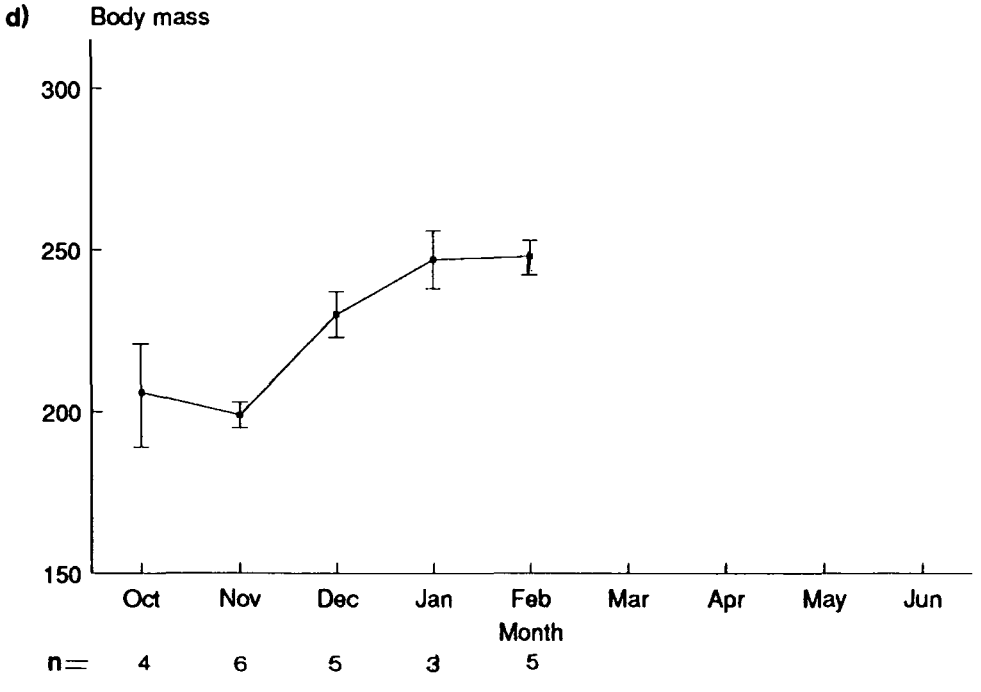


Fig 1.19 c & d


c)
Body mass
300
250
200
150
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Month
n=
4
5
4
3
4
5
4
4
1


d)
Body mass
300
250
200
150
Oct
Nov
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Apr
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n=
4
6
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Bird No.5, caught and held over the 1989/90 winter season varied in a similar way to those of birds held during 1988/89 winter period although not with the same synchrony. Unlike Birds 1 2 & 3, which regained mass rapidly following capture Bird No.5 took over 10 weeks to regain capture mass. This animal escaped on March 1st and returned to the Tees estuary and has subsequently been seen many times. All Grey Plover held in captivity were juveniles.

1.4: DISCUSSION AND CONCLUSIONS

In the wild, Grey Plover, Redshank and Sanderling appeared to follow a pattern of seasonal change in body mass during the non-breeding season which is common to most shorebird species wintering in N.E England. It is clear in the case of Sanderling, from direct measurements and in Redshank by inference from body mass) that mid-winter fat levels are not the maximum physiologically sustainable levels, as both these species show pre-migratory increases in mass which are greater than those obtained in mid-winter. In the case of one Sanderling a pre-migratory lipid index as high as 47% was recorded. Such high levels of pre-migratory fat reflect the long distance non-stop movements made by these shorebirds whilst on migration, as do the high values for SMI recorded in May. These high values are associated with pre-migratory muscle hypertrophy. It is generally considered (Davidson and Evans, 1990) that muscle hypertrophy occurs in order to: 1) increase the power available for flight in order to maintain performance with considerably increased loads of fat; 2) provide a protein reserve to guard against severe

weather that may be encountered on the breeding grounds and which otherwise may lead to starvation (Mascher and Marcstrom, 1976); 3) allow the earliest possible laying date by providing a protein reserve and thus enhance the chance of breeding success (Byrkjedal, 1978).

It has been suggested that adaptive pre-migratory dehydration may occur in long distance migrants (Fogden, 1972; Davidson, 1984 and Johnson *et al*, 1989). No evidence to support this hypothesis was found in this study. Indeed it is possible that dehydration could constrain flight duration by causing increased blood viscosity and thus a decrease in oxygen supply to the muscles. Water loss has been shown to constrain flight duration in the Pigeon *Columbia livia* (Biesel and Nachtigall, 1990). I suggest that the dehydration observed by some authors is probably due to poor measurement techniques and that the theory of adaptive pre-migratory water loss has evolved to explain the inadequacy of equations formulated to predict flight range (Davidson, 1984).

Davidson (1981a) suggested that interspecific differences in mid-winter peak lipid index can be explained by variation in foraging ecology and behaviour. Those species which feed using visual cues and/or those which undertake movements induced only by the onset of severe weather carry proportionately more fat than do those species which feed by probing and those which do not undertake 'severe weather' movements. Sanderling were shown by this study to carry slightly larger amounts of fat reserves at mid-winter, when compared to a similar sized tactile feeder such as Dunlin. Sanderling feed in open shore habitats and, with

relatively short bills, cannot obtain those prey items which dig deeper into the substrate during cold weather (Smith, 1975; Evans, 1979 and Pienkowski, 1980ab). In addition their feeding habitat is more prone to unpredictable change caused by storms when compared to the mud-flat habitat of estuaries. These factors taken together lead to the prediction that Sanderling should carry relatively large fat reserves, as found.

Grey Plover appear to store proportionately more fat during mid-winter than do most other shorebirds wintering on the Tees estuary, with only Lapwing and Golden plover showing greater fat levels (Davidson, 1981a). It should be noted however that the predicted peak lipid index of 22%, for Grey plover (Fig 1.15), is based on the formula of Davidson (1981a) for predicting lean mass from bill and wing length. These formulae do not take into account any changes in lean mass that may occur during the winter period. Neither in Davidson's study or in this has it been possible to corroborate this figure with direct measurement of lipid index during midwinter. Plovers feed using visual cues and during high winds feeding becomes impossible or extremely energetically expensive. Thus in general Plovers are more likely to experience periods when they are unable to feed and these periods will last longer than those experienced by Scolopacid Sandpipers, which can switch from visual to tactile feeding, (Goss-Custard, 1976 and Evans, 1976). A few individual Grey Plovers may move to different estuaries during very cold winters although both Dugan *et al*, (1981) and Townsend (1981) found that the majority of individually marked Grey Plover on the Tees were resident throughout the whole of the winter period.

Davidson (1981a&b) suggested that Redshank are unable to maintain their fat reserves during the wintering period on estuaries in N.E England, as the decline in mid-winter fat reserves and SMI is more rapid than in any other species and they seem unable to recover reserves lost during severe weather. There is good evidence to show that feeding efficiency declines in Redshank as the weather deteriorates (Goss-Custard 1969, 1976) but a satisfactory reason for this inability of Redshank to lay down sufficient reserves before the wintering period has not been put forward. It is possible that the apparent decline in nutritional reserves in Redshank between January and February is due to migration away from Teeside of birds of superior condition compared to those that remain. Certainly there is a decline in the numbers of Redshank wintering on the Tees estuary between January and February in most years,(Davidson, 1981a).

The occurrence of mid-winter peaks in the body masses of those species studied here support the general hypothesis that Shorebirds wintering at northern latitudes experience similar constraints on foraging at a similar time of year and is consistent with the severe weather theory discussed above *ie.* that, like passerines, shorebirds lay down fat in mid-winter as insurance against periods when maintaining a positive energy balance becomes impossible either due to lack of food supply or inability to feed due to environmental factors. Individuals which winter at more southerly latitudes where storms are less frequent show greatly reduced mid-winter peaks in both body mass and fat reserves, (Blem, 1973 and Pienkowski *et al*, 1979). Grey Plover

wintering in South Africa show mean mid-winter lipid indicies of 6.4% (Summers and Waltner, 1979) and in Pacific Golden Plover wintering on Hawaii there was no evidence of a mid-winter peak in fat reserves (Johnson *et al*, 1989). Decline in the level of protein reserve being carried also occurs as birds winter further south (Davidson, 1981a; Davidson and Evans, 1986).

As discussed previously two hypotheses have been evoked to explain the mid-winter peak fat reserve and its subsequent decline. These are: 1) that mid-winter peak size and subsequent decline are internally regulated and; 2) that mid-winter levels are the maximum which the environment will allow and that subsequent decline is a result of negative energy budget. The internal control hypothesis has been substantiated to a large extent for Dunlin and Grey Plover, (Davidson 1981a&b). As both species tend to be able to recover nutritional reserves following a period of severe weather (Dugan *et al*, 1980). For Grey Plover the "internal regulation" hypothesis has also been substantiated by data presented in this study, as those Grey Plover held in captivity showed a pattern of mass change which is similar to those in the wild even though food was supplied *ad libitum*. If, as suggested by Davidson (1981a&b), Redshank cannot maintain their body reserves throughout the wintering period, captive Redshank supplied with food *ad libitum* should not follow the pattern seen in the wild. As discussed earlier, the apparent rapid decline seen in the captive population from January to February was due largely to the addition into the population of lighter individuals from the wild. The January to February decrease in mass seen in those captives caught before January was

slight compared with the decrease in those in the wild, thus supporting the claim that Redshank in the wild are unable to regulate their nutritional reserves, *ie.* external regulation Note, however that Figure 1.13 indicates that Redshank do not display unusually low levels of fat reserves for their size at mid-winter.

The captive Sanderling held during this study maintained relatively stable masses during the winter period. This suggests that the external regulation hypothesis may be correct for Sanderling. Within Britain, Sanderling appear to avoid apparently suitable sandy beaches and most large concentrations are found on or close to the sandier parts of estuarine areas (Pratter and Davies, 1978). This may be due to the enhanced levels of invertebrate biomass that are found in association with estuaries and the Sanderlings dependence on this in order to winter in northern England. Alternatively Sanderling may only lay down fat reserves when feeding in an unpredictable area. It is also possible that the lack of correlation between the patterns of seasonal body mass variation in the populations of wild and captive Sanderling is due to the inability of Sanderling to adapt to captive conditions. When Knot were held in captivity the pattern of seasonal mass change in the captive group also did not correlate with that of the wild population (Goss-Custard, 1983). Knot were probably a poor choice of shorebird to use to compare seasonal body mass flux of captive and wild populations, as a large proportion of the wintering Knot population in Britain is known to undertake long distance inter-esturine movements during the wintering period (Dugan 1981).

Interspecifically size is strongly correlated with the amount of fat carried. This is as expected since larger animals require more energy and thus more reserves to avoid starvation. From my data it appears that starvation endurance varies with a mass exponent of 0.29. If increased endurance is a strong evolutionary pressure towards size change as suggested by Millar and Hickling (1990), selection within a species should favour larger birds where short term absolute shortages of food are common but smaller birds where chronic but not absolute shortages occur. This may explain why more Redshank die than Dunlin during severe winters (which are periods of chronic food shortage). Larger species of shorebirds carry more reserves than would be predicted on the basis of energetics alone, although the extent to which additional fat is carried is less than for mammals. This may reflect the heavy increase in expenditure that is incurred when flying under increased load. Increased load would also cause a decline in manoeuvrability which may increase the probability of an individual being taken by a predator. As smaller birds are more likely to be predated it would be anticipated that small species should carry depressed levels of fat. This would cause an increase in the slope of the fat/total body mass relationship above that predicted by energetic considerations alone, but would not explain why the slope of this relationship is more shallow with respect to birds than mammals.

The criteria discussed above should apply at the species level also. Contrary to the interspecific relationship, however a linear relationship could not be found between size and fat

reserve in Sanderling for any period of the non-breeding season. There are two broad reasons why this may be so: 1)The Samples tested may not have been physiologically homogeneous and thus any relationship was over shadowed by seasonal effects; 2) The effect of size on the overall energy expenditure of Sanderling during the non-breeding season may be insignificant when compared to individual variation. Therefore a size-dependant effect on fat reserve carried is not present or is obscured by other factors. Individual variation in fat reserve could be caused by such factors as individual variation in feeding efficiency, digestive capability or disturbance levels at feeding sites. It is important that, for this relationship to be investigated more fully, measurement of body mass and reserve must be carried out on a large number of birds from each catch.

The value of body composition analysis by destructive methods is limited by the inherent inability to follow individuals throughout a season, which leads to the need to make often unsubstantiated inferences concerning population stabilities and origins of individual birds. The methods used are slow and time consuming and the method often leads to difficult ethical decisions concerning the sacraficing of samples which tend to lead to small sample sizes and patchiness within the data set. Chapter 2 discusses two new methods which may serve to alleviate these problems and permit the measurement of body composition of live birds.

CHAPTER 2

AN EVALUATION OF THE USE OF TOTAL BODY ELECTRICAL CONDUCTANCE AND ULTRASOUND AS METHODS FOR ESTIMATING THE TOTAL LEAN MASS OF LIVE BIRDS IN THE FIELD.

2.1:INTRODUCTION

As shown previously, body composition of many animals changes seasonally. In wild birds this is primarily a result of the deposition or utilization of fat reserve, but changes in muscle mass also occur particularly in relation to migration and reproduction (Davidson and Evans, 1988). Traditionally these changes have been studied using destructive analysis of dead birds. Such methods are time consuming, do not permit the study of species that are rare or endangered, give rise to ethical dilemmas and do not allow the study of seasonal changes in body composition of individual birds. Estimates of total lean mass and fat content derived from a combination of measurements of total body mass with various biometrics tend to be imprecise (Perdeck, 1985; Rising and Somers, 1989 and Freedman and Jackson, 1990) and are satisfactory only in comparisons between groups of individuals.

During the past four decades medical science has developed a number of techniques for the determination of body composition non invasively in human subjects. These techniques include nuclear magnetic resonance, ultrasound and neutron activation analysis (See Ellis *et al*, 1986 and Foster and Fowler, 1988 for reviews). Most of these techniques are not appropriate for field

use by the biologist as they require either large bulky equipment or a high level of subject compliance or in some cases both.

In this chapter I present an evaluation of two non-invasive techniques which are readily usable under field conditions: 1) Total body electrical conductance (TOBEC) and 2) Ultrasonic probing. The TOBEC technique is used to estimate the TLM of a subject and hence, by subtraction from total body mass, the fat content. Walsberg (1988) reported preliminary studies of the TOBEC method and its use in the estimation of TLM of a range of sizes of small birds and mammals. He established separate second order polynomial equations relating TOBEC values to TLM for the two vertebrate classes and briefly examined the importance of body temperature as an influence on the TOBEC value. I have extended his approach to focus particularly on the relationship between the TOBEC value and fat-free mass (TLM) of individuals of the same species of bird, to test whether the interspecific relationship derived by Walsberg (1988) also holds intraspecifically. Many of the results presented here for the evaluation of the TOBEC technique are already in press (Scott Grant and Evans 1991).

In addition to the information presented for the purposes of calibration of the TOBEC method I have also included predictions of TLM and LI of several species of shorebird caught in the wild and subsequently released. These data are shown in order to give further indication as to the accuracy and variation in the predictions of the various calibration equations presented.

Predicted TLM and fat mass from 10 Redshank and 1 Grey Plover (based on TOBEC indices) which were held in captivity during the 1989/90 non-breeding season (See Chapter 1) are shown. These data are presented so that an indication of the applicability of the TOBEC method for following seasonal variation in body composition can be gained. The predictions of seasonal variation in body composition of captive individuals, shown in this chapter, has subsequently been used, in the the analysis of variation in BMR with body mass (See chapter 4).

Ultrasonic probing is used to measure the thickness of pectoral muscle and hence give an estimation of protein reserve. I report here my preliminary investigation into the potential usefulness of this technique for my studies.

2.2 SPECIALIST EQUIPMENT

a) TOBEC

The TOBEC apparatus, known as the SA-1 small animal body composition analyser, (formerly known as the ME-100) was supplied by EM-SCAN inc (3420 Constitutional Drive, Springfield, Illinois 62707 U.S.A). The apparatus measures 400x315x265mm and weighs 9 Kg. The cylindrical sample chamber measures 385mm in length by 75mm diameter and can accommodate birds of elongated shape up to 0.3 Kg. in mass. For use in the field, power was provided from a 12v accumulator via an Oertling PC-01 converter to provide 240 volts 50 cycles A.C.

b) ULTRASOUND

The ultrasound apparatus was model 731c supplied by Scanco Inc. 2776 Triphammer Rd. New York 14850) and kindly by Cobb International Inc. The transducer probe transmits sound waves at 20 MHz The apparatus weighs 1.5 Kg and is readily portable.

2.3 PRINCIPLE OF OPERATION

a) TOBEC

The TOBEC method uses technology developed in the early 1970's and has recently been exploited by the medical and agricultural fields, (Cochran *et al*, 1989; Keim *et al*, 1988 and Herenroede, 1989). The TOBEC method employs the Harker principle (Harker, 1973), namely that when a conductor is placed inside a solenoidal coil producing a time varying electromagnetic field, power is dissipated by the current induced in the conductor. The power dissipated can be measured as a change in the coil's impedance. This change is related principally to the electrical conductivity of the sample inserted in the coil. e.g. a live bird. The conductivity of living organisms is primarily determined by their lean mass (as the electrical conductivity of lipids is only 5% that of lean tissues), although the output from the apparatus is also affected by body geometry in a complex manner, as described by Fiorotto *et al* (1988).

b) ULTRASOUND

Ultrasound testing has been used in industry for the accurate measurement and detection of flaws in plastics and metals. It has also been used widely in agriculture for the measurement of fat and muscle depth and the detection of pregnancy in livestock. The technique is also used for the detection of pregnancy in humans and was recently employed by Sears (1988) to measure the thickness of Mute Swan *Cygnus olor* breast muscle.

The probe used in this study employs the pulse-echo method which relies on the principle that sound waves require a mechanical medium to act as a carrier. As they pass through the carrier medium the ultrasonic sound wave is propagated. Each differing type of medium generates a unique oscillating signal. In the case of the Scanco-apparatus ultrasonic waves generated by the probe head pass through the tissue until the sound wave reaches an interface with a different medium where the wave signal is disrupted. Waves of ultrasound are then reflected as an echo which is detected by the probe head. The length of time between input and output signal is proportional to the distance that sound waves travel. In practice the Scancoprobe gives a direct reading in mm.

2.4 MATERIALS AND METHODS

a) TOBEC

In order to obtain an index of total body electrical conductivity birds were wrapped in a soft plastic cylindrical jacket with velcro fastenings prior to insertion into the apparatus. Birds were positioned in the cylindrical sample chamber so as to secure a maximum reading (See Results). Four readings were taken with the bird in position (B) alternating with four readings with the chamber empty (E). Before and after each set of readings a reference number (R) was obtained from the apparatus (a measurement which characterizes the sensitivity of the measurement chamber by sensing the response to a stable, internal reference device). The index of total body electrical conductivity (TOBEC index) was calculated from the formula below.

$$I = \frac{E-B}{R.a}$$

Where *a* is a normalization constant provided by the manufacturer (0.9883 for our apparatus) and other terms are the mean values of the repeated determinations for the same individual test animal. In all cases birds were weighed and TOBEC indices recorded as soon as possible after capture.

For determination of the relationship between TLM and the TOBEC index of a species small sample of birds were collected under licence and sacrificed by thoracic compression. The body composition was determined using the method described in Chapter 1 (Section 1.2a) except that Chloroform was used as solvent in

order to maintain consistency with Walsberg (1988). For studies of the effects on the TOBEC index of body temperature, positioning of specimens in the measurement chamber and the effects of contamination of plumage by salt, I used dead specimens that had been collected over several years and stored in a deep freezer.

Effect of change in body temperature on TOBEC index

Samples of seven dead Dunlin and five Knot *Calidris canutus* were tested at six temperatures between 15°C and 40°C. Each sample was allowed to equilibrate at the required temperature in a thermoregulated cabinet before being tested in the SA-1 apparatus.

Effect of salt water contamination on the TOBEC index

The TOBEC indices of five dead Dunlin were taken prior to and immediately after immersion in sea water and after 12 hours drying at normal room temperature.

b) ULTRASOUND

The accuracy of the ultrasound method was evaluated by comparing the depth of pectoral muscle recorded by ultrasound with that determined by insertion of pins into the muscle of a sample of dead birds. The muscle thickness was measured at various positions overlying the sternum, using a sharp needle that was inserted up to the bone of the sternum at an angle approximately

45° to the vertical. This angle was chosen to maintain consistancy with Sears (1988). A crocodile clip was placed around the needle at the point of entry into the skin to serve as a marker.of the muscle thickness. This was measured to the nearest 0.5mm from the tip of the needle to the crocodile clip using dial callipers. At each position the thickness of the pectoral muscle was also measured using the ultrasound probe positioned at the same angle to the vertical. Before each measurement was taken, the feathers covering the breast muscle were moved aside and oil was applied to the probe head to prevent air from interfering with the transmission of the ultrasonic waves. The probe was then placed in contact with the skin, care was taken not to compress the muscles beneath, and the depth of muscle was then read directly. Measurement positions were determined using the anterior end of the keel as a fixed reference point.They were taken at points moving towards the tail longitudinally and laterally using the keel's ventral edge as a reference point. The bird species used were Pigeon *Columbia livia*, Knot and Dunlin.

2.5 RESULTS: TOBEC

2.5a Position of bird within chamber

Using a range of specimens of dead birds of body masses between 40g and 160g it was found that neither the height of the specimen above the sample chamber base, nor its orientation (dorsal or ventral side uppermost) affected the signal output. However the horizontal position, measured with respect to the half way mark on the cylindrical chamber made a significant difference to the

signal obtained. To obtain consistent readings each sample was positioned so that a maximum difference between the chamber empty and the chamber full was obtained. This corresponded approximately to the mid-sternum of the bird lying close to the half way mark on the chamber (which corresponds to the most uniform and strongest part of the magnetic field generated by the coil). The optimum positioning varies slightly from species to species, depending on the anatomical distribution of lean tissues and overall body geometry.

b) Effect of Body temperature on TOBEC index

The relationship between mean TOBEC index of each sample(species) and temperature was linear, but the slope of the relationship differed significantly between species (ANCOVA, $\underline{F}$=20.72 df 1,8 $\underline{P}$=0.01) (Fig 2.1).

For Dunlin I=0.80 T^{o}C +20.21 (r=0.99 n=6)

For Knot I=3.50 T^{o}C +102.7 (r=0.96 n=6)

The slope term for the species of higher TLM (Knot) was found to be steeper than that for Dunlin. The interspecific difference in slope is almost eliminated if the temperature co-efficient is expressed in proportional terms. At 40^{o}C (close to the normal body temperature of most birds) the TOBEC index changed by 1.53% for Dunlin and 1.44% for Knot for each ^{o}C of change. This equates to a change in estimated TLM per ^{o}C of about 0.7g for Dunlin and 1.6g for Knot. Within each species, the slope of the relationship

Fig 2.1 Relationship between temperature and TOBEC index for a sample of dead Knot and Dunlin. Lines have been fitted using least squares regression analysis. See text for equations and statistics.

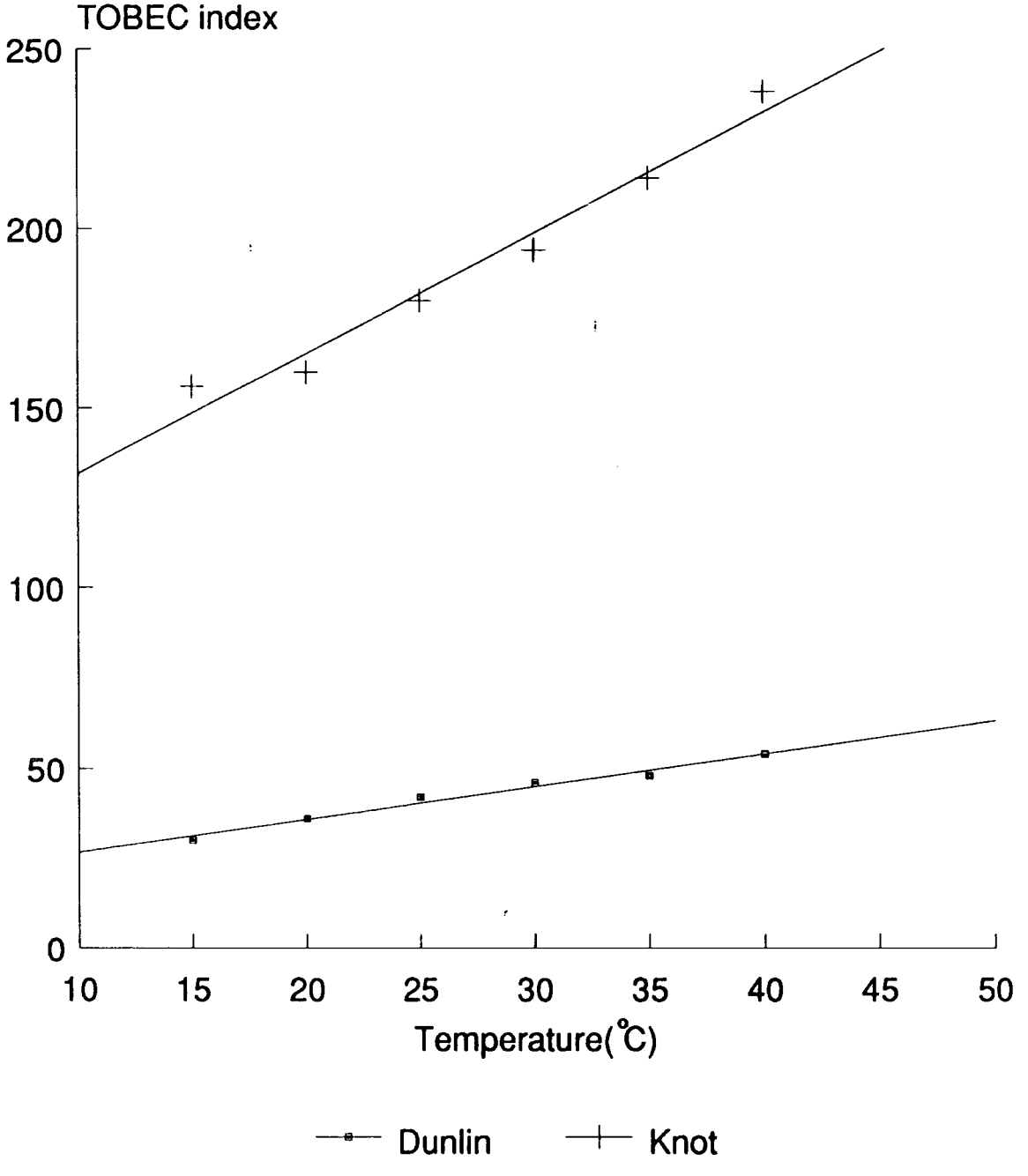
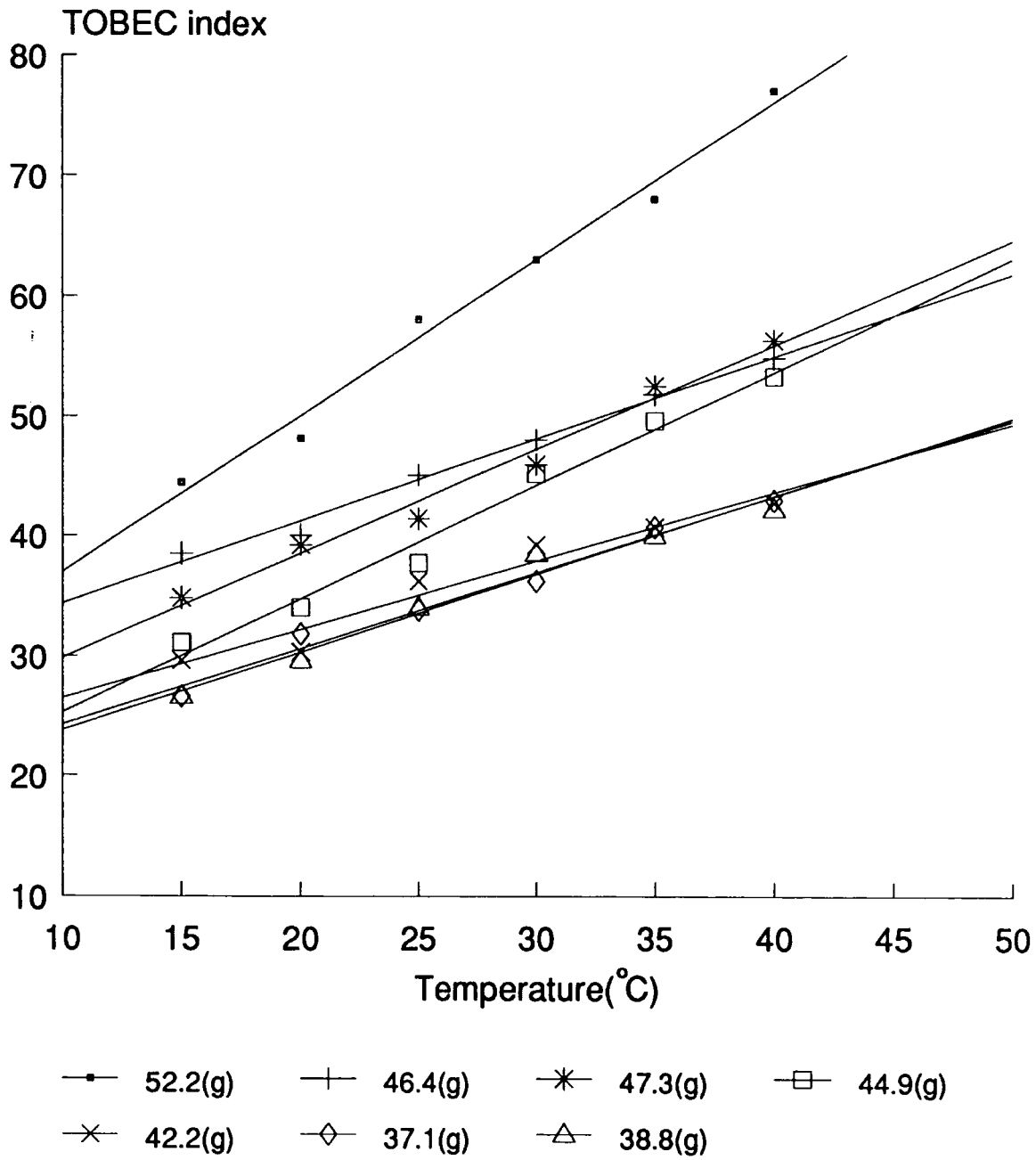


Fig 2.2 Relationship between temperature and TOBEC index for a sample of seven Dunlin. Each line represents the relationship for one individual. The slope of the relationships are dependant on the total lean mass of the individuals.



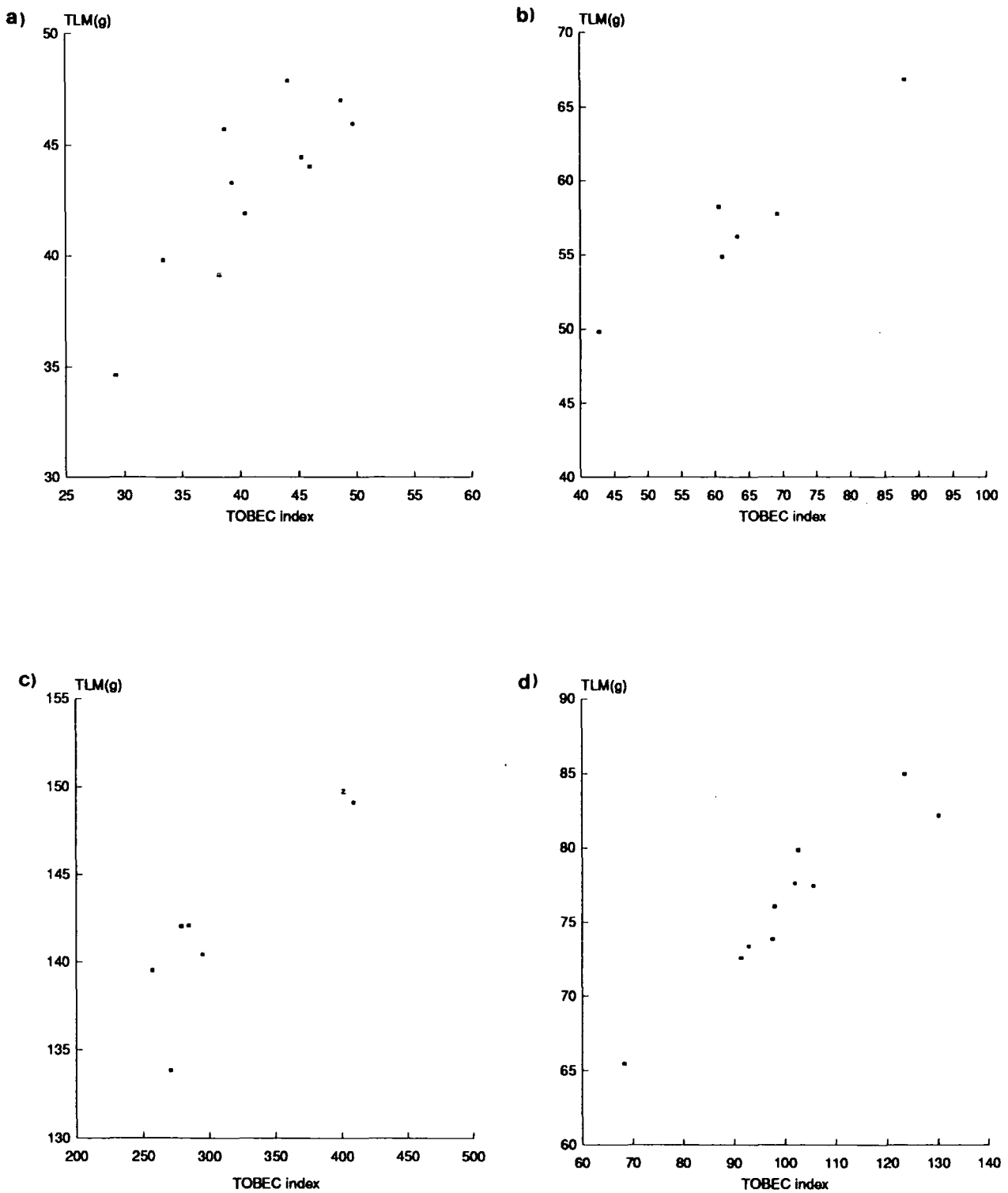
varied amongst individuals. Subsequent analysis of body composition showed that Dunlin of high fat free mass had TOBEC indices that increased more steeply with temperature than birds with lower total lean mass (Fig 2.2). Again, most of these difference disappear if the temperature coefficient is expressed in proportionate terms, or if the TOBEC index is divided by the TLM. (This latter correction, cannot of course, be applied under field conditions as the TLM is not known.) As found by Walsberg (1988), no effect of environmental temperature and thus operating temperature of the equipment on the TOBEC index was found.

c) Intraspecific variation in TLM: effect on TOBEC index

11 Dunlin, 6 Ringed Plover and 6 Redshank captured under licence at the Tees estuary in 1989 and 15 Starlings *Sturnus vulgaris* caught in Durham in mid-winter 1989/90 were used to evaluate the effect of TLM on TOBEC index. Body composition was determined as described previously. The relationships between TOBEC index and TLM for the four species are illustrated in Figures 2.3 *a-d*. Although specimens were selected to span a wide range of total body mass within each species, the range of TLM (revealed after fat extraction) was not so large, particularly in the Redshank.

Relationships between the TOBEC index and TLM were explored by least squares regression according to three models: Linear; Second order polynomial; and modified linear with TOBEC index x Body length square rooted as the independent variable (body length being taken as the distance from base of neck to the cloaca). The third model incorporates an adjustment for body

Fig 2.3 Intraspecific relationship between TOBEC index and TLM for four bird species a) Dunlin b) Ringed Plover c) Redshank d) Starling. See Table 2.1 for equations and statistics.



geometry (Fiorotto *et al.* 1987). The polynomial model was fitted using the constrained non-linear regression program of the SPSS^X statistical package. The constraints applied were that a) that the coefficients identified in the linear model could not be used in the quadratic model and b) that the quadratic description would not be developed if the quadratic term did not contribute significantly to the explanation of the overall variation. The results are summarized in Table 2.1. The data for three species fit best to the simple linear model, but for Dunlin the fit to the Fiorotto model is slightly better. Nevertheless the error of the intercept is larger in the Fiorotto model, so that the simple linear model has narrower confidence limits. (The large scatter about the regression line may be a result of contamination of some of the Dunlin sampled by sea-water, see section 2.5(g) below) The slopes of the regression lines differed significantly between pairs of species (Dunlin and Ringed Plover ANCOVA $\underline{F}$=8.7 df=1.12 $\underline{P}$=0.01; Dunlin and Redshank ANCOVA $\underline{F}$=10.7 df 1,13 $\underline{P}$=0.006; Ringed Plover and Starling ANCOVA $\underline{F}$=29.7 df 1.12 $\underline{P}$=0.001 Dunlin and Starling; $\underline{F}$=16.44 df1,17 $\underline{P}$=0.001). None of the intra-specific relationships could be described satisfactorily by a second order polynomial equation constrained as above.

Table 2.1: Relationship between TLM and TOBEC index for each individual species studied

Species	n	Equation	r	Signif of regression	SE of slope	SE of intercept
1) Linear model						
Dunlin	11	M= 0.53x + 21.4	0.84	P<0.005	0.11	4.66
Redshank	6	M= 0.07x + 119	0.82	P<0.05	0.02	7.75
Ringed Plover	6	M= 0.36x + 33.7	0.96	P<0.02	0.05	3.15
Starling	10	M= 0.35x + 45.6	0.95	P<0.0001	0.02	2.41
All 4 species	33	M= 0.35X + 34.2	0.97	P<0.0001	0.015	2.19
2) Independant variable = xL						
Dunlin	11	M= 0.81z + 3.25	0.89	P<0.001	0.14	6.70
Redshank	6	Not significant	--	--	--	--
Ringed Plover	6	M= 0.82z + 7.29	0.94	P<0.005	0.13	8.27
Starling	10	M= 0.81z + 6.78	0.81	P<0.01	0.25	19.06
All 4 species	33	M= 0.82z + 7.74	0.98	P<0.0001	0.03	2.70

Where L=length of bird x=TOBEC index
M=TLM z=xL

d) Interspecific relationship between TOBEC index and TLM

The total data set for all four species is plotted in Fig 2.4. As found by Walsberg (1988), the interspecific relationship between the two variables is best described by a second order polynomial. The equation derived from my data (Table 2.2) differs significantly from the equation derived from Walsberg's Fig 2 in the squared term but not in the other two parameters. It is not clear whether this results from a difference in performance of the apparatus between experimenters or from the less wide range of TLM's studied in the work presented here.

e) Effect of fat content on TOBEC index

No correlation was found between the residual variation from the regression of TLM against TOBEC index and the lipid indices (Mass of fat as a % of total body mass) of specimens. This result applies to species treated individually (Linear models) and taken together (polynomial model). This indicates that variation in fat content did not affect the TOBEC index as Walsberg(1988) had already reported for the interspecific polynomial relationship.

Fig 2.4 Interspecific relationship between TOBEC index and TLM. The line of best fit is a second-order polynomial for both graphs. (a) Relationship found by the present study (b) The relationship found by Walsberg (1988) see table 2 for equations and statistics.

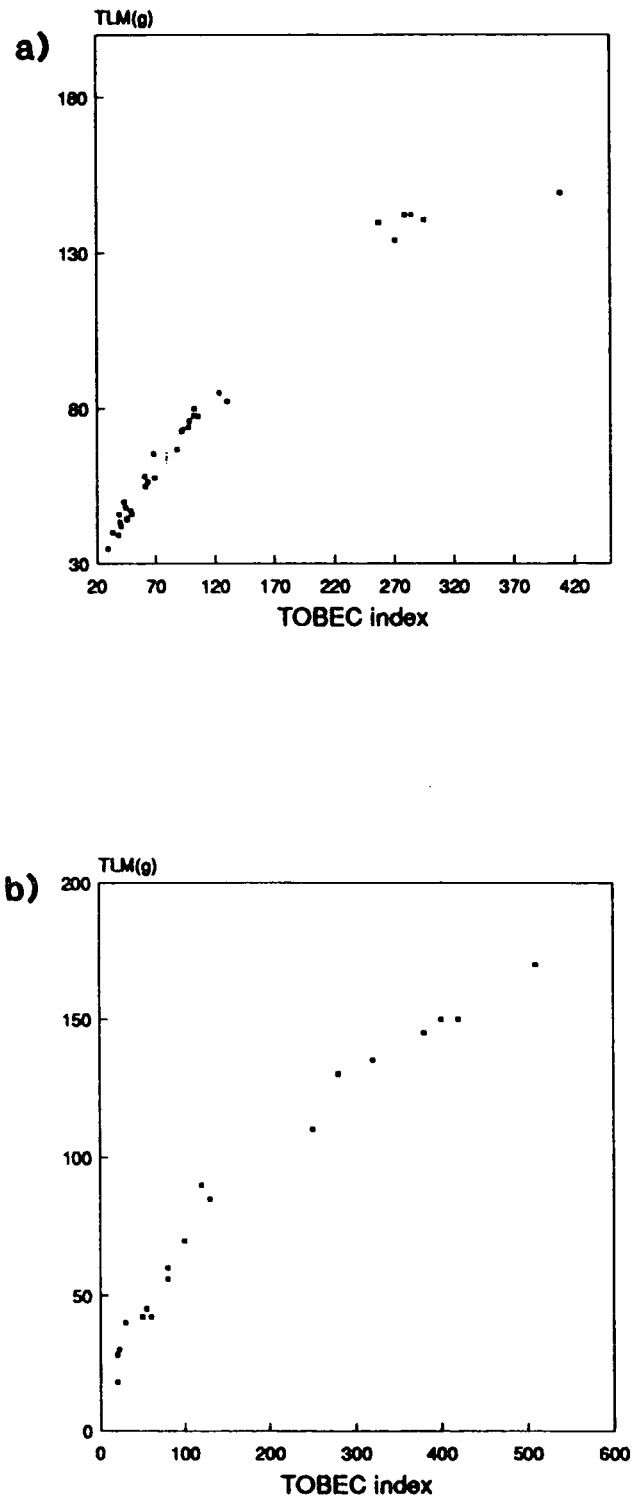


TABLE 2.2 Relationship between TLM and TOBEC Index for all species studied

	Equation parameter			Standard error				
	(a)	(b)	(c)	(a)	(b)	(c)		n
All species used in present study	M= 17.56	+0.67x	-0.0008x^2	1.46	0.02	0.00006	(r^2=0.99)	33
Walsbergs study(1988)	M= 15.70	+0.58x	-0.0006x^2	2.88	0.04	0.00008	(r^2=0.98)	25

Where M= TLM x =TOBEC index

TABLE 2.3 Accuracy of predictive equations for estimation of TLM of Starlings

Bird No.	Actual TLM(g)	Prediction using species-specific equation	% error	Prediction using species-specific equation	% error	Prediction using species-specific equation	% error
1	74.57	74.32	0.32	73.87	0.94	65.63	11
2	77.09	76.84	0.32	78.09	1.3	69.47	10
3	74.65	77.99	4.4	73.93	7.1	71.16	4.7
4	74.49	74.13	0.48	73.54	1.3	65.33	12
5	74.30	74.61	0.42	74.36	0.10	66.08	11

f) Accuracy of predictive equations

To date I have been able to test the accuracy of the descriptive equations(Table 1) for predictive,purposes only with a sample of 5 Starlings which were randomly selected from the original group of 15 caught. The TLM's (as determined by solvent extraction) and the predicted values for TLM given by the species-specific and two interspecific equations are shown in Table 2.3 The results indicate that the species-specific equation produces a much more accurate prediction of TLM than either the interspecific curve developed during the present study or that derived from Walsberg's (1988) data. Prediction from the species-specific equation produced a maximum error of 3.34g but a mean error of only 0.9g ie 1.2% of a body mass of about 75g.

g) Effects of salt water contamination on TOBEC index

The TOBEC indices of five dead Dunlin specimens increased by a mean of 30% (from 32.98 to 42.82) following immersion in sea water and even after 12 hours drying at room temperature were still 10% (significantly) higher (paired t-test $t=5.39$ $P<0.01$) than before immersion. Some of the Dunlin caught for the study of TOBEC index/TLM relationship (see Section 2.5c above) were thought to have been splashed with sea water before they were extracted from the net in which they were trapped. This may explain why the intra-specific relationship for the Dunlin Fig 2.4a showed more scatter than for species caught in dry

conditions i.e Redshank and Ringed Plover or away from salt water (Starlings).

h) Effect of the presence of metal identification rings

TOBEC indices were recorded on live birds before and after ringing. The mean increase was 13% for Dunlin (n=7) 40% for Redshank (n=8) and 45% for Turnstone (n=6). The ring size for both Turnstone and Redshank is significantly larger than that used for Dunlin and the alloy composition of the metal ring is believed to differ.

i) Measurements in the field

In addition to those birds sacrificed in order to establish calibrations I have recorded the TOBEC indices of 71 live shorebirds 32 Dunlin 6 Redshank 23 Ringed Plover and 10 Turnstone in the field between September 1989 and May 1990. From these TOBEC indices, I calculated predicted TLM's according to three equations (Table 2.4). The equation derived from Walsberg (1988) consistently gave lower predictions of TLM than either the interspecific or the species specific equations produced in this study (Table 2.4). Values for TLM which were predicted using equations which were derived from interspecific data are generally lower than would be predicted from previous work carried out by destructive analysis. The interspecific equation derived during present study predicts a higher level of variation in TLM between individuals than do the species specific equations. The coefficient of variation of the prediction of TLM

for each catch of birds for the interspecific and species-specific equations derived from this study are respectively 0.06 and 0.04 for Ringed Plover, 9.6 and 9.1 for Turnstone, 0.085 and 0.076 for Dunlin and 7.4 and 1.8 for Redshank. This indicating that those predictions based on interspecific equations are more sensitive to variation in the TOBEC index, Which may be a reason why predictions based on intraspecific calibrations were shown to be more accurate than those based on calibrations derived interspecifically (See section 2.5(f). Comparison between predictions of TLM from TOBEC indices and those figures obtained previously by destructive analysis are given in Table 2.4

Table 2.4 Mean predicted TLM and LI of adult birds caught in the field during the non breeding season.

Month	n	BM	PTLM(W)	PLI(W)	PTLM(SI)	PTLI(SI)	PTLM(SS)	PLI(SS)	LI from Davidson (1981)
Dunlin									
Sept	25	51	37.7	26	42.7	16	42.4	17	3
Oct	5	51	43.1	15	48.8	4	47.9	6	6
Nov	2	44	36.6	17	41.5	6	41.3	6	6-14
Redshank									
Oct	4	136	113.3	16	124	9	134.4	1	7
Feb	2	153	129.5	16	139	9	138.7	9	2
Turnstone									
Oct	3	99	82.7	16	92	7	-	-	NA
Dec	4	121	96.7	20	107	11	-	-	NA
Feb	3	123	92.9	24	103	16	-	-	NA
Ringed-Plover									
Sept	4	64	48.5	24	54	14	55.5	13	NA
Oct	1	63	52.6	16	60	6	58.4	7	NA
Nov	3	66	52.2	21	60	7	59.0	11	30(n=1)
May	4	61	46.5	23	53	13	54.1	11	22

Where **PTLM**:refers to predicted **TLM** PLI:refers to predicted LI
(W):indicates Walsberg's interspecific equation was used
(SI):indicates that the interspecific equation derived by the present study was used
(SS):indicates that the species-specific equation was used

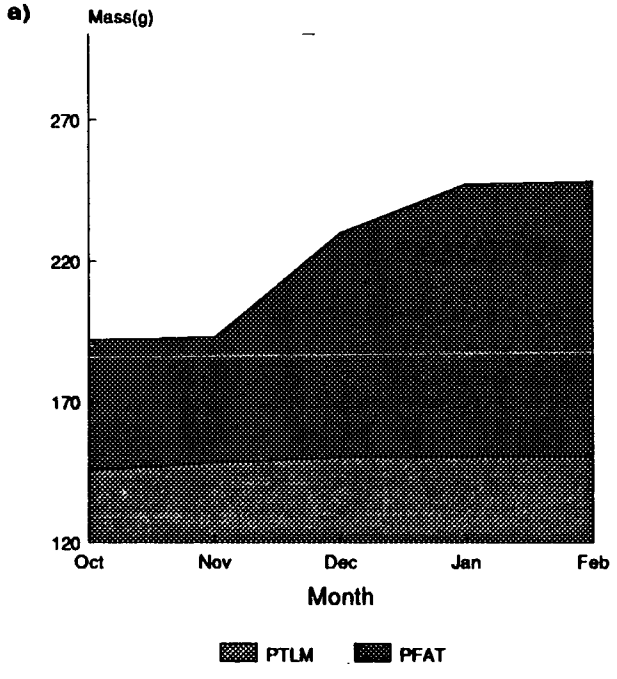
J) Measurements from captive birds

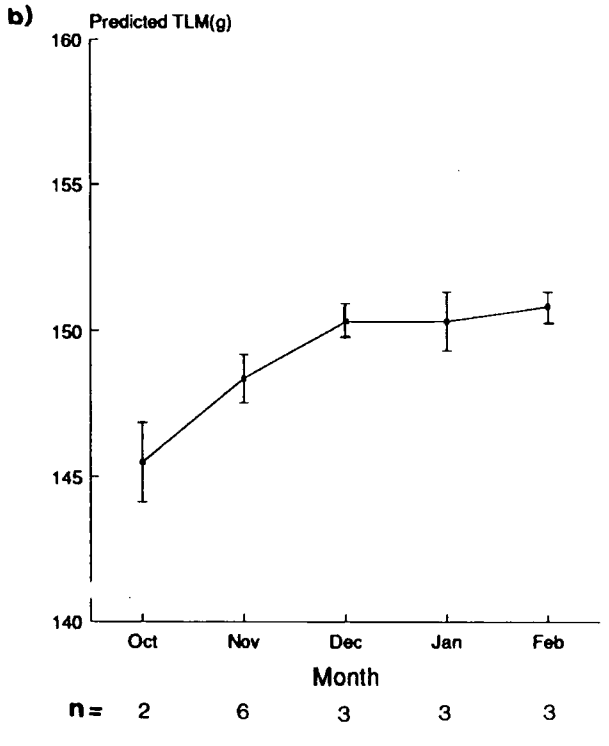
During the non-breeding season of 1989/90, 118 TOBEC measurements were taken from ten individual Redshank and one Grey Plover. Measurements were taken between 10am and 11am G.M.T. Predicted TLM has been derived for Redshank from the species-specific equation (Table 2.1) and for Grey Plover, from the interspecific equation derived by this study (Table 2.3). For both Grey Plover (Fig 2.5) and Redshank (Fig 2.6) monthly variation in predicted TLM was small. For Redshank the coefficient of variation of monthly predicted TLM was 0.017 but of predicted fat content was 0.54. Figures 2.7 and 2.8 show seasonal variation in predicted TLM and fat mass for those Redshank of the captive popoulation caught in Nov. 1989 (2.8) and those caught in Feb. 1990 (2.9). Predicted TLM of the Grey Plover was lower throughout the season than would be anticipated for a bird of it's size (based on wing and bill measurement). The overall Mean predicted lipid index was 34% with values as high as 39% for January. This may be a reflection of both muscular atrophy and obesity which may occur in captive birds.

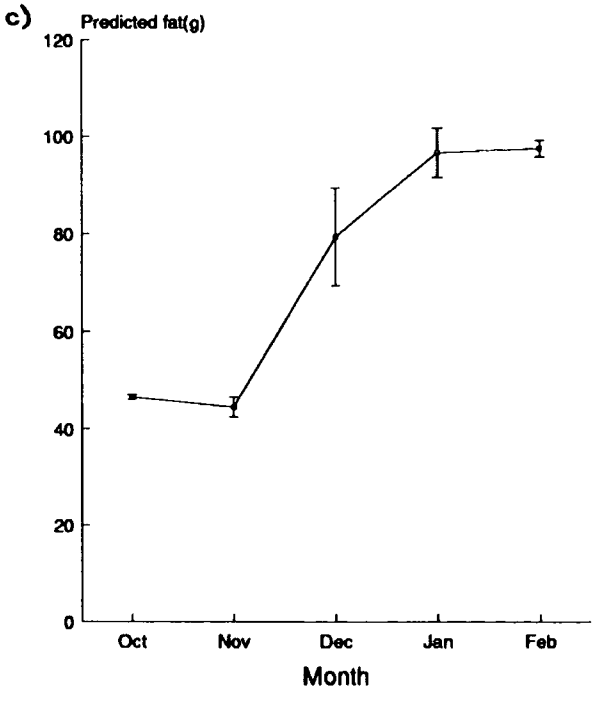
RESULTS: ULTRASOUND

Least squares regression analysis was used to compare the results of ultrasound estimates of muscle thickness with those determined by needle. (The latter were taken as the independent variable.) For the samples of Pigeon (n=5) and Knot (n=3),

Fig 2.5 Seasonal variation in body composition predicted using TOBEC of captive Grey Plover No.5. a) shows variation in predicted lean mass and fat mass in relation to total body mass. b) Variation in predicted total lean mass c) Variation in predicted fat mass. Horizontal bars represent 2x the SE. n= sample size.

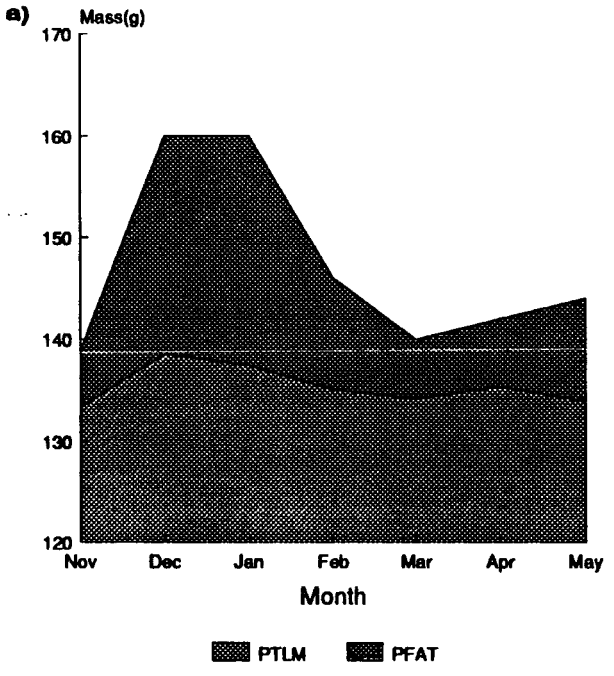


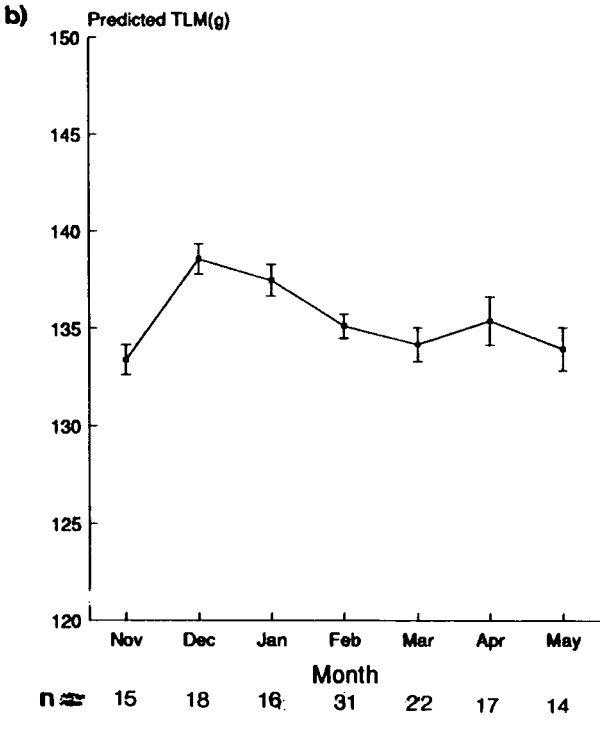


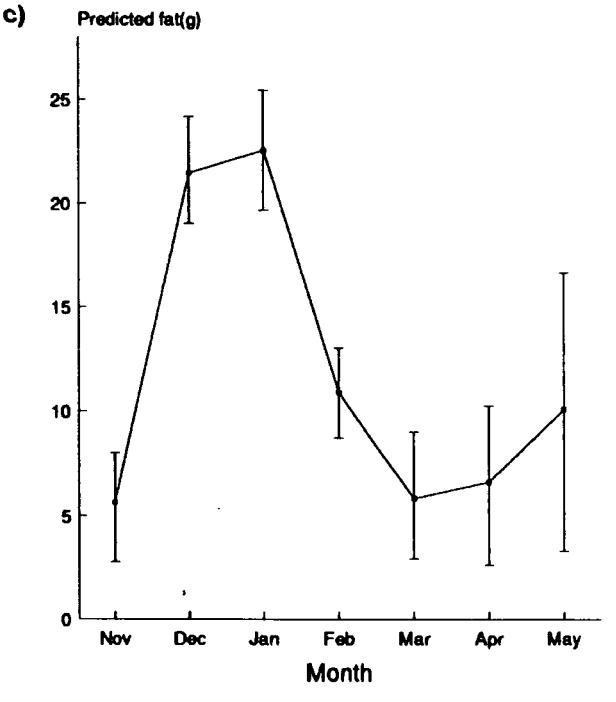


PTLM= Predicted total lean mass & **PFAT**= Predicted fat mass

Fig 2.6 Seasonal variation in body composition predicted using TOBEC of those Redshank held in captivity a) shows variation in predicted lean mass and fat mass in relation to total body mass. b) Variation in predicted total lean mass c) Variation in predicted fat mass. Horizontal bars represent 2x the SE. n= sample size.

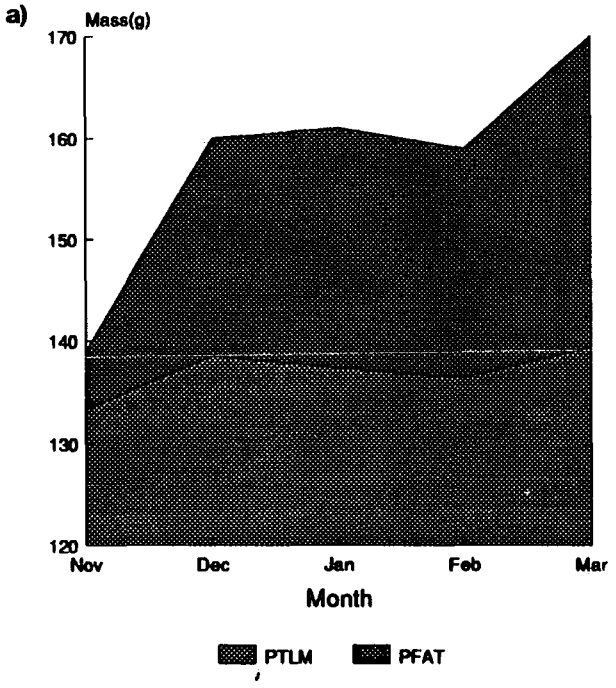


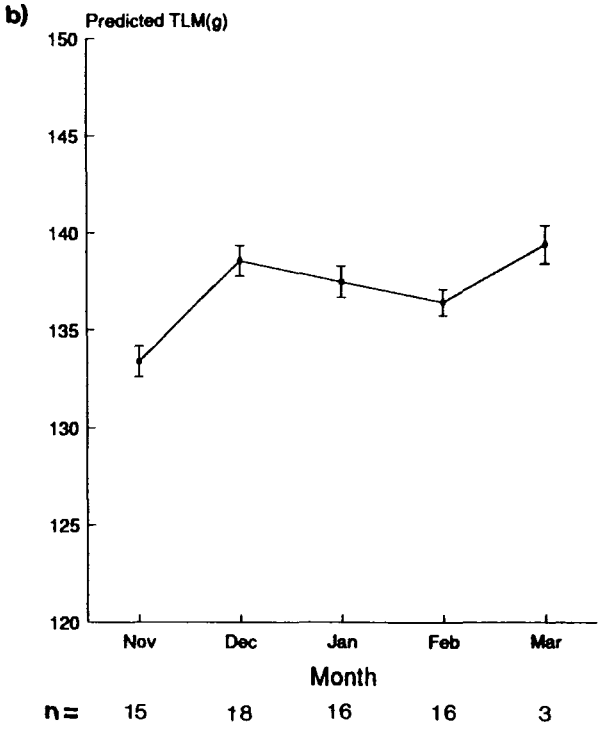


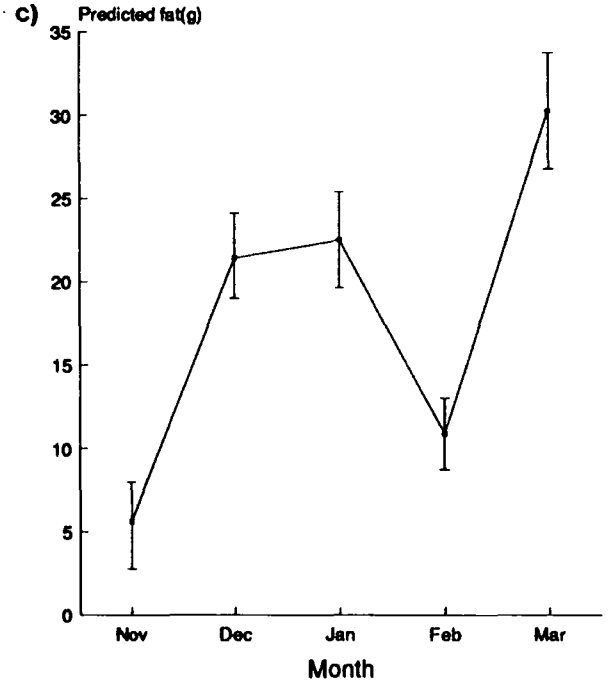


PTLM= Predicted total lean mass & **PFAT**= Predicted fat mass

Fig 2.7 Seasonal variation in body composition predicted using TOBEC of those Redshank held in captivity which were caught during November 1989 a) shows variation in predicted lean mass and fat mass in relation to total body mass. b) Variation in predicted total lean mass c) Variation in predicted fat mass. Horizontal bars represent 2x the SE. n= sample size.

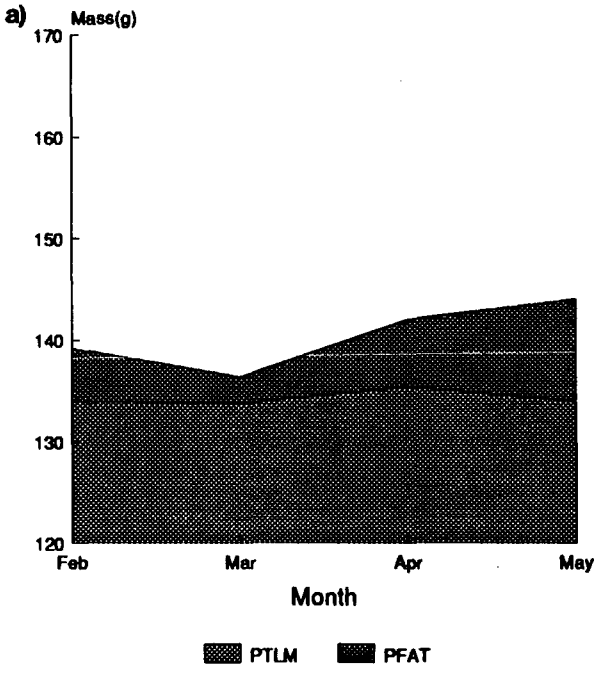


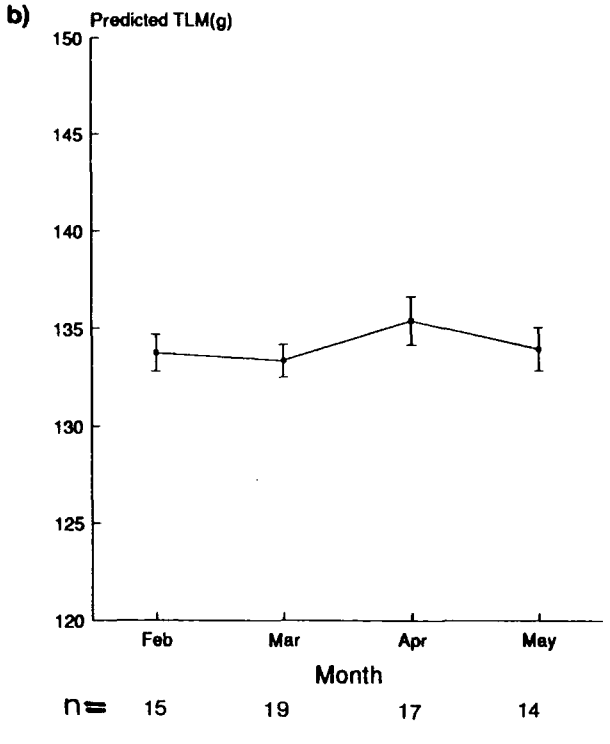


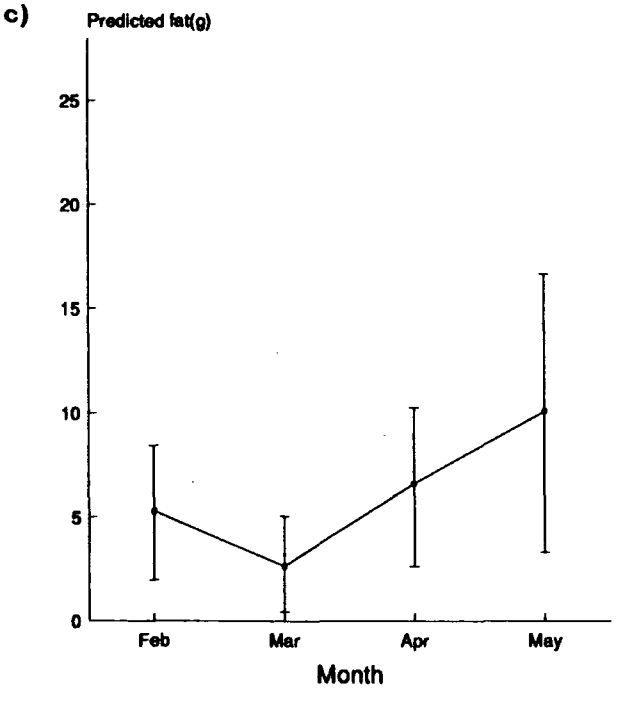


PTLM= Predicted total lean mass & **PFAT**= Predicted fat mass

Fig 2.8 Seasonal variation in body composition predicted using TOBEC of those Redshank held in captivity which were caught during February 1990. a) shows variation in predicted lean mass and fat mass in relation to total body mass. b) Variation in predicted total lean mass c) Variation in predicted fat mass. Horizontal bars represent 2x the SE. n= sample size.







PTLM= Predicted total lean mass & **PFAT**= Predicted fat mass

measurements taken with the probes positioned towards the centre of the pectoral muscle were more accurate than those taken towards the sides. For the Pigeon the optimum test area was along a strip of muscle 2cm below the ventral edge of the keel (r=0.95, n=28, Y=X.0.93+1.25) For Knot the optimum position was similar but only 1cm down below the edge of the keel (r=0.93, n=9, Y=X.1.07-0.31). These analyses do not take into account the limits of accuracy claimed by the manufacturer ie ±1mm. When this limit is taken into account 32 out of 37 estimates lay within this limit, the other 5 were outside by no more than 1mm. For Dunlin only two measurements were made for each bird (n=3), The size of the probe in relation to the surface area of the pectoral muscle does not allow for many measuring positions. Nevertheless, all measurements taken by ultrasound were within the limits of accuracy claimed by the manufacturer.

DISCUSSION AND CONCLUSION: THE VALUE OF MEASUREMENTS FOR PREDICTION OF TLM BY TOBEC

The results confirm that the TOBEC index of a bird is related to its TLM. The best description of this relationship is curvilinear when calculated interspecifically but linear within a species for the four species examined. As the slope of the relationship for prediction of TLM from TOBEC index decreases with increasing TLM, prediction of TLM's of larger birds will be less affected by errors in the measurement of the TOBEC index than those of smaller birds. Unfortunately the slope of the TLM/TOBEC relationship index is not predicted by the tangent to the interspecific curve at the mean TLM of a species. This suggests

that calibration lines may need to be established empirically for each species studied, if accurate estimations of TLM are to be obtained. As shown in Table 2.3 for Starlings, the species-specific equation is a better predictor of TLM than interspecifically derived equations. Castro *et al*, (1990) have claimed that over a restricted range of body masses (between 18 and 90g) TLM can be predicted from the tobec index by using a linear interspecific relationship (rather than the polynomial relationship established by Walsberg, (1988)). They infer that the relationship they have established is also true intraspecifically. Two points arise from this: 1) As with other studies (eg Bracco *et al*, 1983 and Fiorotto *et al*, 1987) the equations derived by Castro *et al* (1990), are merely descriptive. The predictive boundaries of such equations can be determined only by testing their predictions against known of body compositions of animals not used to produce the descriptive equations; (for further discussion of this problem see Perdeck 1985); 2) The high coefficient of determination associated with the descriptive equation of Castro *et al*, (1990) is a result of the large range of TLM's included, a far wider range than found normally within any one species. Examination of their Figure 1 indicates that for any one species their interspecific linear equation would be a poor fit.

In this study I have used the TOBEC index as the independent variable in order to develop predictive equations for TLM. This is contrary to Walsberg, (1988) and Castro *et al*, (1990) but follows Bracco *et al*, (1983) and Fiorotto *et al*,(1987). I

have used TOBEC index as the independent variable because it can be measured with high reproducibility in the SA-1 machine if proper attention is paid to the positioning of the bird in the chamber. Also the same TOBEC index can, be obtained from birds with different TLM's for good biological reasons, eg. variation in the proportions of skeletal mass, muscle mass, and water in the body or variation in body temperature. Under field conditions when predicting TLM the TOBEC index is used as if it is the independent variable.

Those data gathered from animals held in long term captivity and those caught and released indicate that for animals with TLM's beyond the bounds of the predictive equations, eg Grey plover, these equations under-predict TLM. In addition the species specific equation derived for Redshank does not apply throughout the whole TLM range of this species. The intercept term for this equation is 119g (Table 2.1) which is higher than the total body mass of some Redshank recorded in this study (eg Redshank No.7 see Chap. 1). I suggest that the high intercept term for the Redshank species specific equation is due to one point (marked z on fig 2.4) that has a higher TOBEC index value than would be expected for it's TLM. This would also lead to low sensitivity in this equation. Nevertheless the accuracy of the species-specific equation for Starlings, tested with only 5 birds so far (Table 3) allows prediction of TLM with 95% confidence to within 2.5g for a TLM of about 80g. The equation was established without incorporation of body temperature or state of hydration, both of which affect the TOBEC index (Walsberg, 1988; and this study). Measurement of deep body temperature would allow correction of

the TOBEC index, eg to a standard 40°C value and provide improved predictive accuracy. This could be particularly important during moult, when increases in deep body temperature and body water content are known to occur.(see eg Newton 1968). I suggest that the lack of effect of the presence of metal bands (rings) on the TOBEC index of birds, claimed by Castro *et al*, (1990) was fortuitous and due to the use of small rings of low magnetic susceptibility metal (probably aluminium as are commonly used in the U.S.A.) My results indicate that larger rings affect the TOBEC index more than smaller rings and that the proportion of ferromagnetic materials (eg Nickel) in the alloy may also probably be very important.(I have found that lead shot has only a small effect on the TOBEC index measured in the SA-1 machine.) It is unlikely that it will be possible to calibrate for the presence of metal bands in a satisfactory manner, as both position of the metal ring within the chamber, and ring ware will need to be taken into account.

The results indicate that the SA-1 apparatus will be useful in investigating seasonal changes in body composition of birds, particularly those which show large changes in fat deposits associated with migration or in winter, such as shorebirds. Whilst predictions of TLM may not be suitably precise over a wide range of sizes it is apparent that relative changes in TLM will be reflected in changes in the TOBEC index. More exploratory work is still required, particularly with regard to quantification of the effect of hydration state on the TOBEC index and of any differences in index which may occur between different sets of apparatus. This would affect the transferability of data and of

calibration equations between researchers. The manufacturers state that all SA-2 systems (a slightly up-dated version of the SA-1) have been standardized to match the response of the same reference instrument at the site of manufacture so theoretically data may be pooled.

CONCLUSION AND DISCUSSION: ULTRASOUND

The results shown above for the Scanco-ultrasound probe are promising. However, due to the level of accuracy stated by the manufacturer at least 2mm change in muscle thickness is required before such a change can be measured with confidence. I have calculated using the formula for standard muscle volume given in chapter 1 that this would require approximately a 1.5g increase in pectoral muscle mass for a bird the size of a Sanderling. Given that the pectoral muscles make up 15% of the TLM for a Sanderling of mass 50g this increase in pectoral muscle mass would require an increase in TLM of over 18g which would be exceptional, although this assumes that change in pectoral muscle mass is associated with similar change in TLM . Whilst this may be overly presumptive, it is apparent that even to detect a change in muscle thickness at the lowest level of resolution of the apparatus would require a substantial change in muscle mass. Thus the Scanco-probe is more suited to investigation of muscle mass changes in large birds and it was decided not to use this apparatus further in this study. It is likely that ultrasound probing will prove useful in ecological studies given that a

suitable probe and operating frequency can be found for the subject being studied.

CHAPTER 3

COMPARISON OF THE METABOLIC OUTPUT OF AVIAN ADIPOSE TISSUE LIVER AND SKELETAL MUSCLE

3.1: INTRODUCTION

The contribution that adipose tissues make to the overall BMR of an animal is frequently considered to be insignificant (Blaxter, 1989). In the 18th century John Hunter described it as 'not animal substance' as the animal is the same with or without it (Owen, 1868). This has led to the suggested use of 'lean mass' rather than fresh mass as a predictor of BMR by some authors (Pullar and Webster, 1977; Toutain *et al.* 1977; Tuite, 1984 and Wood, 1984). However contrasting views occur (Blaxter *et al*, 1982; McCraken and McNiven, 1983 and McNiven, 1984), suggesting that, at least in domesticated mammals increase in the fat reserves carried does add significantly to the overall energy demands of the animal.

Three principal types of adipose tissue are recognized, these are structural metabolically inactive deposits, highly active brown adipose tissue (BAT) and storage white adipose tissue (WAT) (Pond and Mattocks, 1985 and Trayhurn and Nicholls, 1986). Changes in overall BMR may be associated with increases in fat mass may be associated in mammals with the amount and activity of brown adipose tissue deposits. BAT is associated with thermogenesis (for reviews see Himms-Hagen, 1983 and Trayhurn and Nicholls, 1986), and is implicated in energy balance regulation

(Rothwell and Stock, 1979 and Andrews, 1989). Such changes in BAT may also account for the observed seasonal variation in BMR reported for some mammals. For example, during pre-hibernatory fattening in Richardson's ground squirrel *Spermophillus richardsonii* BAT mass is increased. The BAT is subsequently used to generate heat during arousal from hibernation (Andrews 1989).

For birds however, no conclusive evidence that BAT occurs has been found. Some histological evidence suggesting the occurrence of BAT has been presented for a few avian species (Oliphant, 1983; Barre *et al*, 1986 and Andrews, 1989). However in a detailed investigation Saarela *et al*, (1988) could not find any appreciable difference between avian adipose tissue (taken from the furcular and abdominal area) and the white adipose tissue (WAT) of mammals, except that the avian tissue contained a higher proportion of multiocular fat cells. It is generally accepted that adipose tissue can be classified as BAT only if an agent capable of causing the uncoupling of the mitochondrial proton conductance pathway from phosphorylation, can be identified (Blaxter, 1989 and Andrews 1989). For mammals this agent has been shown to be a 32,000M protein (Nicholls and Lockie, 1984). The presence of an uncoupling agent has not been demonstrated in avian adipose tissue.

Avian adipose tissue (AAT) is typically widely distributed within the body, although several distinct sites with large accumulations of fat occur in most species. It is also well vascularised (Pond and Mattocks, 1985 and Sarrela *et al*. 1989). AAT is formed of numerous adipocytes which range between 50-150μm

in diameter. The adipocyte is typically occupied by a large triglyceride droplet which displaces all other cellular elements to the periphery of the cell. When isolated most avian adipocytes are spherical though some may be multiocular (see above).

Growth of the adipose tissue occurs via either cell multiplication (hyperplasia) or by increase in the amount of lipid stored by individual adipocytes (hyperphagia). The latter is presumably to be less of an energetic burden than the former as the overall increase in cell organelles is less. It is widely believed that in adult birds, all increase in adipose tissue result from hyperphagia (Blem, 1976; Hood, 1982 and Leclerq, 1984). However the results of Pond and Mattocks (1985), who observed that fatter birds of the same species (or of related species with similar dietary habits) generally had more adipocytes in total than less fat individuals, are not consistent with hypertrophy accounting for all AAT growth in adult birds.

From the above, it can be seen that there remains a considerable amount of uncertainty concerning the overall physiology of avian adipose tissue. This leads to an inability to predict the contribution that variation in fat stores carried has to the overall metabolic rate of a bird.

The aim of the work presented in this chapter was to establish the relative metabolic activity of AAT when compared to skeletal muscle and liver tissue *in vitro* and thus predict the impact of body composition variation on BMR. These tissues were choosen for comparison because, in mammals, it has been shown that liver is

one of the most metabolically active tissues on a gram specific basis, whilst skeletal muscle appears to contribute most to the overall metabolic rate (Krebs, 1950). Metabolic activity was measured using tissue slices and a polarographic oxygen sensor.

3.2: SPECIALIST EQUIPMENT: POLAROGRAPHIC OXYGEN SENSOR

The oxygen uptake of tissue slices was determined using a Clark type polarographic oxygen sensor to measure dissolved oxygen pressure in aqueous solutions. The model used was Yellow Springs Instrument model 53. The apparatus is based on the principle that, when a polarizing current is passed across an electrolytic cell, oxygen will react with the cathode causing current to flow through the cell. The amount of current produced is proportional to the amount of available oxygen at the cathode. The YSI model 53 uses a platinum cathode, a silver anode and KCL as the electrolyte, which are incorporated into a sensor unit. The sample is separated from the sensor by a teflon membrane which allows the diffusion of oxygen through to the sensor. In practice oxygen is rapidly consumed at the cathode and it can be assumed that the oxygen pressure inside the membrane (at the sensor tip) is zero. The force causing oxygen to diffuse through the membrane (from the sample) is proportional to the absolute pressure outside the membrane. If the oxygen pressure decreases less oxygen passes through the membrane and *vice-versa*. The oxygen diffusion rate across the membrane is directly proportional to the oxygen pressure and the oxygen cell current relationship is stoichiometric, thus a linear relationship exits between external oxygen pressure and the cell current. Thus by monitoring the size

of the current generated by the cell the concentration of oxygen in solution can be followed. The apparatus output is given via a potentiometric recorder (0-10mV) calibrated to read between 0-100% oxygen saturation. This reading was converted into an actual amount of oxygen from the solubility of oxygen in the solution used at one atm. and 40°C of 0.0195ml O_2/ml. This figure is derived from that given by Davison (1970) for 37°C and converted to a value for 40°C.

3.3: MATERIALS AND METHOD

Tissue slices were taken from a sample of five Starlings and two Dunlin caught during the winter of 1988/89. The animals were held in captivity for a short period and then killed by cervical dislocation. Following death the liver, pectoralis muscle and adipose tissue from the clavicular region were dissected out and the tissues placed in ice-cold ringers solution ((in mM) Na^+, 125; K^+, 5; Mg^{++}, 1; Ca^{++}, 1; Cl^-, 129; buffered with 10mM Hepes to pH 7.4) and put on ice.

Thin slices of each tissue were prepared using an ice cooled stadie-riggs hand microtome following the method of Wheeler (1984). The microtome blade was carefully drawn through the tissue with steady pressure avoiding any shearing action that would rupture or damage cells. Slices were trimmed to a maximum of $5mm^2$ with a cooled razor blade. The tissues were held in chilled incubation medium (Ringers solution as above plus 10mM and 5mM glucose and sodium pyruvate respectively as substrate). Before being placed in the measurement chamber of the apparatus,

the slices were removed from the storage medium, blotted between filter paper and weighed to the nearest mg. Prior to each determination the incubation medium in the chamber (as above) was saturated with 100% oxygen. The tissue sample was placed in the chamber and after an equilibration period of 5mins the electrode was placed into the chamber. The volume of the sample chamber was 5ml for each experiment. During the experimental run oxygen uptake by the test tissue was not replaced, giving a fall in oxygen concentration. Oxygen uptake was recorded for each tissue for between two and ten minutes. For each determination a control chamber was also run, filled only with incubation medium. This allowed the residual fall in oxygen concentration in the chamber due to consumption by the electrode to be taken into account. The entire procedure, from death of the subject to a complete set of determinations for each of the three chosen tissues was carried out within 30mins.

The order in which determinations were made from each of the tissue types from each bird was rotated in order to avoid introducing systematic bias into the results. All apparatus and solutions used were autoclaved prior to use, to avoid the artificial elevation of oxygen uptake due to bacterial growth.

3.4: RESULTS AND DISCUSSION

The rate of oxygen consumption by the three tissues examined is given in Tables 3.1 a & b and Figure 3.1. The results are expressed per gram fresh mass so that they may be compared easily to the *in vivo* situation. The rate of oxygen consumption by adipose tissue was approximately 10% that of skeletal muscle and 2% of liver. Two of the Starlings killed showed no appreciable development of adipose tissue. The ratio of oxygen uptake rate between liver and skeletal muscle shown here (Table 3.1b), i.e 4:1 is similar to that reported by Wheeler, (1984) for small mammals although higher than that reported for these tissues by Field *et al*, (1939) for the laboratory rat *Rattus norvegicus*. The Mean uptake rate however of both liver and muscle is higher than that reported for slices of these tissues taken from *Mus musculus, Rattus norvegicus* and *Mesocricetus auratus* by Wheeler (1984). This may reflect the higher overall metabolic rate observed in birds when compared with mammals. HSD tests based on one way ANOVA of oxygen consumption rate with tissue type (Table 2) indicate that the difference in uptake rate between the tissues is highly significant.

Table 3.1a Oxygen uptake from avian skeletal muscle,liver and adipose tissue.

Species:	Dunlin		Starling				
Bird ID	A	B	C	D	E	F	G
Tissue	Oxygen uptake ml/O_2/g/h						
Adipose	0.08	0.04			0.05	0.06	0.06
Liver	2.95	2.74	2.56	2.96	2.54	2.74	2.52
Skeletal Ms.	0.60	0.70	0.51	0.64	0.54	0.85	0.75

Table 3.1b: Mean Oxygen uptake from tissues of Dunlin and Starling

Species	Dunlin	Starling	Both species together
Tissue	Oxygen uptake $ml/O_2/g/hr$		
Adipose	0.06	0.06	0.06
Liver	2.84	2.66	2.71
Skeletal Ms.	0.65	0.66	0.66

Variation in the oxygen uptake rate between individuals may reflect a slight difference in tissue slice thickness, (which effects the oxygen diffusion rate), or inherent individual variation in tissue respiration rate.

Table 3.2: ANOVA Of rate of oxygen uptake with tissue type as an independant variable.

Source of variation	Sums OF squares	df	Mean Squares	F	Signif of F
Tissue type	24.59	2	12.29	662	P<0.001
error	0.297	16	0.185		
Total	24.88	18			

The oxygen uptake rate of tissue slices has been shown to scale negatively with body mass by some authors (Klieber, 1941; Krebs, 1950; Girard and Grima 1980). No evidence of this phenomenon was found during the study presented here for any of the tissues

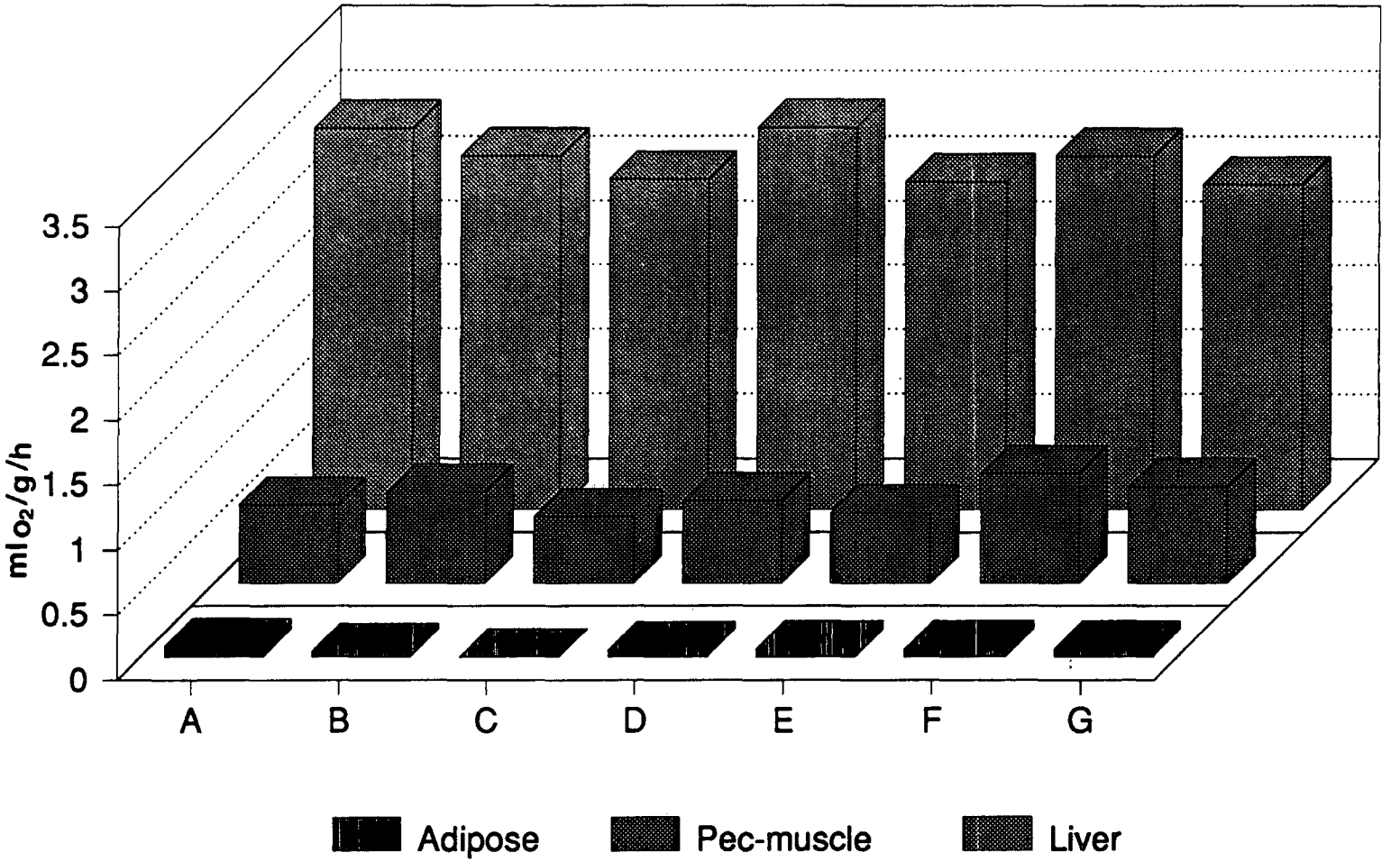


Fig 3.1 Oxygen uptake of avian adipose, muscle and liver tissues *in vitro*. Letters refer to individual birds. Species are, A-B Dunlin C-G Starling.

studied. The difference between the O_2 uptake rate of a particular tissue for Dunlin (mean total body mass=55g) and Starling (mean total body mass=77g) was not statistically significant. A mass effect may be occluded because, as discussed in the (General introduction) the mass specific BMR of passerines is considered to be higher than that of non-passerines. Thus tissue slices from a passerine should be expected to have a higher respiration rate than that of a similarly sized non-passerine.

The rate of oxygen uptake obtained for adipose tissue indicates that any increase in BMR associated with fat deposition within an individual would be lower than that predicted by interspecific allometric equations. This is assuming that all the extra cost of carrying extra fat is derived from the respiration of the adipocytes alone. Using the interspecific equation of Kendeigh *et al*, (1977) for non-passerines, the BMR of a hypothetical 100g bird which lays down an additional 30g of fat, would increase by 0.16W. From the figures of oxygen uptake from tissue slices, however, the predicted increase would be 0.01W. It is obvious therefore that predictive BMR/mass equations must take into account changes in body composition.

For the calculations above it has been assumed that tissue slices *in vitro* respire at a basal level. In studies where the oxygen uptake rates of tissue slices of all major tissue types in the subject have been summated in order to produce an overall metabolic rate, virtually no assessments add up to give total measured oxygen consumption of the whole animal *in vivo* (Grande,

1980). The result of 105% of BMR for the dog, *Canis domesticus* is the closest to date (Martin and Fuhrman 1955), although the same study produced a result of 72% of BMR for Laboratory mice. Whilst Blaxter (1989) maintains that the errors produced in such calculations are probably due to experimental technique, this view is difficult to quantify. It is certain that tissue is damaged during the preparatory techniques, giving rise to errors, but the results obtained from *in vitro* studies of tissue respiration do allow us to conclude that tissue slices give a reasonable approximation of the respiration rate of intact organs. Future studies of tissue respiration would be greatly aided by the use of non-invasive techniques such as NMR spectroscopy which has the obvious advantage of leaving both animal and organ intact. The results of the study presented here, show that the oxygen uptake rate of Avian adipose tissue, whilst low, is not zero as is implied by some authors and thus must be taken into account studies which aim to examine the overall BMR of birds.

CHAPTER 4

THE INTRASPECIFIC AND WITHIN-INDIVIDUAL ALLOMETRY OF BASAL METABOLIC RATE AND BODY MASS IN BIRDS

4.1 INTRODUCTION

The aim of the work presented in this chapter was to establish the intraspecific relationship between BMR and body mass for a number of species of birds (concentrating on *Charadrii*) and to examine the BMR/mass relationship within individuals which undergo seasonal changes in body mass. To facilitate comparison, the allometric relationship between BMR and body mass has been determined interspecifically using data drawn from both the work presented in this chapter and from the literature.

The theoretical explanations put forward to explain such allometric relationships, which will be tested in this chapter, have been discussed at length in the General Introduction. Where possible those data gathered for body composition using TOBEC (Chapter 2) have been incorporated into the work presented below. This has allowed predictions to be formulated concerning the role of body composition in determining the BMR/mass relationship. The original hypothesis under test is that the mass exponent of BMR within an individual should be less than the value for the exponent for the data viewed interspecifically or intraspecifically. This is because as discussed previously, within an individual bird, most of the variation in body mass can

be attributed to variation in mass of fat and, as shown in Chapter 3, it is highly probable that the energy output per gram of adipose tissue is much lower than that of lean tissue.

The study presented in this chapter concentrates on 3 shorebird species (Redshank; Grey plover; Sanderling), and one species of wildfowl, Wigeon. BMR was determined using indirect calorimetry. Oxygen consumption was measured (using a paramagnetic oxygen analyser) and CO_2 production (using an infared gas analyser) in an open circuit system.

4.2 SPECIALIST EQUIPMENT

4.2a Paramagnetic oxygen analyser

The oxygen analyser used was model OA/272 supplied by Taylor Servomex Limited Crowborough, Sussex. It operates on the principle that oxygen can be distinguished from most other gases by virtue of its paramagnetism. The OA/272 relies on the linear relationship between concentration of O_2 and degree of displacement displayed by a body of oxygen when forced to pass through a magnetic field.

4.2b Infrared gas analyser

The CO_2 analyser used was Lira model 3000 supplied by the Mine Safety Appliances Company, Pennsylvania, U.S.A. The apparatus operates on the principle that molecules which contain more than one type of atom can selectively absorb infrared energy. Each

compound absorbs infared energy at a unique frequency. The Lira model 3000 detects the amount of energy absorbed at the frequency which is unique to CO_2. Thus the apparatus is essentially an absorption spectrophotometer. The extent of the absorption is directly proportional to the concentration of the gas present.

4.3 ANIMALS

The shorebirds used were the same individuals as described in section 1.3 of chapter 1. The Wigeon used were borrowed from the Wildfowl Trust centre at Washington on the day of BMR determination, The birds kept by the centre are pinioned and kept in large enclosurers in wildfowl gardens. They are fed principally on grain.

4.4 MATERIALS AND METHODS

The metabolic heat production was measured by determination of oxygen consumption and CO_2 production in an open circuit system (See Fig 4.1). Birds were placed in a metabolic chamber measuring 16cm x 36cm x 32cm for Wigeon and Grey Plover; 24.5cm x 21cm diameter for Redshank and 16cm x 15.5 diameter for Sanderling. The metabolic chamber was then in turn, placed inside an environmental cabinet, ensuring that all measurements were made in complete darkness and at a constant temperature. Dry air was drawn through the sealed chamber at a rate of 240 $l.h^{-1}$ for Wigeon, 210 $l.h^{-1}$ for Grey Plover, 150 $l.h^{-1}$ for Redshank and 120 $l.h^{-1}$ for Sanderling. Gas analyses were performed on samples

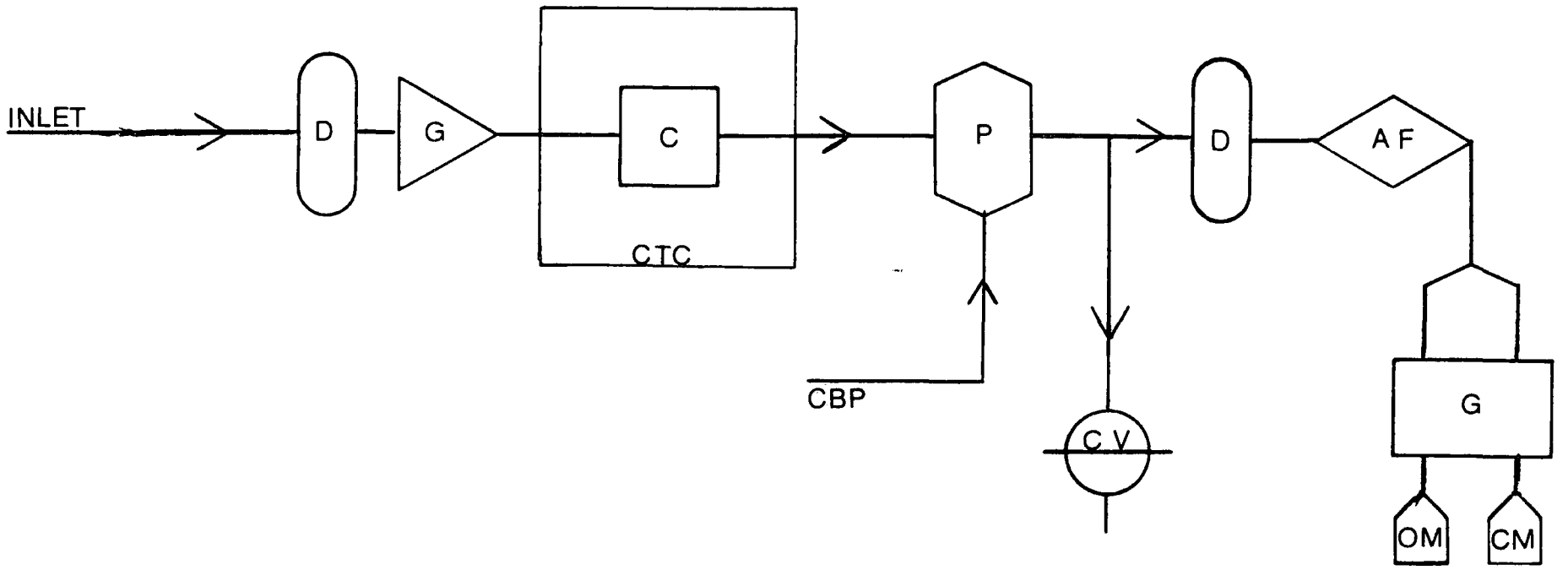


Fig 4.1 Schematic diagram representing the apparatus used to measure the metabolic rate of birds during the course of this study. **D**= air drier **G**= gas flow meter **C**= measurement chamber **P**= pump **CTC** = constant temperature cabinet **CBP**= chamber by -pass **CV**= control valve **AF**= air filter **OM**= oxygen meter **CM**= carbon dioxide meter. **>** indicates direction of air flow.

taken from the inlet and outlet gases via gas mass flow controllers (9 $l.h^{-1}$ for O_2 and 60 $l.h^{-1}$ for CO_2). Both inlet and outlet gases were dried prior to measurement, by passing them over columns of dried coarse mesh silica gel. Gases were also cleaned prior to analysis, in order to prevent damage to the apparatus, by passing them through two five micron membrane filters.

The accuracy of the measurements according to the manufacturers specifications was 0.01% for CO_2 and 0.05% for O_2. Calibration was performed prior to each experiment using dry, oil-free gas mixtures of 100% N_2; 100% O_2; 95% O_2 5% CO_2 mix; and a certificated air mixture of 21% O_2, 0.03% CO_2 in N_2, supplied by S.I.P Analytical LTD.

The oxygen and carbon dioxide concentrations of the outflow gases were monitored continuously except for a 10 minute interruption each hour, during which inlet air was led directly through the gas analysers, by-passing the metabolic chamber. The output from the analysers was registered on a potentiometric chart recorder.

For a BMR determination, the bird under investigation was removed from its cage between 09.30 and 10.00 GMT. It was weighed (to the nearest gram) and, when the apparatus became available, a TOBEC measurement was recorded. The bird was then placed in the appropriate metabolic chamber and measurement was commenced at 14.00 GMT, after a period of at least 3 hours of acclimatization to the metabolic chamber environment and 4 hours of fasting. Conditions were said to be basal only when the RQ had fallen to

0.70 ± 0.04. When measurements were taken at night, the timing of experiments was adjusted so that the whole of the measurement period occurred during the hours of darkness, although the period of acclimation and fasting were the same as stated above. The measurement period lasted for 4 hours. In practice, determinations were made from two birds during a each four hour measurement period. On completion of the determination the bird was re-weighed and returned to its cage. The mass used in subsequent analyses of the BMR/mass relationship is the mean value of the mass prior to and after determinations of BMR.

If during the course of the experiment there was a drift in the measured baseline inlet oxygen concentration of more than 2.5% of full scale, the experiment was abandoned. Drift in the concentration of CO_2 was generally negligible. Results have been used in subsequent analyses only When the mean RQ is equal to 0.70 ± 0.04 as this was deemed to be a good indicator of birds in a post-absorptive state (Blaxter, 1989), ie 0.70 is the RQ value associated with the catabolism of fats (Schmidt-Nielsen, 1985). The error in RQ permitted was that which would be caused by a simultaneous maximum error in both CO_2 and O_2 gas analysers. All gas volumes were converted to standard temperature and pressure (273K at 1 atms). Mean RQ values were $0.72 (\pm 0.05$ SE) for Grey Plover; $0.71 (\pm 0.002$ SE) for Redshank and $0.72 (\pm 0.006$ SE) for Sanderling. A conversion factor of 20.1 $KJ.l^{-1}O_2$, the energy equivalent of the combustion of fat in 1 litre of oxygen, was used throughout the study.

Values recorded for BMR are not the mean rates throughout the test period. During the course of a determination, short periods of elevated BMR sometimes occured. These were assumed to represent bouts of activity and were typically followed by metabolic rate falling to a minimum level. When such a level had lasted for more than 10mins the metabolic rate was said to be basal. The values quoted represent the average of these basal levels.

As this study was primarily concerned with measurements of BMR, the temperature of the environmental chamber was held within the thermal neutral zone (TNZ) of each bird species. The thermal neutral zones for the shorebird species studied were determined in sets of preliminary experiments, in which the environmental chamber temperature was increased progressively from 0°C until the thermal neutral zone was established (ie BMR did not decline with further increases in temperature). The lower critical temperature was found to be 17°C, 18°C and 7°C for Grey Plover Redshank and Grey Plover respectively. The upper critical temperature was not found for any of the species studied. (The maximum test temperature was used was 40°C due to licence restrictions) For the shorebirds studied, the temperature used during determinations was 25°C. For Wigeon, determinations were made at 20°C on the assumption that this value is within the TNZ based on data given by Kendeigh *et al*, (1977).

4.5 STATISTICAL ANALYSIS

Historically, least-squares regression (LSR) has been the favoured method in biology for calculating so called " lines of best fit." This stems from several reasons including ease of calculation, familiarity and availability of computer packages with LSR facilities. Unfortunately this has led to LSR being applied erroneously in a number of cases. LSR should be used only where one of the variables (x axis) is measured without error or can be measured in such a way that the error can be accounted for (Mandel, 1964). Error is also taken to be that resulting from a lack of dependence i.e. a third interlinking variable is involved such that variation in the x variable does not directly cause effects along the y axis (Harvey and Mace, 1982). LSR should not be employed to examine the relationship between two continuous variables which are distributed according to the bivariate normal distribution (Sokal and Rohlf, 1981). This is because LSR adjust the position of the line to take into account sampling error on the y axis only.

In practice errors in application of LSR analysis become most important when functional significance is being inferred about the linear relationship between two variables, as is frequently the case when examining the BMR/mass relationship. As well as LSR, other methods are available for calculating the slope of the relationship between such variables, reduced major axis (RMA) or geometric regression being one such technique. RMA assumes that the error variance is the same proportion of the total variance on each axis (Clutton-Brock and Harvey, 1984). With respect to

the BMR/mass relationship it is possible in practice to say that, in terms of physiology only, it is much more probable that body size will dictate BMR than the converse and that errors associated with the measurement of BMR will be greater than those associated with the measurement of body mass (Calder, 1984). It is likely therefore that the functional slope of the BMR/mass relationship (if there is one), lies somewhere between that derived by LSR and that by RMA. Thus for the study presented in this chapter, where appropriate, results obtained by use of both LSR and RMA are shown. For comparative purposes, however, those results obtained by LSR are used, to maintain consistency with the literature.

4.6 RESULTS

4.6a Interspecific variation in BMR

I have re-analysed the BMR/mass relationship for birds using those data given in a compilation by Gavrilov and Dolnik (1985). All values used were measurements taken during the circadian rest phase (n=263). The logarithmic linear expression of this relationship is given below for (1) all birds (2) passerines and (3) non-passerines. The equations in brackets represent those derived by RMA. Numbers in brackets are the standard error of the slope and intercept terms respectively. BMR is expressed in Watts and Mass(M) in grams (See Fig 4.2).

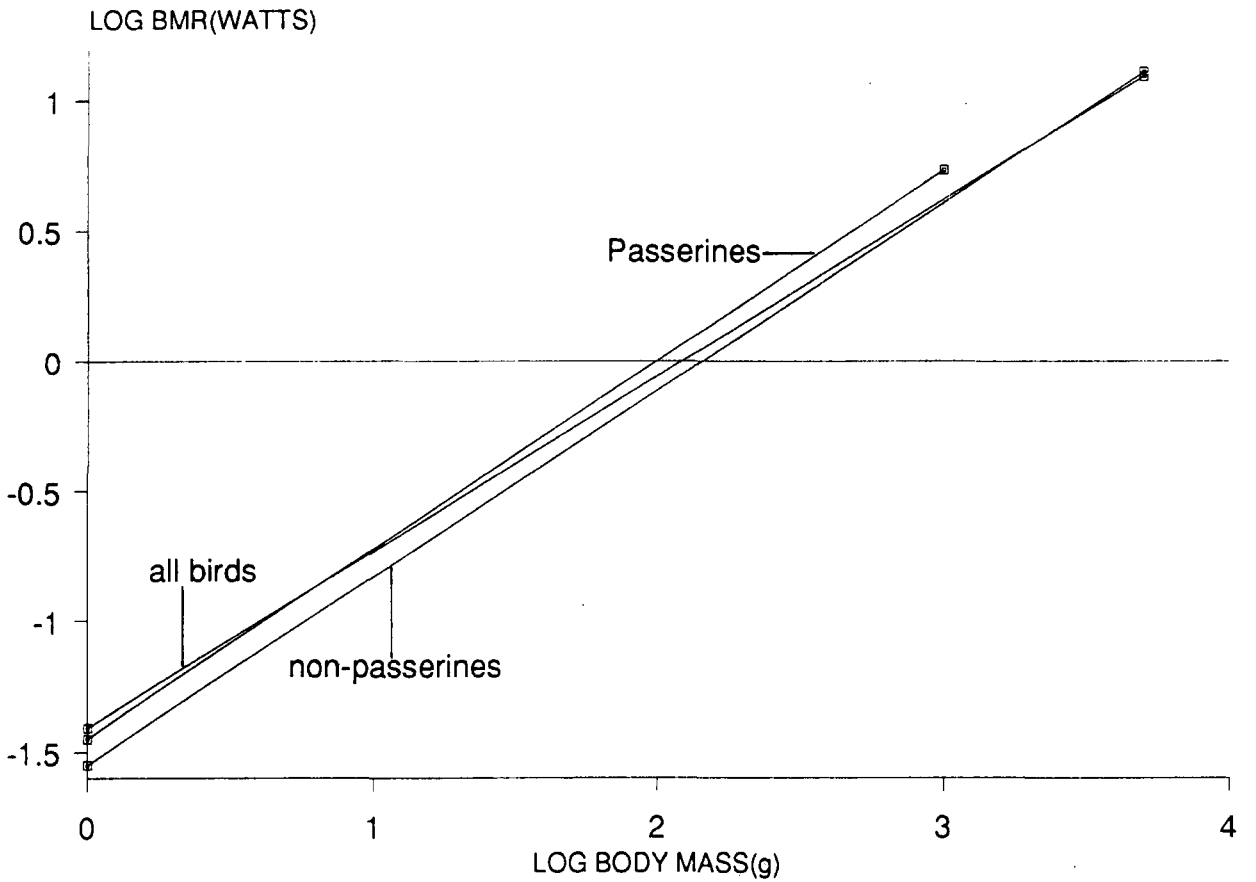


Fig 4.2 Comparison of the regression lines of BMR with body mass for passerines non-passerines and all birds. Regression equations are given in the text, (Data are taken from Gavrilov and Dolnik, 1985).

All birds

$LogBMR = Log0.039(0.0009) + 0.677(0.0081) LogM$ $r^2=0.96$ n=263 (1)

$(LogBMR = Log0.036 + 0.69 LogM)$

Passerines

$LogBMR = Log0.035(0.0007) + 0.727(0.021) LogM$ $r^2=0.92$ n=105 (2)

$(LogBMR = Log0.031 + 0.761 LogM)$

Non-passerines

$LogBMR = Log0.028(0.0006) + 0.721(0.0136) LogM$ $r^2=0.95$ n=158 (3)

$(LogBMR = Log0.023 + 0.758 LogM)$

The mass exponent of equations 1, 2 and 3 are not statistically distinguishable from those for equivalent groups of birds given by Aschoff and Pohl, (1970) and Kendeigh *et al*, (1977).

For shorebirds (Sub-order *Charadrii*) I have obtained values of BMR for 9 species. The equation relating BMR to body mass for this sub-order is given below (See also Fig 4.3) Body mass and BMR values used are the mean values obtained for each species during the reported study.

$LogBMR = Log0.032(0.002) + 0.737(0.045) LogM$ $r^2=0.95$ n=15 (4)

$(LogBMR = 0.031 + 0.722 LogM)$

The sources of the data used to compile equation 4 are given in Table 4.1. I obtained values for Turnstone, Oystercatcher and Dunlin during the preliminary studies for the work presented in this chapter. Where two or more values were obtained for the same species from separate studies, each value has been used as a separate datum point. This was done to avoid the removal of

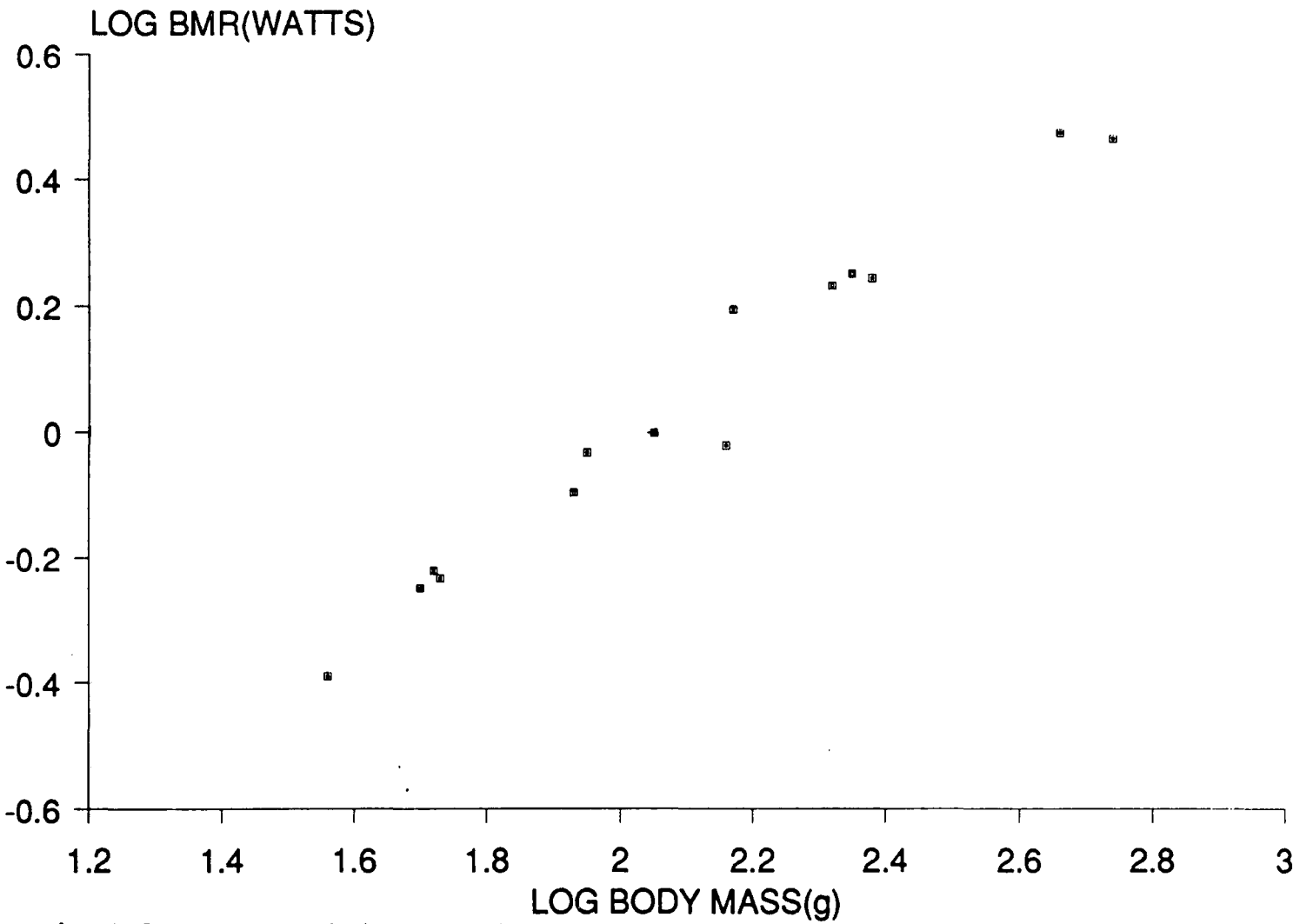


Fig 4.3 Interspecific relationship between BMR and body mass for nine species of shorebird. Data sources are given in table 4.1 Regression equation and statistics are given in the text.

variation in BMR which may be due to factors such as geographical location or inherent population variability. Whilst the intercept term is higher than that for the general non-passerine equation (3) see earlier, it is not significantly so ($P>0.05$ $t=0.498$). This result is also in accord with that obtained by Kersten and Piersma (1987). Similarly the slope of the regression for the Charadrii (Equation 4) is not distinguishable from that for non-passerines (Equation (2) $P>0.05$ $t=0.130$). It is worthy of note that the value obtained by Speakman (1984) for the Redshank is 1.6 times higher than that found by the study presented here.

Table 4.1: BMR and body mass of nine species of shorebird.

Species	Body mass(g)	BMR(W)	n	Source
Oystercatcher	554	2.92	2	K&P
Oystercatcher	467	2.98	2	This study
Redshank	149	1.56	6	Speakman(1984)
Redshank	147	0.95	10	This study
Grey Plover	226	1.78	3	K&P
Grey Plover	244	1.75	5	This study
Grey Plover	210	1.71	4	Wood(1984)
Green Sandpiper	90	0.93	?	G&D
Sanderling	50	0.56	9	Castro(1987)
Sanderling	53	0.56	6	This study
Dunlin	54	0.58	2	This study
Turnstone	114	0.99	3	K&P
Little Ringed Plover	36	0.41	1	K *et al*
Pacific. Golden Plover	127	1.31	12	J et al

n refers to the number of individuals used of each species from which the averages for BMR and body mass are based.

k&P refers to Kersten and Piersma (1987)
G&D refers to Gavrilov and Dolnik (1985)
K et al refers to Kendeigh *et al* (1977)
J et al refers to Johnson *et al* (1990)

The conditions used for metabolic rate determination used by Speakman (1984), were similar to those used in the present study. As Speakman (1984) also reports values for Oystercatcher (mean Body mass 596 BMR 313 watts n=2), which are in accord with those

reported in the present study, it is unlikely that variation in experimental technique can account for the observed difference in the result obtained for the Redshank by Speakman (1984) and that obtained during the present study. It is possible that the difference is due to a high level of individual variation and or differences in body composition between those individuals measured by Speakman and those measured during the present study (see section 4.6d).

However the interspecific equation for the relationship between BMR and body mass for *Charadrii* is changed little by the exclusion of the result of Speakman. The corrected equation is given below.

$$LogBMR = Log0.032(0.005) + 0.733(0.073)LogM \quad r^2=0.92 \quad n=14$$

Except in the case of the Redshank the results obtained during the course of the study presented here are in broad agreement with those found in the literature (Table 4.1).

4.6b Intraspecific variation in BMR

I have been able to compute allometric equations for the BMR/mass relationship for four species, given below (See also Fig 4.4a-d). The regression for Grey Plover has been compiled using values given by Wood (1984), Kersten and Piersma, (1987) and from BMR determinations made during the present study. With respect to Wigeon, metabolic rate determinations were of fasting metabolic rate (ie during the active phase of the day). For shorebirds,

Fig 4.4a Relationship between BMR and body mass for Grey Plover. Regression equation and statistics are given in the text.

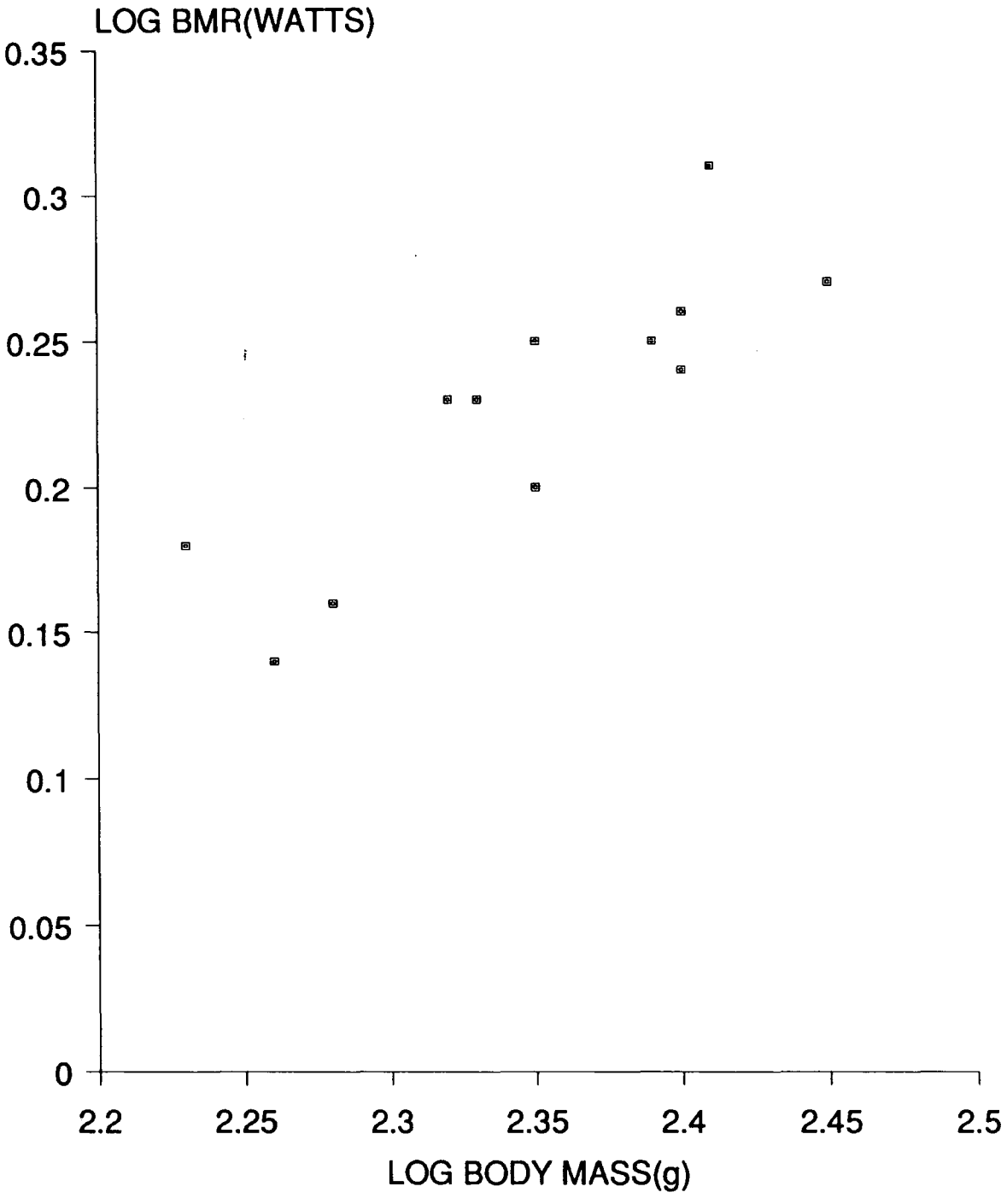


Fig 4.4b Relationship between BMR and body mass for Kestrel Regression equation and statistics are given in the text. Data are taken from Daan *et al*(1987).

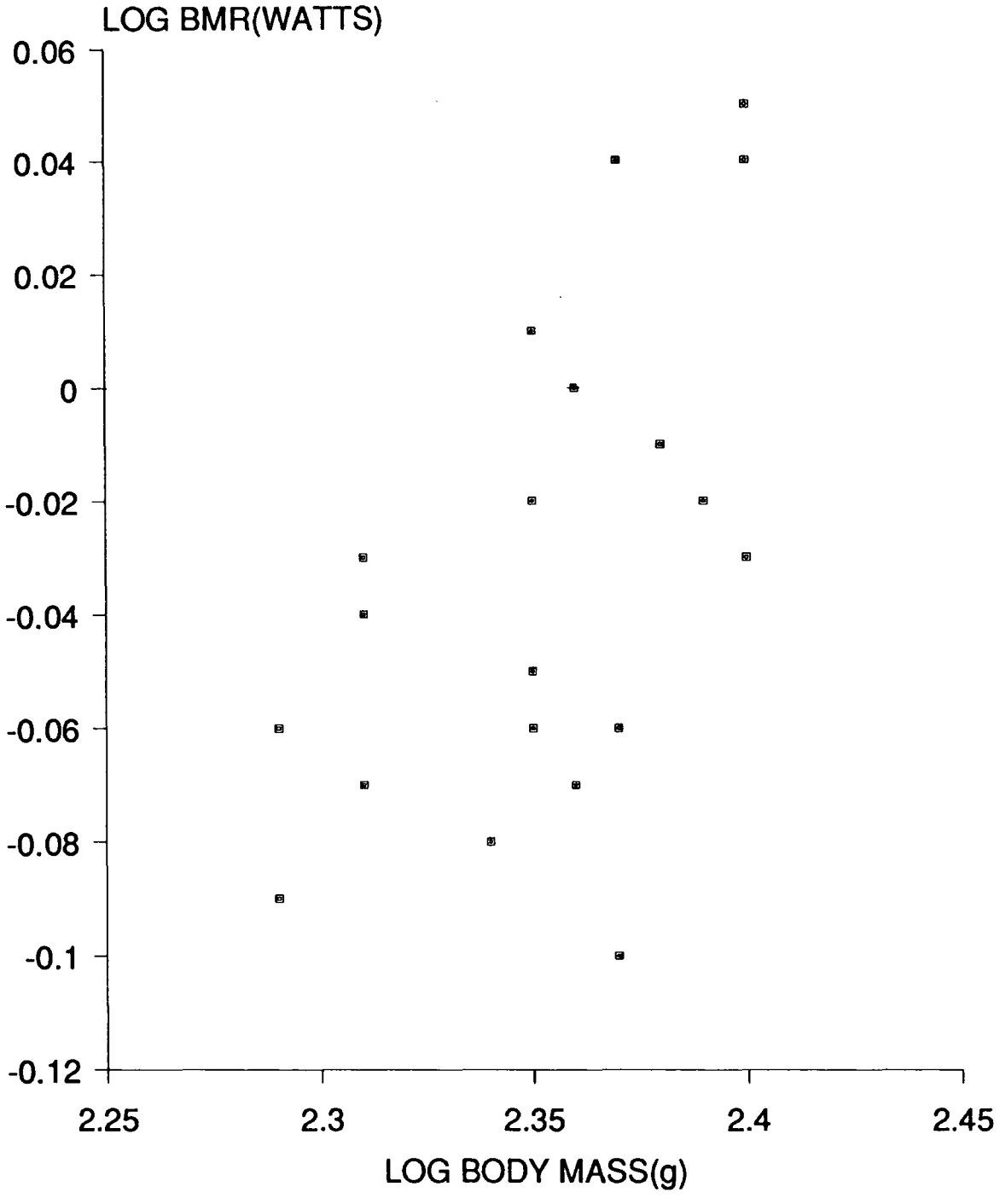


Fig 4.4c Relationship between FMR and body mass for Wigeon. Regression equation and statistics are given in the text

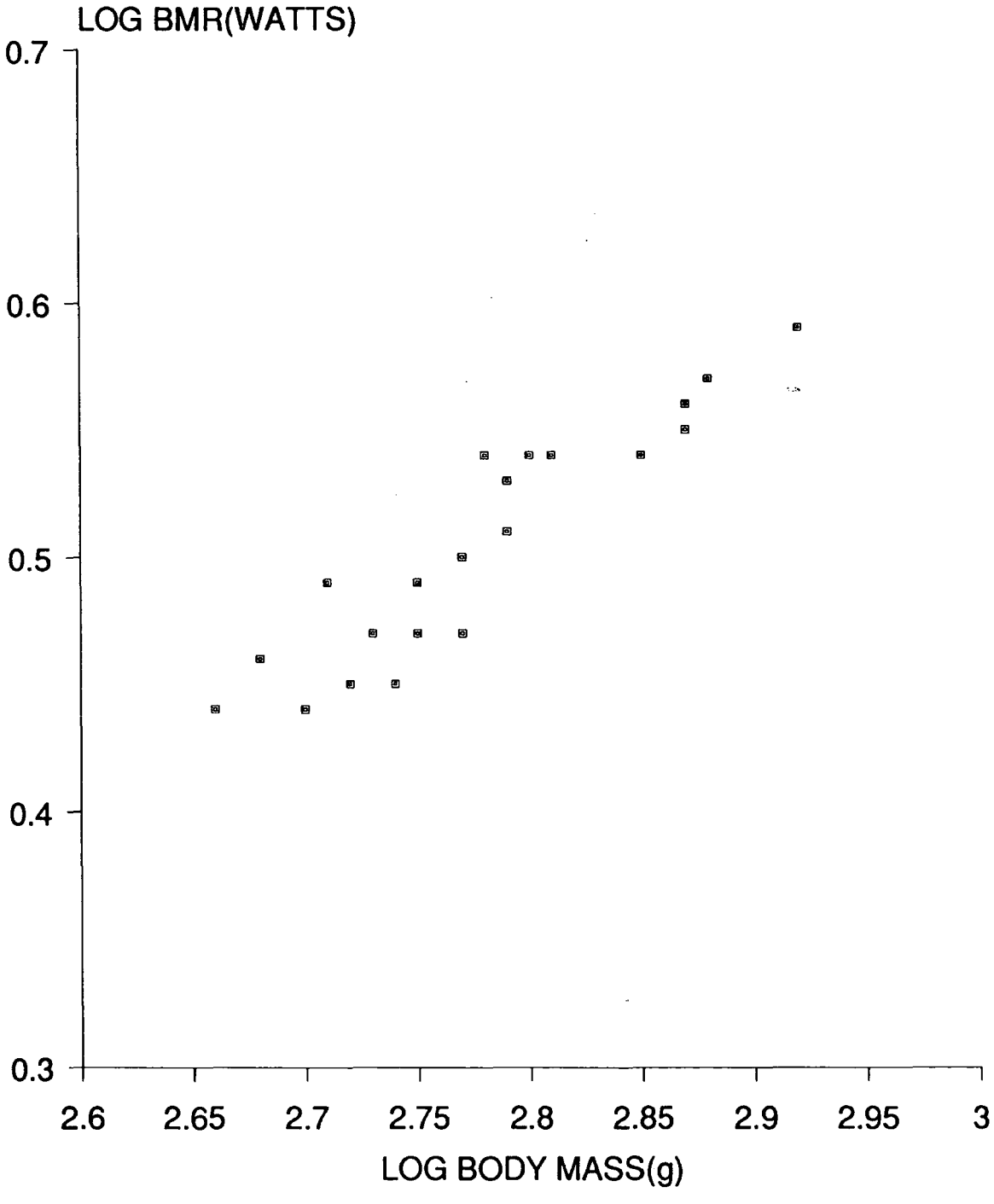
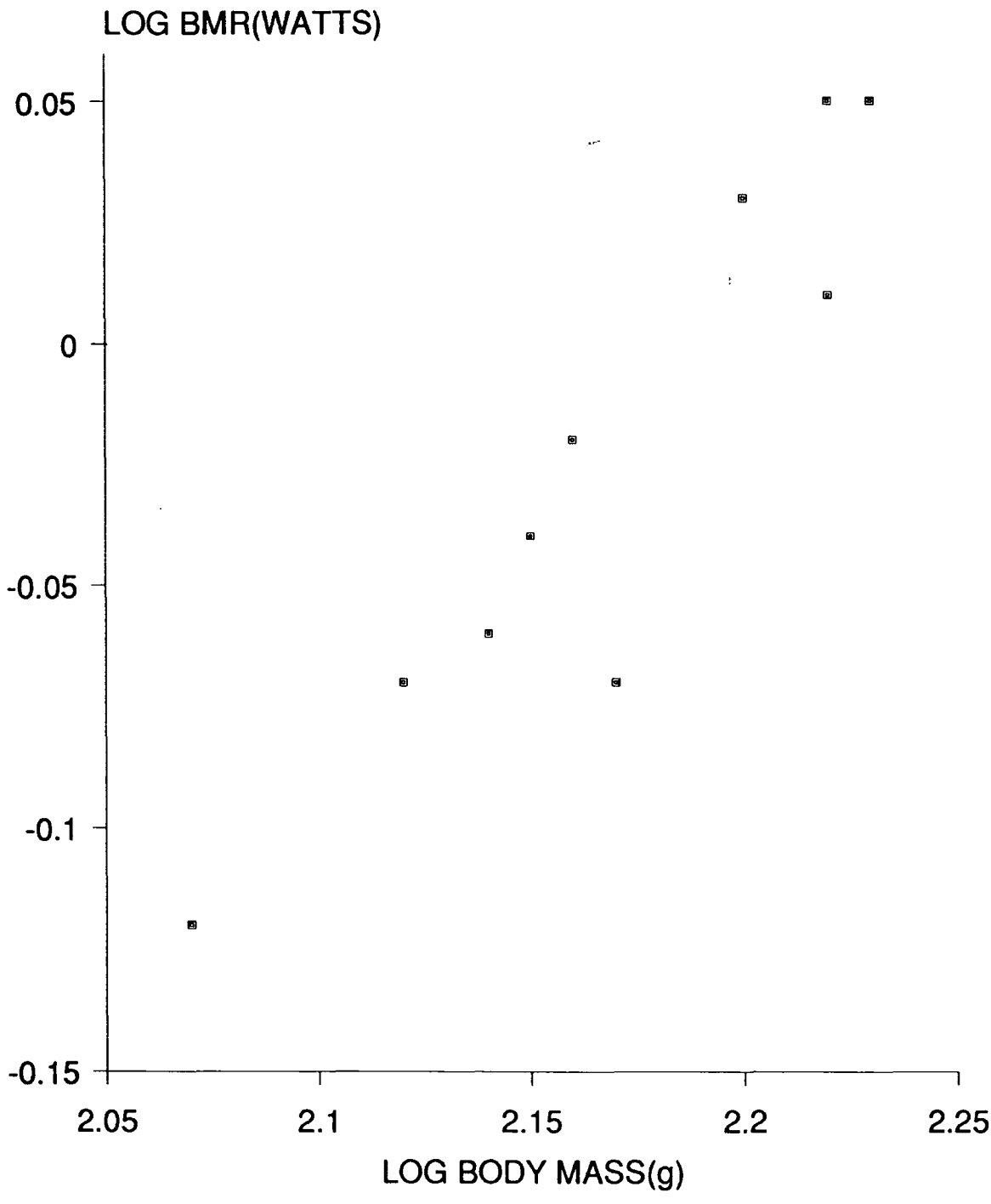


Fig 4.4d Relationship between BMR and body mass for Redshank
Regression equation and statistics are given in the text



determinations were made during the day, as I believe these represent measurements at basal conditions (see section 4.6d). Each point plotted on Figures 4.4a-d represents the BMR and Mass values for an individual averaged over the entire study period

Wigeon

$$LogBMR=Log0.063(0.008)+0.61(0.06)LogBM \quad r^2=0.83 \ n=22 \ P<0.001 \quad (5)$$

$$(LogBMR=Log0.044+0.67LogBM)$$

Grey Plover

$$LogBMR=Log0.057(0.012)+0.62(0.11)LogBM \quad r^2=0.72 \ n=12 \ P<0.001 \quad (6)$$

$$(LogBMR=Log0.041+0.73LogBM)$$

Redshank

$$LogBMR=Log0.0054(.0006)+1.03(0.13)LogBM \quad r^2=0.88 \ n=10 \ P<0.001 \quad (7)$$

$$(LogBMR=Log0.0040+1.1LogBM)$$

For Sanderling no significant relationship between metabolic rate and body mass could be found. Variation in metabolic rate was large (CV=0.14) and in mass relatively small (CV=0.08).

Values for slope terms for the intraspecific relationship between mass and metabolic rate are summarized in Table 4.2 for both birds and mammals studied to date.

Table 4.2 The Intraspecific allometry of BMR and body mass, based on LSR equations

Birds	Slope	n	Source
Species			
Kestrel (1987)	0.78(0.23)	20	Daan et al
Wigeon(FMR)	0.61(0.06)	22	This study
Grey Plover	0.63(0.11)	12	This study
Redshank	1.03(0.13)	10	This study

Mammals			
Species			
Human	0.60	7000	Schofield (1985)
Sheep(d)	0.75	43	Blaxter (1989)
Sheep(d)	0.72(0.08)	13	Heusner (1982)
Rat (l)	0.82(0.08)	10	Wheeler (1984)
Rat (l)	0.64(0.13)	45	Heusner (1982)
Mice(L)	0.72(0.03)	18	Wheeler (1984)
Mice(L)	0.72(0.03)	28	Heusner (1984)
Peromyscus M	0.91(0.23)	23	Heusner (1984)
Apodemus-sylvaticus	0.57(0.09)	20	Wheeler (1984)
Guinea-pig(l)	0.67	?	Wilkie (1977)
Cat(d)	0.58(0.16)	7	Heusner(1982)
Cattle(d)	0.52(0.07)	11	Heusner(1982)
Dog(d)	0.58(0.11)	16	Heusner(1982)

Numbers in brackets are the standard errors of the slope
(d) indicates that the animals studied were from domestic stock
(l) indicates that the animals studied were laboratory bred.

The slope terms derived for Wigeon of different sex are not statistically distinguishable from each other, this is also true for the intercept term. However, both the slope and the intercept term for all wigeon taken together are significantly different from those terms expressed in the overall BMR/mass equation(3) for non-passerines ($P<0.002$ $t=3.18$(intercept) $P<0.05$ $t=2.58$ (slope)). The converse is true for both Grey Plover ($P>0.05$ $t=1.64$(intercept) $t=1.48$ $P>0.05$(slope)) and Kestrel (Daan *et al* 1989) however, conform to equation(3). Analysis of covariance (ANCOVA) indicates, perhaps unexpectedly, that the slopes of the regression lines for Kestrel, Redshank, Grey Plover and Wigeon do not differ significantly from each other (Table 4.3). (The mean slope for the intraspecific regressions for these four species is 0.76 ($\pm$0.09 SE)). The intercept terms for the intraspecific regressions, however, differ significantly from each other as shown by ANCOVA (Table 4.3).

Table 4.3 ANCOVA of the Interspecific variation in the BMR/body mass relationships amongst four species of birds.

Assuming parallel slopes do intercepts differ?

Source of variation	Sums of squares	df	Mean squares	F	Signif. of F
Variation about the overall slope	0.05	59	0.001		
Variation due to the overall slope	0.09	1	0.09	109.87	P<0.001
Variation due to species deviation from overall slope	0.59	3	0.20	244.98	P<0.001

Do slopes differ?

Source of variation					
variation about individual slopes	0.04	56	0.001		
Variation due to body mass	0.08	1	0.08	97.05	P<0.001
Variation between slopes	0.001	3	0.0003	2.03	P>0.05
Co-variation between body mass and species slopes	0.001	3	0.0003	1.56	P>0.05

It is apparent that there is considerable variation amongst species in the intraspecific relationship between BMR and body mass. For the species analysed above variation in the elevation of the slope may be due to differing levels of physiological heterogeneity (such as variation in body composition) across the test period between individuals. Consistency in the extent of physiological variation amongst the test sample is an implicit assumption which must be made before a functional interpretation can be placed on the regression described above.

With respect to Redshank the intraspecific mass exponent of the BMR/mass relationship is not statistically distinguishable from month to month (ANCOVA F=0.81 df=27,6). The means of the slope and the intercept of the across month regression are 1.19 ($\pm$0.09

SE) and -2.61 ($\pm$0.21 SE) respectively, these values are not statistically distinguishable from those given in equation 7.

The relatively high value obtained for the intraspecific slope term for Redshank could have arisen because the sample consisted of individuals of differing race (see Chapter 1). When the individuals considered to be of the race *T.t robusta* are excluded from the analysis, the mass exponent of the BMR/mass relationship falls to 0.85, although the regression is no longer statistically significant. A further complication with the sample of Redshank is that the extent of their fat reserves varied both between individuals and seasonally (as measured by TOBEC). I shall show below, that the amount of fat carried may have a strong influence on the BMR of an individual.

The sample of Wigeon used during the present study were all tested within a 25 day period in late winter and had spent most of their lives in a captivity. As such, the sample should of been more physiologically homogeneous than those of other species examined. As some of the points used to produce the regression lines for Grey Plover and the Kestrel came from diverse sources, it is not possible to asses the level of physiological homogeneity within each sample.

The mass coefficient (intercept term or *a*, see the General introduction) is equal to the basal metabolism divided by the mass raised to the mass exponent. To maintain consistency with Heusner (1982) I have used the mean value for the mass exponent (0.76) in order to calculate standardized values for the mass

coefficients for the species studied in this chapter. This calculation maintains that the slopes of each of the species of bird studied lie parallel to each other and that the slope is equal to 0.76 (ie. the mean slope of the four species studied). The mass coefficients given in equations 5, 6, 7 and that of the Kestrel given by Daan *et al* (1987), are thus adjusted to yield a slope 0.76. These values are given in table 4.4. As can be seen there is little evidence to support the view put forward by Heusner (1982) that the mass coefficient *a* increases with body mass. The value given for Wigeon is for fasting metabolic rate and as such is probably higher than that which would be predicted for BMR. Nevertheless it is very close to that for Grey Plover. The value for the Kestrel is low, which is consistent with the finding of low BMR amongst Falconiformes in general. Falconiform BMR values, for those species studied to date, all lie below the allometric predictions based on all species of bird (Zar, 1968; Wijnandts, 1984 and Daan *et al*, 1989).

Table 4.4 Mass coefficients (*a*) expressed as Watts/$g^{0.76}$, for four species of bird.

Species	Mean body mass(g)	Mass coefficient(*a*)
Redshank	145	0.022
Grey Plover	222	0.028
Kestrel	224	0.015
Wigeon	598	0.025

The *a* values for Redshank and Grey Plover do appear to scale with mass, but it is not possible to draw any meaningful conclusions from only two points.

Multiple regression analysis of BMR with mass and biometrics as independant variables(commonly invoked as size parameters) indicates that for Wigeon, head plus bill accounts for 3.6% of the variation in BMR in addition to the 82% explained by mass (multiple r^2=0.86 $\underline{P}$<0.001) and that for Redshank, tarsus plus toe accounts for 6.5% of the variation in BMR in addition to the 88% explained by mass (multiple r^2=0.95 $\underline{P}$<0.001). It is important to note that biometrics excluded by the analysis (wing and tarsus plus toe for Wigeon and wing and bill for Redshank) are excluded because of their high correlation with mass and therefore variation in BMR which is explained by mass is also that which would have been explained by the excluded variables.

4.6c Within-individual variation in BMR

For the purposes of analysis of variation in BMR with body mass and body composition it has been implicitly assumed that the results are not effected by systematic seasonal variation in BMR that is independant of body composition. Seasonal variation in BMR is examined in Chapter 5. Table 4.5 gives the equations for the logarithmic LS-regressions of BMR on body mass for 10 Redshank and four Grey Plover I studied. No significant regressions were found for any of the Sanderling studied. The plots of Log BMR against Log body mass are shown in figures 4.5a-d for Grey Plover; figures 4.6a-j for Redshank and figures 4.7a-f for Sanderling. The maximum absolute change in mass of any one Sanderling was 21g the mean mass change was 10.66 ($\pm$2.76 SE). Daily variation in BMR in Sanderling may be so great as to have obscured any mass effect. Maximum variation in BMR for an

Fig 4.5a-d Within individual relationship between BMR and body mass for four Grey Plovers. Regression equations and statistics are given in text a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.5

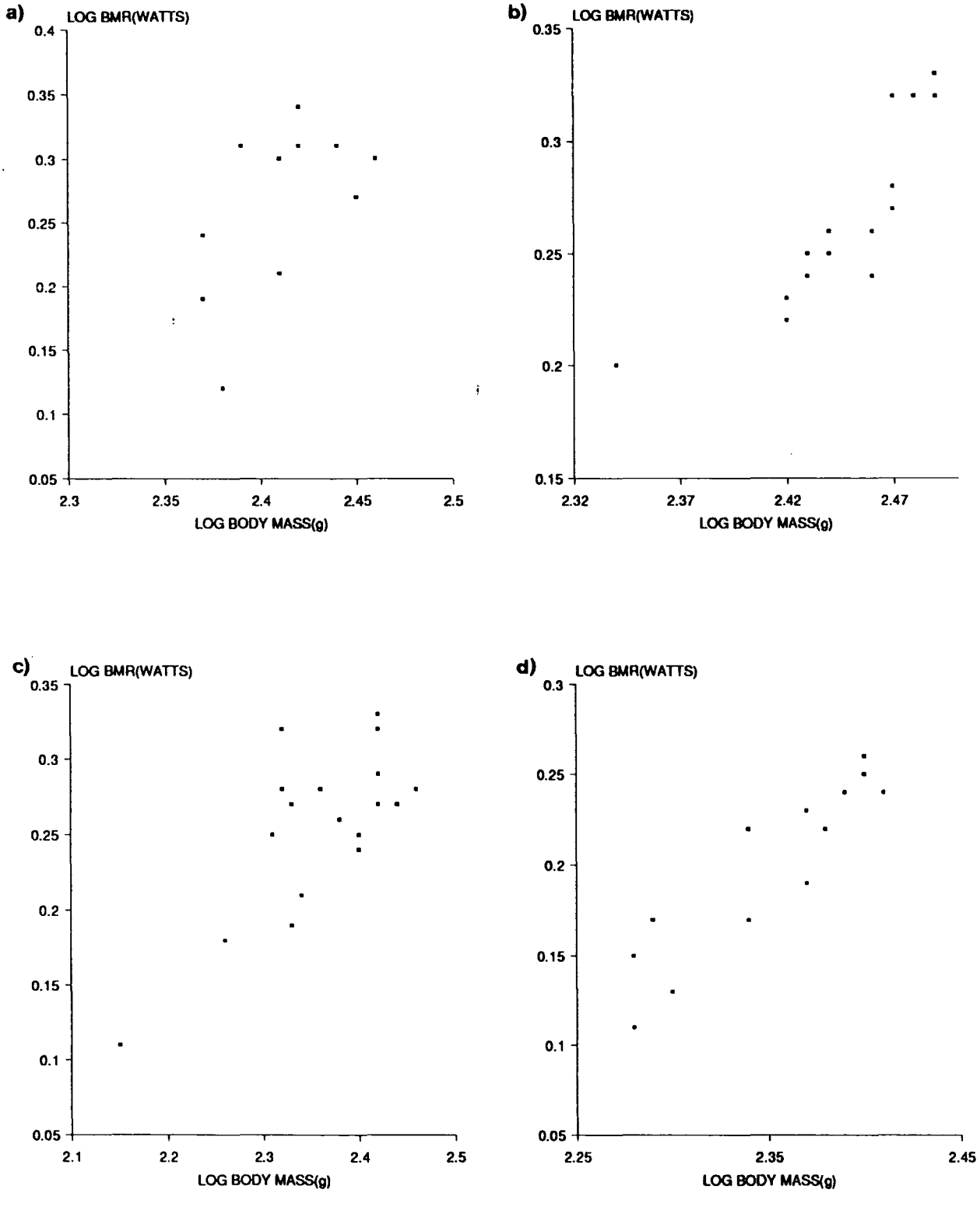
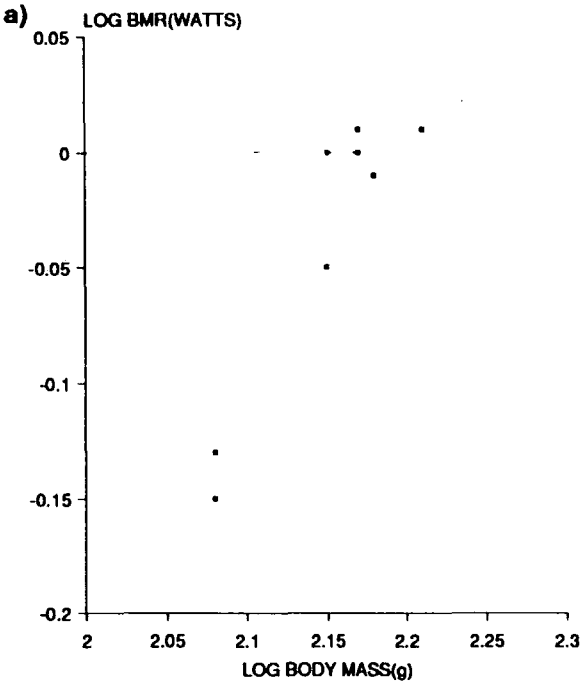
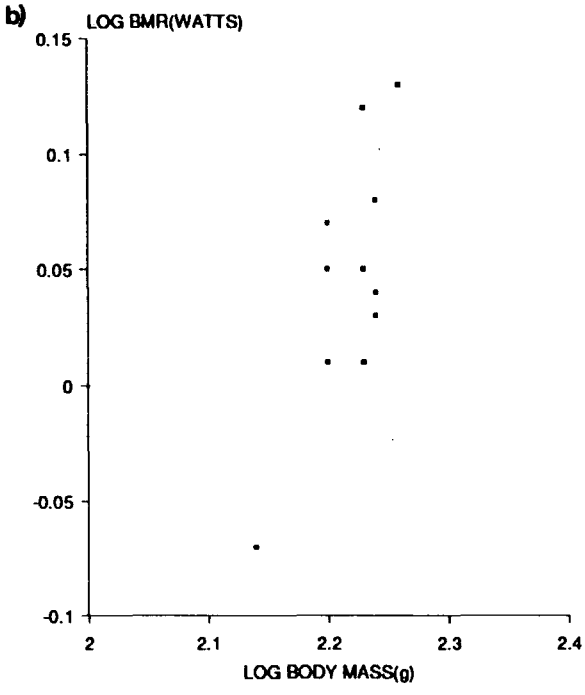
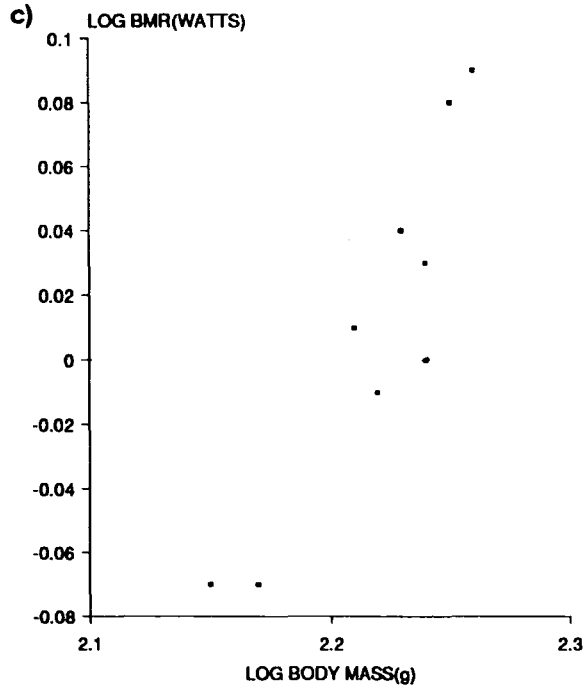


Fig 4.6a-j Within individual relationship between BMR and body mass for ten Redshank. Regression equations and statistics are given in text. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)= bird No.4 e)=bird No.5 f)=bird No.6 g)=bird No.7 h)=bird No.8 i)=bird No.9 j)=bird No.10







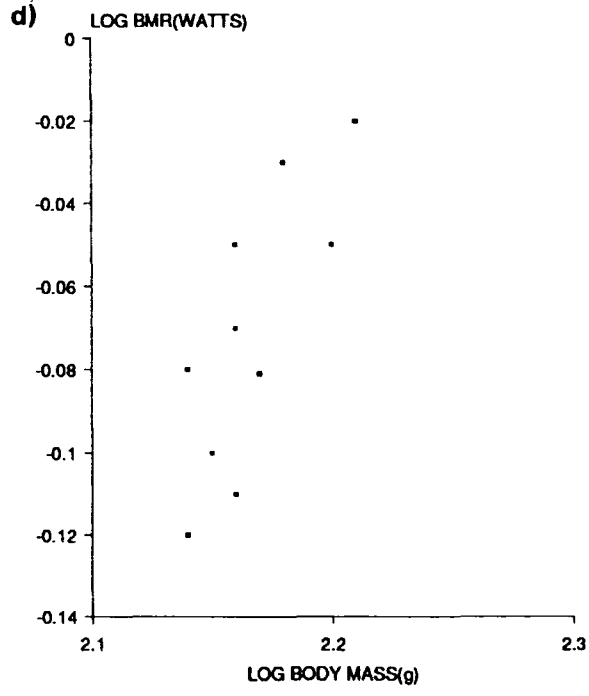
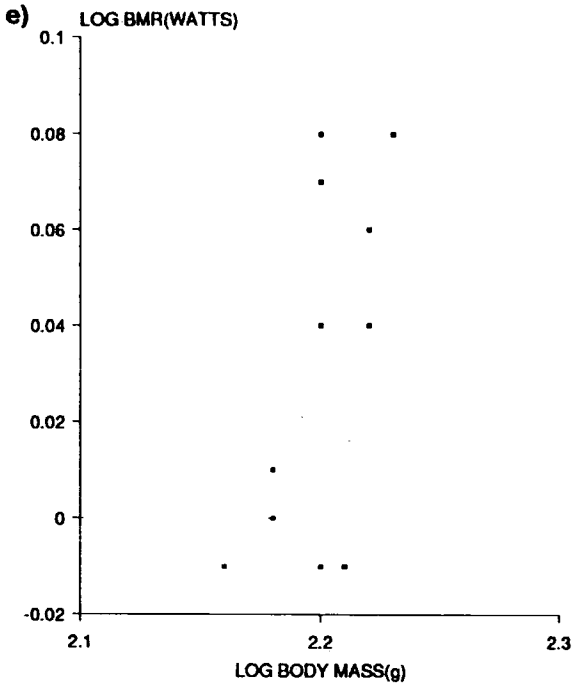
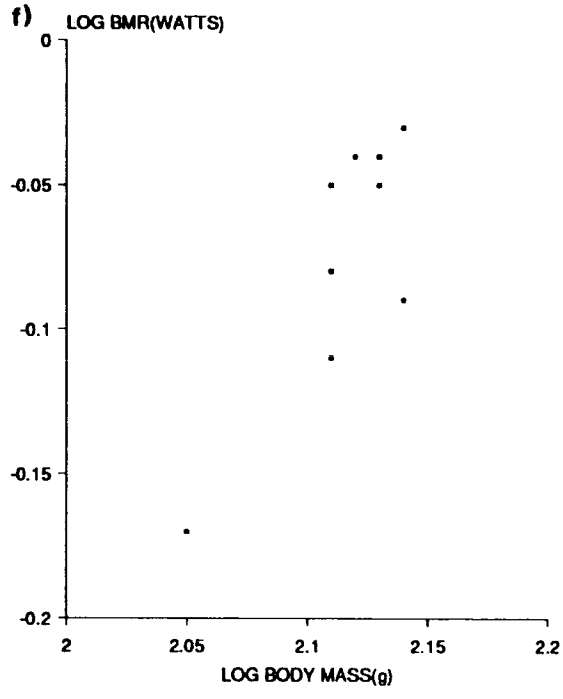
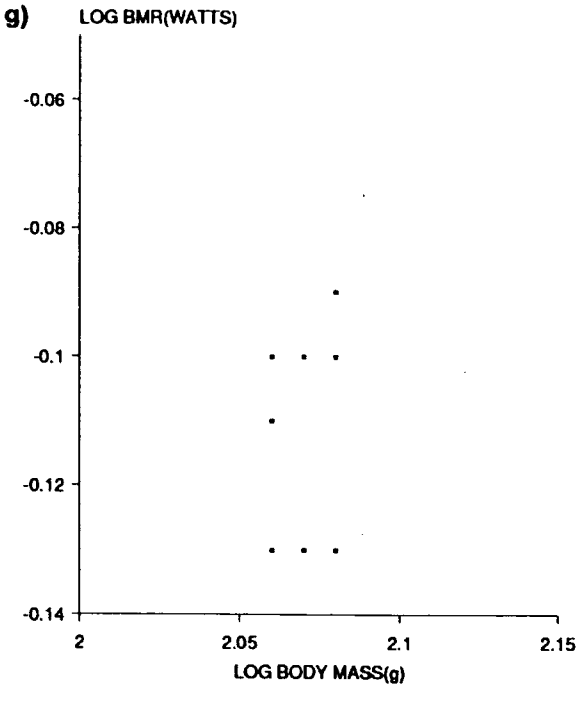


Fig 4.6 e-h


e)
LOG BMR(WATTS)
0.1
0.08
0.06
0.04
0.02
0
-0.02
2.1
2.2
2.3
LOG BODY MASS(g)


f)
LOG BMR(WATTS)
0
-0.05
-0.1
-0.15
-0.2
2
2.05
2.1
2.15
2.2
LOG BODY MASS(g)


g)
LOG BMR(WATTS)
-0.06
-0.08
-0.1
-0.12
-0.14
2
2.05
2.1
2.15
LOG BODY MASS(g)

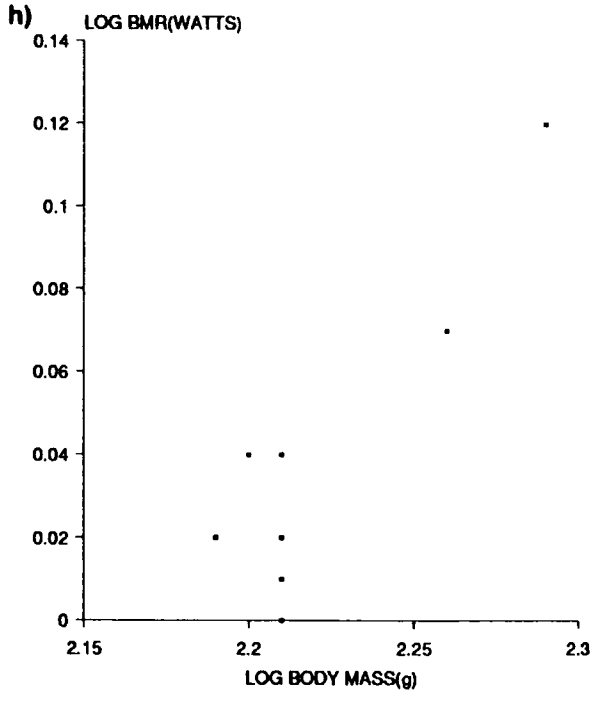
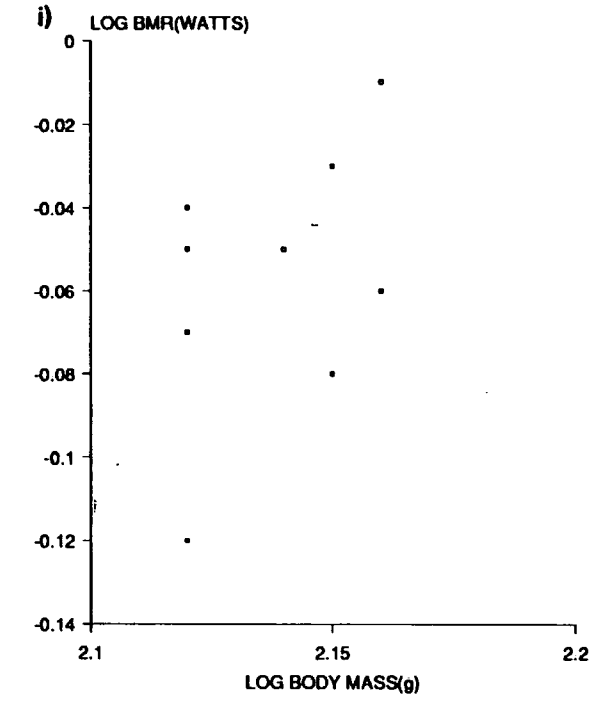

h)
LOG BMR(WATTS)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2.15
2.2
2.25
2.3
LOG BODY MASS(g)

Fig 4.6 i-j


i)
LOG BMR(WATTS)
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
2.1
2.15
2.2
LOG BODY MASS(g)

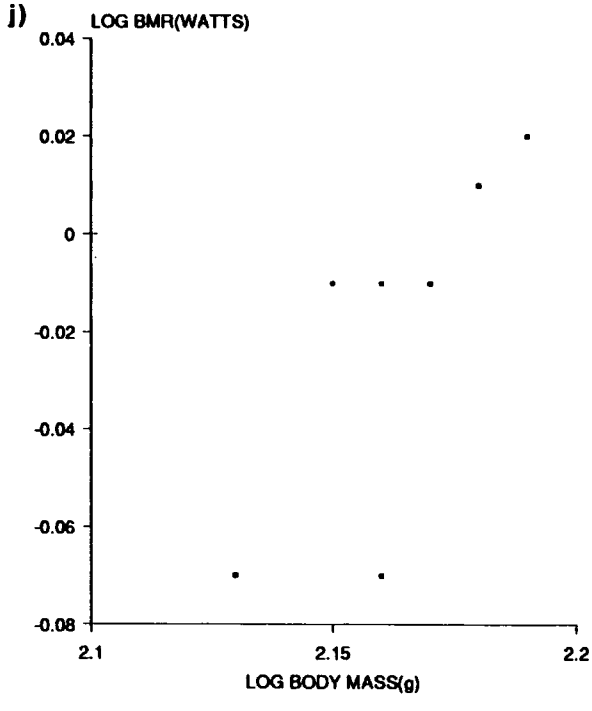
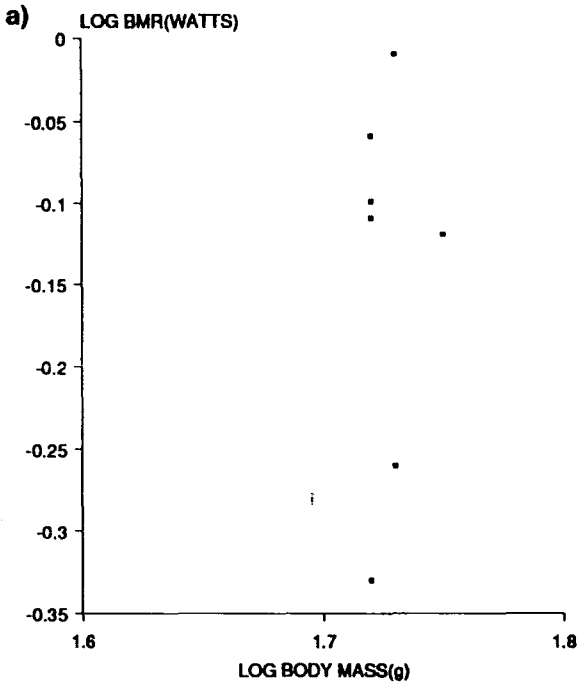
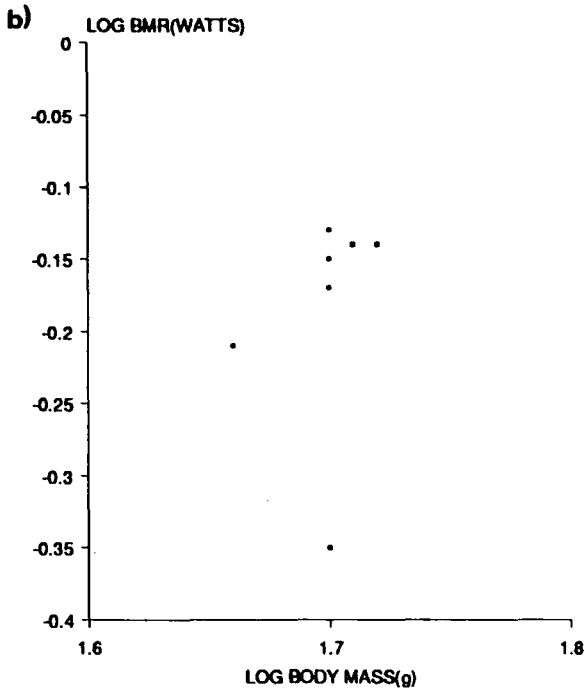
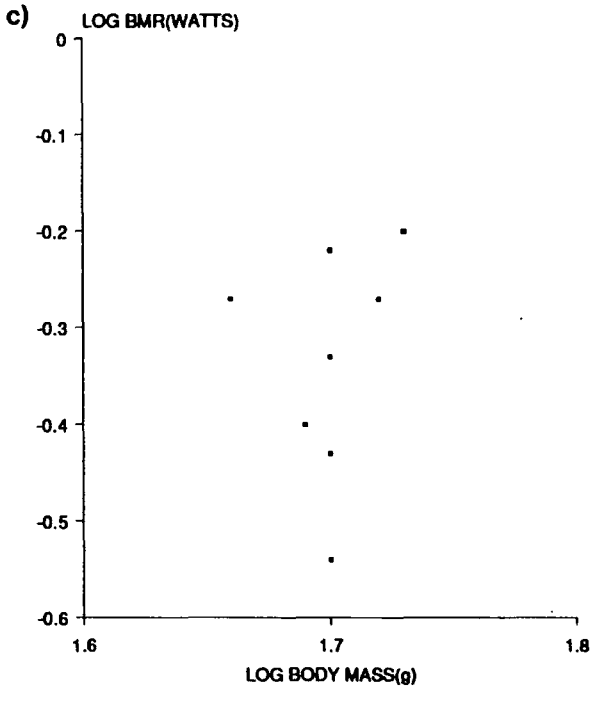

j)
LOG BMR(WATTS)
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
2.1
2.15
2.2
LOG BODY MASS(g)

Fig 4.7a-f Within individual relationship between BMR and body mass for six Sanderling a)=bird No.1 b)=bird No.2 c)=bird No.3 d)= bird No.4 e)=bird No.5 f)=bird No.6







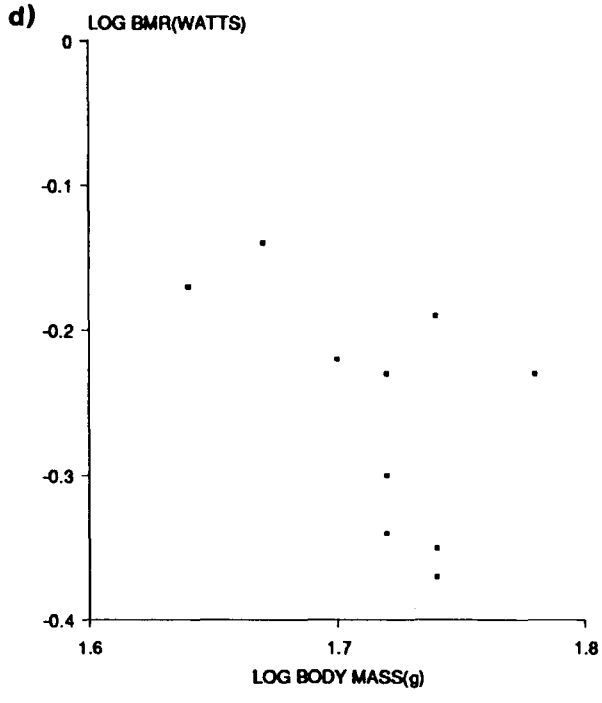
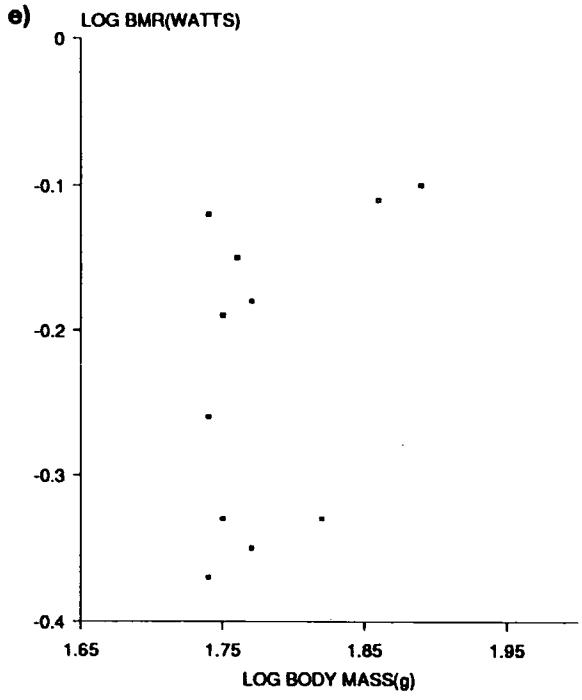
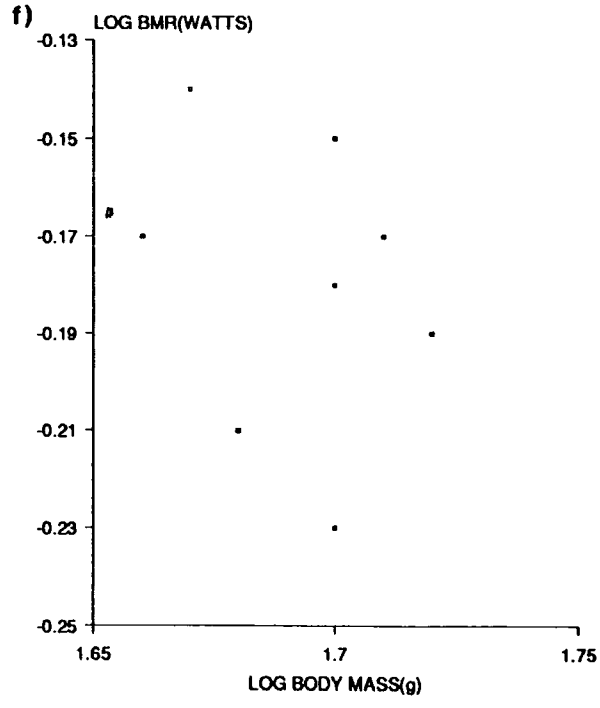


Fig 4.7 e & f


e)
LOG BMR(WATTS)
0
-0.1
-0.2
-0.3
-0.4
1.65
1.75
1.85
1.95
LOG BODY MASS(g)


f)
LOG BMR(WATTS)
-0.13
-0.15
-0.17
-0.19
-0.21
-0.23
-0.25
1.65
1.7
1.75
LOG BODY MASS(g)

individual was 0.49W with mean variation of 0.37 ± 0.03W which is very large considering that the data suggest that such variation is mass independent.

Table 4.5 BMR/body mass relationships within individual Redshank and Grey Plover

Species	Regression equation	n	r^2	Significance of regression	Corrected (a)
Redshank					
No.1	LogBMR=Log0.0012(.00015)+1.31(0.18)M	8	0.90	P<0.001	0.0025
No.2	LogBMR=Log0.0013(.00031)+1.27(0.31)M	13	0.61	P<0.01	0.0025
No.3	LogBMR=Log0.0005(.00007)+1.40(0.23)M	9	0.85	P<0.001	0.0023
No.4	LogBMR=Log0.0050(0.0014)+1.02(0.31)M	10	0.58	P<0.05	0.0022
No.5	LogBMR=Log0.0023(0.0009)+1.21(0.48)M	11	0.42	P<0.05	0.0025
No.6	LogBMR=Log0.0015(0.0004)+1.26(0.36)M	11	0.58	P<0.01	0.0025
No.7	LogBMR=Log0.064(0.060)+0.523(0.5)M	9	0.26	P>0.05	0.0021
No.8	LogBMR=Log0.0063(.0013)+1.01(0.19)M	10	0.70	P<0.01	0.0025
No.9	LogBMR=Log0.044(0.041)+0.61(0.59)M	10	0.11	P>0.05	0.0024
No.10	LogBMR=Log0.0007(.0002)+1.37(0.44)M	8	0.64	P<0.05	0.0025
Grey Plover					
No.1	LogBMR=Log0.0017(.00078)+1.23(0.53)M	11	0.38	P<0.05	0.016
No.2	LogBMR=Log0.009(0.0015)+0.94(0.14)M	17	0.74	P<0.001	0.015
No.3	LogBMR=Log0.102(0.029)+0.59(0.12)M	18	0.53	P<0.001	0.018
No.4	LogBMR=Log0.009(0.0012)+0.95(0.11)M	13	0.85	P<0.001	0.016

Where: BMR= basal metabolic rate M= Log10 of body mass and numbers in brackets are standard errors

For Redshank and Grey Plover, variation, in BMR with mass yielded significant regressions for all birds except for Redshanks No.7 and No.9, both of which showed relatively low variation in body mass during the course of the study. The slopes of the individual regressions are not statistically distinguishable either amongst the four Grey Plover (ANCOVA F=2.19 P>0.1 df=54,3 see Table 4.6) or the ten Redshank (ANCOVA F=0.55 P>0.05 df=83,9 see table 4.7), although the intercept terms are distinguishable. Variation in the latter represents individual variation in metabolic intensity.

Table 4.6 ANCOVA of variation between individuals in the BMR/mass relationship for four Grey Plovers.

Assuming parallel slopes do intercepts differ?

Source of variation	Sums of squares	df	Mean squares	F	Signif. of F
Variation about the overall slope	0.07	54	0.001		
Variation due to the overall line	0.09	1	0.08	59.86	P<0.001
Variation due to individual deviation from overall line	0.03	3	0.01	6.72	P<0.001

Do slopes differ?

Source of variation	Sums of squares	df	Mean squares	F	Signif. of F
Variation about individual slopes	0.06	54	0.001		
Variation due to mass	0.07	1	0.08	59.92	P<0.001
Variation between slopes	0.01	3	0.003	2.66	P>0.05
Co-variation between slope terms and mass	0.01	3	0.003	2.49	P>0.05

Table 4.7 ANCOVA of variation between individuals in the BMR/mass relationship for ten Redshank

Assuming parallel slopes do intercepts differ?

Source of variation	Sums of squares	df	Mean squares	F	Signif. of F
Variation about the overall slope	0.06	88	0.001		
Variation due to the overall line	0.11	1	0.11	164.68	P<0.001
Variation due to individual deviation from overall line	0.04	9	0.005	6.95	P<0.001

Do slopes differ?

Source of variation	Sums of squares	df	Mean squares	F	Signif. of F
Variation about individual slopes	0.06	79	0.001		
Variation due to mass	0.06	1	0.04	55.76	P<0.001
Variation between slopes	0.01	9	0.0001	0.53	P>0.05
Co-variation between slope terms and mass	0.01	9	0.0001	2.49	P>0.05

Mean slope of the BMR/mass relationship for Grey Plover was 0.92 ($\pm$0.13 SE) and for Redshank 1.23 ($\pm$0.23 SE). For both species the corrected mass coefficients *a* (see Section 4.6b and Table 4.5) do not correlate with mass. Although within-individual variation in the slope term did not significantly vary between individuals, it is worthy of note that within the sample of Grey Plover, the slope of the relationship for one individual, No.3, was much shallower than for the rest of the group (Table 4.5). I can offer no reason why this was so other than to suggest that this result may indicate a low of variation in metabolic response to body mass change within the individual. Within the sample of Redshank, low slope values are associated with individuals of low body mass range.

4.6d Individual variation in BMR and Body composition

I have performed stepwise multiple linear regression analyses on the mean metabolic rate of individual birds with their mean fat mass and mean total lean mass (as predicted by TOBEC see chapter 2) as independent variables. For Redshank, intraspecifically, the analysis indicates that lean mass is overwhelming the most important variable (Table 4.8), as mass of fat is not included in the multiple regression equation produced. (Expressed in another way fat mass is not correlated with the residual variation to a significant level (PIN=0.05) after variation due to lean mass is accounted for).

Table 4.8 Results of multiple regression of intraspecific variation in metabolic rate on TLM and fat free mass for ten Redshank

		Independants	
Dependant		**Log TLM**	**Log Fat**
Log BMR			
	Beta	0.955	0.101
	T	9.21	0.195
	P	<.001	>0.05

Multiple r^2= 0.91

Significant equation= LogBMR= -11.00+5.15LogTLM
F=84.75 P<0.001
Co-variance of LogTLM with LogFat r=0.83

However, a similar analysis performed with all BMR determinations on all individuals treated as individual datum points (Table 4.9) indicates that more of the variation in BMR is associated with variation in fat mass than lean mass. That is to

say, when a component of individual variation is included in the analysis, fat mass becomes the more important correlate of BMR.

Table 4.9 Results of stepwise multiple regression of within-individual variation in metabolic rate on TLM and fat mass for ten Redshank

		Independants	
Dependant		**Log TLM**	**Log Fat**
Log BMR			
	Beta	0.49	0.41
	T	5.09	5.02
	P	<.001	<0.001

Multiple r^2= 0.75

Significant equation= LogBMR= -5.35+0.10LogFat+2.43LogTLM
F=147.884 P<0.001
Co-variance of LogTLM with LogFat r=0.83

The inference that more of the variation in within individual BMR is associated with change in fat mass than in TLM is also supported by within-individual multiple regressions of BMR with lean and fat mass of Redshank and Grey plover No.5. (Table 4.10). All, except Redshank No.5 yielded multiple regression equations which included only fat mass as a predictive variable. (That of Redshank No.5 conversely included only TLM, which suggest that both TLM and fat mass are important in determining BMR but that most body mass variation within an individual is due to change in fat mass).

Table 4.10 Results of stepwise multiple regressions of basal metabolic rate against TLM and fat mass for individual Redshank and Grey Plover
(Variables are given only if they were significant)

Grey Plover

	Independants	
Dependant	**Log Fat**	**Log TLM**
Log BMR		
Beta	0.661	0.37
T	4.83	2.70
P	<.001	<0.05

Multiple r^2= 0.89

Significant equation= LogBMR= -1.14LogFat+0.008LogTLM
F=45.56 n=13 P<0.001
co-variance of LogTLM with LogFat r=0.59

Redshank

Bird No.1
Significant equation= LogBMR= -0.39+0.25LogFat
Multiple r^2=0.94 n=8 P<0.001
Co-variance of LogTLM with LogFat r=0.86

Bird No.2
Significant equation= LogBMR= -0.57+0.37LogFat
Multiple r^2=0.65 n=12 P<0.001
Co-variance of LogTLM with LogFat r=0.51

Bird No.3
Significant equation= LogBMR= -0.93+0.56LogFat
Multiple r^2=0.81 n=9 P<0.001
Co-variance of LogTLM with LogFat r=0.85

Bird No.4
Significant equation= LogBMR= -0.47+0.27LogFat
Multiple r^2=0.59 n=10 P<0.05
Co-variance of LogTLM with LogFat r=0.58

Bird No.5
Significant equation= LogBMR= -10.24+4.80LogTLM
Multiple r^2=0.46 n=10 P<0.05
Co-variance of LogTLM with LogFat r=0.67

Bird No.6
Significant equation= LogBMR= -0.20+0.10LogFAT
Multiple r^2=0.63 n=10 P<0.05
Co-variance of LogTLM with LogFat r=0.24

Bird No.8
Significant equation= LogBMR= -0.41+0.27LogFAT
Multiple r^2=0.73 n=10 P<0.05
Co-variance of LogTLM with LogFat r=0.29

Bird No.10
Significant equation= LogBMR= -0.65+0.62LogFAT
Multiple r^2=0.62 n=7 P<0.05
Co-variance of LogTLM with LogFat r=0.57

4.6e Diurnal variation in BMR

Resting metabolic rate was measured by day and night in individual Redshank, Grey Plover and Sanderling. The value obtained at night was compared with a subsequent value recorded during the day, not less than 18 and not more than 48 hours after the night-time determination. Paired t-tests indicate that there is no difference between resting metabolic rate recorded by day and at night (Table 4.11). Thus determinations performed during the day for these three species may be said to be at basal conditions. The results are in marked contrast to those for passerines in which there is a diurnal rhythum of BMR with the lowest value at night (Kendeigh *et al*, 1977).

Table 4.11 Circadian variation in BMR

Species	n	BMR(W) during day	BMR(W) during night	Paired--t	Signif. of t
Sanderling	6	0.52(.32)	0.53(.28)	0.131	P>0.05
Grey Plover	5	1.75(.13)	1.76(.23)	0.301	P>0.05
Redshank	15	0.82(.16)	0.82(.13)	0.361	P>0.05

Numbers in brackets are the standard errors of the sample means.

4.7 DISCUSSION

4.7a Diurnal variation in BMR

There is no *a priori* reason why the shorebird species I investigated should show depressed levels of BMR at night. Grey Plover Sanderling and Redshank are all known to feed by night (Cramp and Simmonds, 1983). Feeding in these species during the non-breeding season is closely linked to tidal cycles and roosting is therefore timed similarly or is opportunistic.

4.7b Interspecific allometry of BMR for the sub-order Charadrii

For the sub-order *Charadrii* BMR appears to be higher than for non- passerines in general. This is in accordance with the results presented by Kersten and Piersma, (1987), who reported elevated BMR, existence metabolism and daily energy expenditure (DEE) in the *Charadrii*. They argued that high BMRs recorded in shorebirds are a result of bias in the samples; which are chiefly drawn from higher latitudes, given that, interspecifically, the ratio between predicted and observed BMR increases from 0.8 in the tropics to 1.8 in arctic breeding sea bird species (Ellis 1984) and that similar latitudinal trends are reported for terrestrial birds (Weathers, 1979 and Hails, 1983). It is further implied by Kersten and Piersma that living in more northern latitudes requires a higher DEE than living in the tropics and that high BMR is an inevitable consequence of high DEE.

A strong correlation between high DEE and high BMR should not be unexpected as the slope of the allometric relationship between

DEE and body mass has been found to be 0.64 (Nagy, 1987), and not statistically distinguishable from that of the BMR/mass relationship for all birds (Equation(1)) given earlier in this chapter. Thus high DEE does indeed correlates with high BMR but, the former cannot be proven to be a cause of the latter.

Kersten and Piersma (1987), also put forward the "energetic margin hypothesis" that a high DEE required at some peak period of demand would lead to a higher than expected BMR all year round. They suggested that for shorebirds such periods of high DEE occur during mid-winter cold spells and possibly periods of migration and early-winter fattening. I do not dispute that these periods represent both potential and actual periods of high DEE. I suggest that as birds are known to be able to adjust BMR levels, at least on a seasonal basis (Davidson and Carey, 1976; Pohl and West, 1973 and Saarela *et al*, 1980), and in response to exposure to low temperatures (Rauteneberg, 1969), that there is no need for them to maintain a high BMR all year round, simply to accommodate peak periods of high DEE. The observed correlation between increased BMR and increased DEE could be a result of scale phenomena and the interdependence of BMR and DEE (ie. BMR is a component of DEE). Explanations for a physiological linkage between BMR and DEE as put forward by Kersten and Piersma appear to be "over adaptive" (*sensu* "the Panglossian paradigm" Gould and Lewontin, (1979)). and as such should be avoided.

4.7c Fasting endurance

By comparing Equation (4) in this chapter with the equation given in chapter 1 for the interspecific allometry of body fat and body mass, for the sub-order *Charadrii*, it is possible to derive an allometric equation for the survival time of the *Charadrii* as follows:

Equation 4 expressed in allometric form = $0.032M^{0.737}$ Js^{-1}

Equation for predicting body fat from body mass expressed in allometric form = $0.169M^{0.98}g$

Taking the energy content for fat as $39.3KJg^{-1}$ (Schmidt-Nielsen 1985) the allometric equation for predicting body fat can be formulated as $6641M^{0.98}J$

An allometric equation predicting survival time is subsequently obtained by dividing the new allometric equation for predicting body fat by that for predicting BMR from body mass.

$$6641M^{0.98}J/0.032M^{0.737}Js^{-1} = 2.07x10^{5}M^{0.243}s$$

which equals $57.64M^{0.243}$ hours

This figure is for basal conditions of energy expenditure and confirms that larger shorebirds have the ability to survive for longer periods of time without food than smaller species.

4.7d Intraspecific scaling of BMR, implications for the 0.75 "rule"

The results presented earlier for the intraspecific scaling of BMR with body mass are difficult to evaluate because of a high level of individual variation leading to a high degree of scatter of points around the regression line. Nevertheless it is possible to discount some of the theories put forward to explain the interspecific BMR/mass exponent of 0.75; although to do this, data must also be drawn from the literature and from both birds and mammals.

Those theories which are holistic in their approach should predict the intraspecific scaling of BMR to be the same as the interspecific, ie. approximately 0.75. Thus the arguments of Economos (1979), based on the energetic cost of resisting the force of gravity can be dismissed, as can that of Blum (1977), and Boddington (1978), based on the inter-relation between mass, longevity and metabolic rate. Though this theory can be dismissed only if longevity scales with mass within a species. Holistic theories demand that the measured intraspecific mass exponent should be at, or within statistical reach of, the overall interspecific mass exponent of 0.75. For both Redshank and Wigeon this is not the case and it is also not the case for some of the mammalian examples given in Table 4.2. The theory of elastic similarity developed by MacMahon, (1973) (see also the General Introduction) has been strongly countered by Heusner, (1987) who demonstrated that the underlying basis from which the theory was developed cannot be supported empirically, ie. Heusner, (1987)

demonstrates that neither trees nor animals are elastically similar.

The theories which stand, therefore, are those which are based on composites of an intraspecific scaling function combined with an interspecific function, or which dismiss the interrpecific mass exponent of 0.75 as a statistical artifact (Heusner, 1982).

Heusner (1982) claimed that "In the 7 animal species studied (see Table 4.2) the mass exponent is the same and equal to 2/3 (0.67)" This statement is not true. The mean value of the mass exponent derived by Heusner, (1982) was 0.67, but the actual values range from 0.48 to 0.91. Even though, as Heusner shows, the actual values are not statistically different from the mean this does not imply that they are equal to the mean value. The conclusion derived by Heusner may also be a result of the low level of sensitivity of the ANCOVA technique (Hays, 1988). For example, amongst those intraspecific exponents of four species of birds (Table 4.2), those of Wigeon and Redshank tested by students t-test were significantly different but ANCOVA indicates that there was no significant difference in the exponents when the four species are viewed together.

As shown in Table 4.2, variation in the intraspecific mass exponent amongst species is large (CV=0.19). I would contend that there is no evidence to suggest that there is only a single value which represents the intraspecific mass exponent of the BMR/mass relationships and as such, all theories which maintain that the

surface to volume ratio is the definitive cause of the BMR/mass relationship at the intraspecific level can be dismissed.

The conclusion above is also reached by Heusner (1987) who states that there is no definitive value for either the intraspecific or interspecific mass exponents. I would suggest that the scaling of BMR with mass is produced by a composite of functions which scale with mass to varying degrees. Natural selection acts on such functions and exerts effects depending on an animal's environment, and physiological and behavioural constraints. The mass exponent of an individual species will thus depend on a combination of factors which may, or may not, have been selected upon to varying degrees. The limits to which the exponent is able to vary may be set by either over-riding physical laws such as the Arrhenius-van't Hoff (see below), or by ecological realities such as the food supply. These constraints are broad but, taken overall, lead to the empirical Brody-Klieber interspecific exponent of 0.75. Blaxter, (1989) suggests that "to maintain stability of body temperature, heat production is set at such a level that heat loss can be facilitated and metabolic acceleration through the Arrhenius-Van't Hoff relationship avoided" He suggests further that the metabolic rate of different species represents the end result of a series of different solutions to the problem of maintaining temperature stability. I would add further that this has led to differing species having differing BMR/mass exponents as they are constrained by differing ecological, morphological and physiological needs.

The multiple regression of logarithmically transformed metabolic rate with mass and other biometrics (commonly used as parameters of size) in Redshank and Wigeon demonstrates that BMR does not scale exclusively with mass. Some size parameters eg bill length may show a high degree of independence from mass within a species. The results of the multiple regressions in Section 4.6c indicate that other non-mass scaling functions may also be important in determining intraspecific variations in BMR.

4.7e Within-individual variation in BMR

The result shown in the study presented here that within-individual metabolic rate increases with mass at a rate, at least as high as found, at the intraspecific and interspecific level, is in accord with the results of Daan *et al* (1989) for the Kestrel. They found that the within-individual mass exponent was 1.29 ($\pm$ 0.8 SE).

The result from the multiple regression of metabolic rate on masses of separate body components (Section 4.6d) indicates that variation in metabolic rate with mass was correlated with change in the size of the fat deposits for the sample of Redshank and one Grey Plover. It is thus possible to reject the original hypothesis that increasing the fat load carried would have little effect on the BMR (Introduction to Chapter4) This result is supported by those of McNiven, (1984) who demonstrated that the mass exponent of BMR of sheep was constant irrespective of the fatness of the animal, McNiven concluded that white adipose

tissue contributed significantly to the maintenance energy requirements of sheep.

Based on the results given in Chapter 3, I conclude that increasing the fat load causes increased maintenance cost, but this cost is probably not associated directly with the cellular energy output of the adipose tissue itself, but with related causes which are discussed below.

If there is a mass/surface area component of the BMR/mass relationship, the laying down of fat would distort this relationship. As discussed in the General Introduction, the concept of geometric similitude which gives rise to a predicted constant relationship between mass and surface area relies on mass being equivalent to volume within the size range concerned. This is true only where density is constant, Fat however has a specific gravity of 0.9-0.92 compared to 1.0 for the remainder of the body (Schmidt-Nielsen 1985). Thus laying down fat would increase the surface area /mass ratio and so may lead to an increase in the slope of the BMR/mass relationship. This effect would however be small. For a 200g bird which laid down 50g of fat with an assumed specific gravity of 0.9 the increase in surface area would be 6.0cm^2 compared with 5.5cm^2, if the specific gravity of the tissue increase had been consistent with the bulk of the tissues.

The increase in BMR associated with active deposition of fat may be considerable. Such an increase may be brought about by the increase in concentration and turnover of the enzymes associated

with lipogenesis and also more significantly an increase in the visceral mass. (although increase in visceral mass would imply increase in TLM, in most of the individual birds reported above there was a high level of correlation between increase in fat mass and TLM). The results of Daan *et al*, (1987) suggest that low BMR in starved Kestrels was associated with low heart, kidney and fat mass. They suggest that low BMR at low mass represents a physiological adaptation to save energy whilst the birds condition is poor.

It can also be argued that, due to the large additional cost associated with carrying extra fat loads (see Introduction to Chapter 1), a large increase in DEE must result when the fat deposits are increased. If we accept the argument that high BMR is an inevitable consequence of high DEE (Kersten and Piersma 1987), then this would "explain" why fat appears to have a "larger than expected contribution to the BMR of birds".

A consequence of the relationships between fat load and BMR is that at a period of fat acquisition, every successive gram of fat becomes more energetically expensive to obtain. For convenience I shall call this phenomenon the "law of diminishing returns" This would explain the discrepancy between predicted and achieved energy intake found for Grey Plover during the pre-migratory fattening period by Kersten and Piersma, (1987). The law would also predict that migratory birds should use several migratory staging posts which were separated by distances such that maximum fat loading was not required in order to fly between them. Of course, in some instances the benefits of maximum loading may

outweigh the energetic cost. An example of might be where arrival at the breeding grounds with substantial reserves enhances the chance of survival or a successful breeding attempt there (Evans and Davidson, 1990).

The higher the value of the slope in the law of diminishing returns, the more the maintenance of high levels of fat reserves becomes energetically unfavourable. As the slope term for Redshank is on average greater than 1 this may explain why Redshank do not maintain, or are unable to maintain, appreciable fat reserves during mid-winter.

I can offer no explanation as to why there was so much variation in the metabolic rates recorded for Sanderling and also no correlation with mass. The lack of correlation with mass does tie in however with the result for the intraspecific allometry of fat with mass given in Chapter 1. In addition, body masses of the captive Sanderling did not correlate with those found in the wild. As discussed in Chapter 1 this may be due to poor adaptation to captive conditions which may also explain high variation in metabolic rate.

CHAPTER 5

SEASONAL VARIATION IN THE BMR OF THREE SPECIES OF SHOREBIRD HELD IN CAPTIVITY DURING THE NON-BREEDING SEASON

5.1 INTRODUCTION

Seasonal variation in BMR independent of changes in body mass (but see note below) has been well documented for both mammals and birds. It has been suggested by Blaxter, (1989) that there is a tendency for depressed levels of BMR to be recorded from individuals of wild species when captured during periods in the annual cycle when food supplies are low. If this is true it may be that depressed levels of BMR are an adaptation to under nourishment (during such periods) and may also reflect a lowering of deep core body temperature (Westerterp, 1976 and Cherel *et al*, 1988). However in passerine birds Kendeigh *et al*, (1977) found that different interspecific allometric equations related BMR to mass in winter and summer, with BMR during winter being higher for the same body mass than in summer. The BMR/mass relationship for non-passerines did not differ significantly with season.

The results of Kendeigh *et al*, (1977) for passerines, obscure the high level of interspecific variation in the change of BMR with season examples of which are given below. In the House sparrow BMR has been shown to increase from September through the winter but to be low over spring and summer (Miller, 1939), but in the Brambling *Fringilla montifringilla* (Pohl, 1971), and Common redpoll *Acanthis flammea* (West, 1972) BMR is at its lowest

in the spring and Autumn. Pigeon *Columbia liva* has been observed to have a higher BMR during winter than Summer (Saarela *et al*, 1980). In the wild Turkey *Meleagris galloparo silvestris* BMR was found to be depressed during the spring only in males (Oberlag *et al*, 1990). For several other species of galliformes BMR has been found to be depressed during winter (Mortenson and Blix, 1986), although it is higher during this period in Black grouse *Lyrurus tetrix* (Rintamaiki *et al*, 1983), Blue grouse *Dendragapus obscurus* showed no seasonal change in BMR (Petkins, 1988).

There is some evidence for internal regulation of seasonal variation in BMR via photoperiod, independent of nutritional status. For sheep it has been found that there is a seasonal component to BMR described by a sine wave with an amplitude of $\pm$14%, with a maximum in summer even when food is supplied *ad libitum,* (Blaxter and Boyne, 1982). Experiments with red deer have shown that variation in BMR was associated with imposed light cycles (Argo and Smith, 1983), as also found for the Blackbird *Turdus merula* (Biebach, 1975).

Experiments have demonstrated cold induced increases of BMR in birds (Rautenberg, 1969 and Dawson *et al*, 1983). This evidence, taken together with the observation discussed previously (Chapter 4) that birds which inhabit colder latitudes tend to have higher BMRs than those of similar species which inhabit the tropics, leads to the suggestion that part of the seasonal variation in BMR may result from the seasonal variation in temperature.

Thus, from the literature it appears that there are at least three main possible external influencing factors that may correlate with seasonal changes in BMR. These are 1: Nutritional state and food availability 2: Day length and 3: Temperature, none of which is necessarily mutually exclusive.

The aim of the work presented in this chapter was to describe the variation in BMR with season for three species of shorebird: Redshank; Grey Plover and Sanderling and to examine the relationship between the variation found and environmental variables. In addition, given that for the shorebird species studied BMR was shown not to vary diurnally (Chapter 4). I have tested the hypothesis that BMR in shorebirds during the non-breding season may vary with tidal cycle.

It is important to note that in many of the examples of seasonal variation in BMR described above, the effect of mass on BMR was controlled for by dividing the BMR by the mass of the subject or subjects. This procedure, however, does not correct satisfactorily for variation that is due to mass, because it assumes that the relationship between BMR and mass is isometric (*ie.* the ratio BMR/mass does not vary with mass). This is not true. The BMR/mass relationship is known to be allometric and thus dividing BMR by mass does not remove all the variation due to mass (Packard and Boardman, 1987). In the work presented in this chapter, seasonal variation in BMR is described in terms of unit mass for the species under consideration, that is $BMR/mass^{x}$, where x is the mean mass exponent of the within-individual

variation of BMR with mass for that species. The values of x are taken from Chapter 4.

5.2 MATERIALS AND METHODS

The animals used were those individuals detailed in Chapter 4. BMR determinations reported in this chapter are those already reported in Chapter 4 except that (unless otherwise stated) variation due to mass has been taken into account by dividing BMR by $M^{1.23}$ and $M^{0.92}$ for Redshank and Grey Plover respectively. For Sanderling, variation in BMR with mass is not taken into account as this variable was not shown to contribute significantly to the overall variation in BMR (See Chapter 4).

The relationship between BMR and day length, temperature and duration of captivity was explored using multiple regression analysis. Day length was calculated from those data given in Whittaker's almanac for the city of Newcastle 54° 52'N 1° 37'E (16 miles North of Durham) and was taken to include "Civil twilight". Meteorological data were obtained from the Durham University observatory (54°46'06''N 01°35'05''E) In order to test for variation in BMR due to tidal cycle, the state of tide at time of BMR measurement was determined for Teeside using admiralty tide tables. Each test BMR determination was then ascribed as having taken place during a period of low, high or mid-tide.

5.3 RESULTS

5.3a: Sanderling

A plot of BMR versus month for the sample of Sanderling held in captivity during the duration of this study is given in Figure 5.1.(Figures 5.2a-f are similar plots for individual Sanderling). An ANOVA of seasonal variation in metabolic rate with individual month and period of tide at time of test as independent variables of BMR indicates that individual variation in BMR accounts for 56% of the explainable variation but that variation with month accounts for only 19% of total variation. There was no significant effect of state of tide at the time of test, (Table 5.1).

Table 5.1: ANOVA of BMR with individual, month and state of tide at time of test as independent variables for 6 Sanderling held in captivity during the non-breeding season

Source of variation	Sums of squares	*d.f*	Mean squares	F	Signif of F
Main effects	0.431	13	0.033	2.750	P<0.01
Individual	0.242	5	0.048	4.009	P<0.01
Month	0.171	6	0.029	2.370	P<0.05
State of tide	0.037	2	0.019	1.538	P>0.05
Explained	0.431	13	0.033	2.750	P<0.01
Residual	0.458	38	0.012		
Total	0.889	51	0.017		

Multiple stepwise linear regressions with day length, experimental day number, minimum temperature and maximum temperature on the day of test as independent variables of metabolic rate did not yield any significant relationships when

Fig 5.1 Seasonal variation in mass corrected BMR of six Sanderling n_1= the number of BMR determinations in each month and n_2= the number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

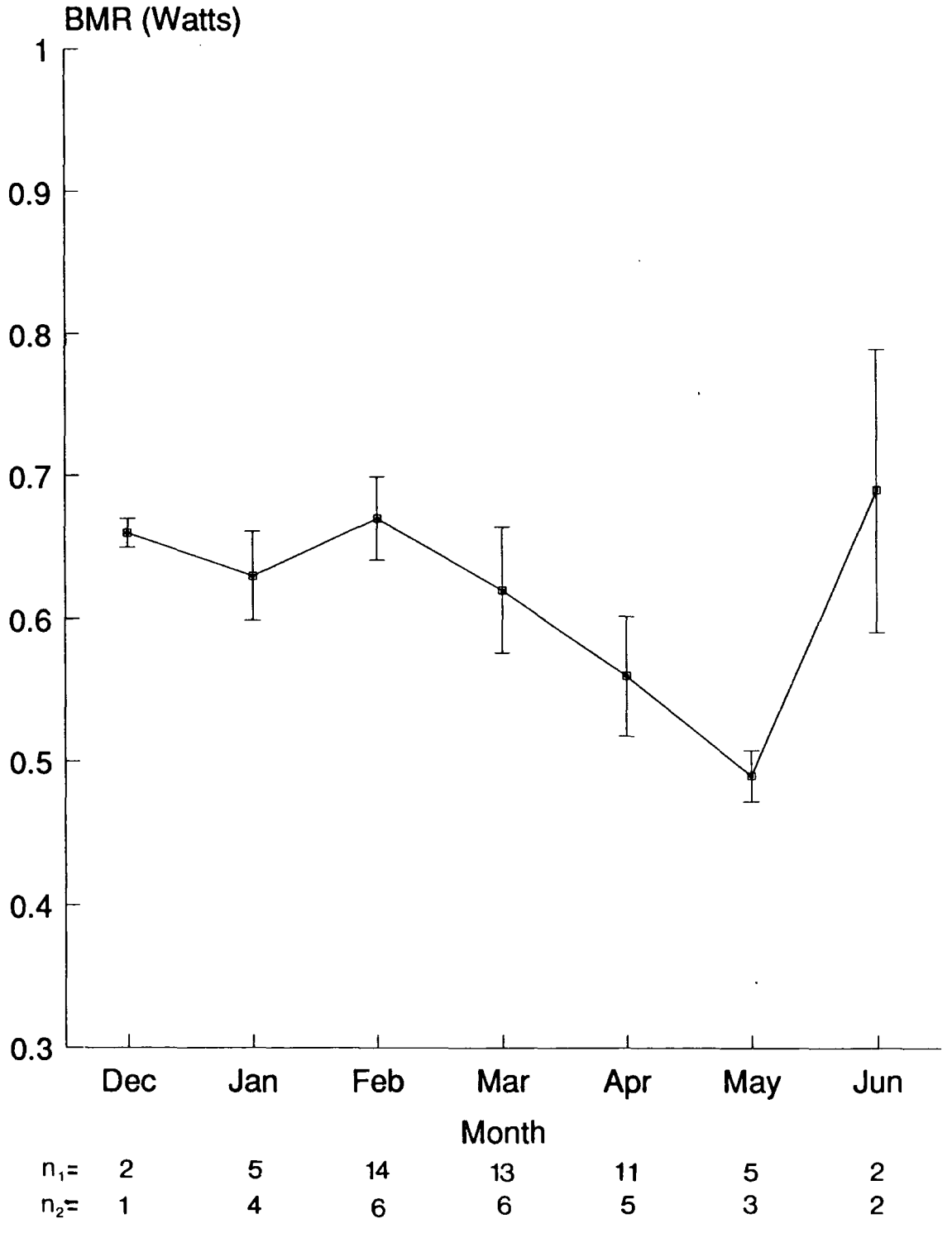
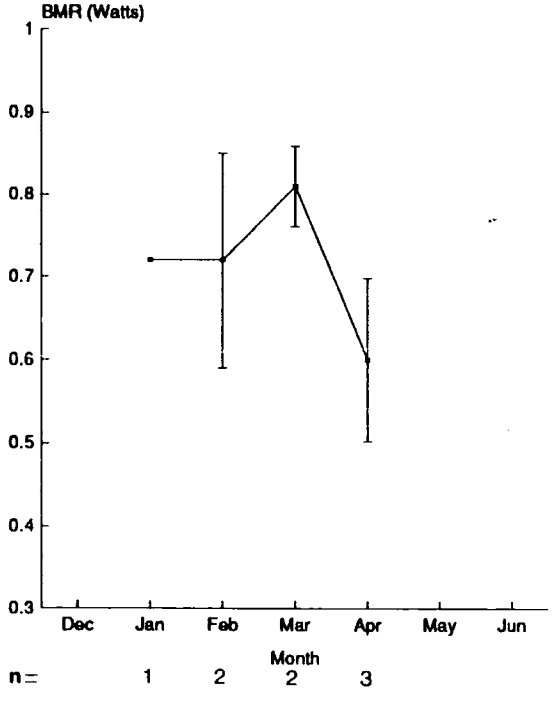
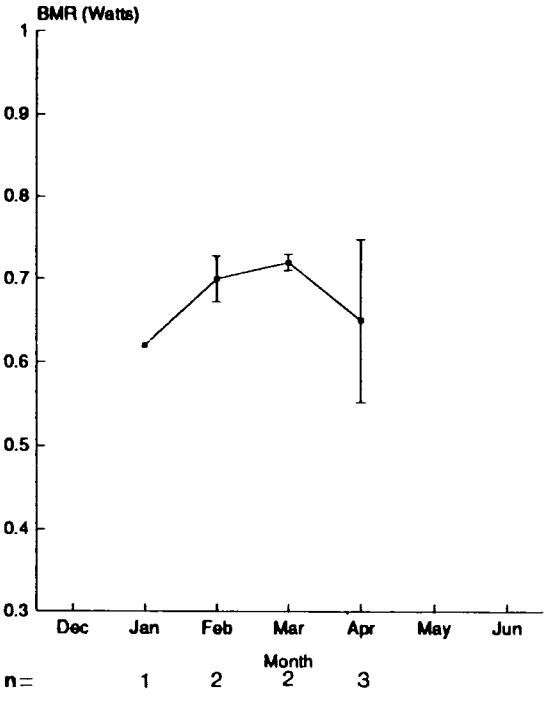
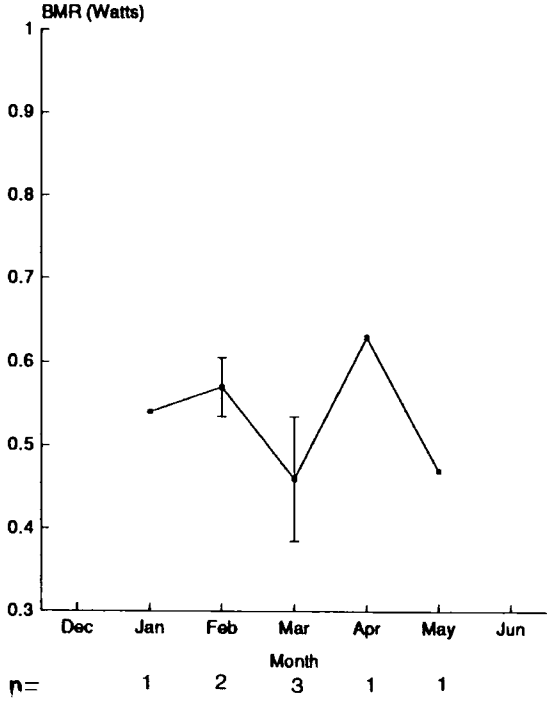


Fig 5.2a-f Seasonal variation in mass corrected BMR for six individual Sanderling. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.4 e)=bird No.5 f)=bird No.6.







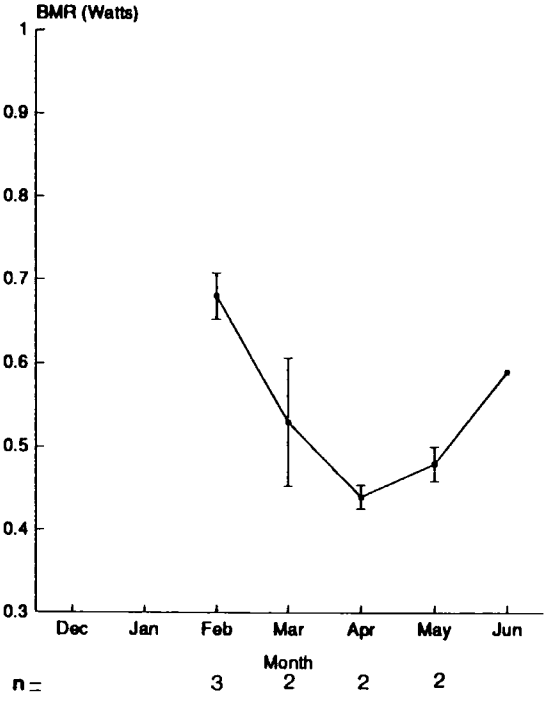
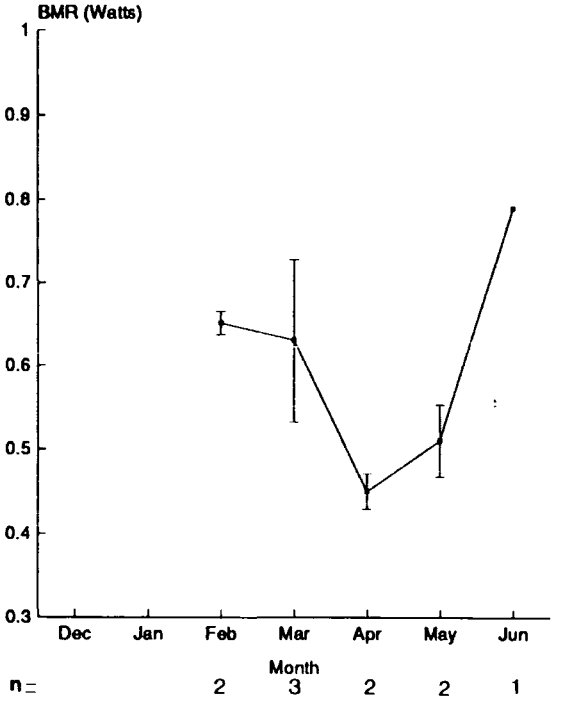
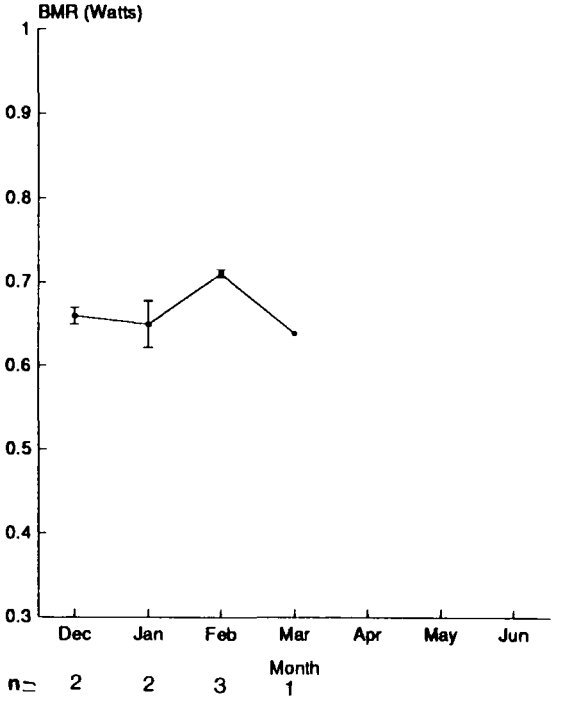


Fig 5.2 e & f


BMR (Watts)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Dec
Jan
Feb
Mar
Apr
May
Jun
Month
n=
2
3
2
2
1


BMR (Watts)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Dec
Jan
Feb
Mar
Apr
May
Jun
Month
n=
2
2
3
1

viewed at the level of the individual. This was also true when the data were pooled by month.

Significant multiple regressions were achieved only when all datum points for all individuals were regressed together. In such an analysis, day length alone, was a significant variable (r^2= 0.11 $P<0.05$). Caution is needed when interpreting the above result as the multiple regression performed could be statistically fallible due to a lack of independence between data points. However, they have been included as such regressions can indicate important trends in the data, which may not be in evidence otherwise due to low sample size. It is also obvious from figures 5.2a-f that there is a high level of variation in BMR between individuals as established by the initial ANOVA.

5.3b: Grey plover

Figure 5.3 shows a plot of $BMR/M^{0.92}$ versus month for Grey Plover and figures 5.4a-d are similar plots for individual Grey Plover. ANOVA of seasonal variation in mass-corrected BMR (with individual, month and state of tide at time of test as independent variables), indicates that variation amongst individual accounts for 49% of the explainable variation (Table 5.2). Variation in body-mass corrected BMR with month and state of tide at time of BMR determination does not contribute significantly to the overall variation.

Fig 5.3 Seasonal variation in mass corrected BMR for four Grey Plovers n_1= the number of BMR determinations in each month and n_2= the number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

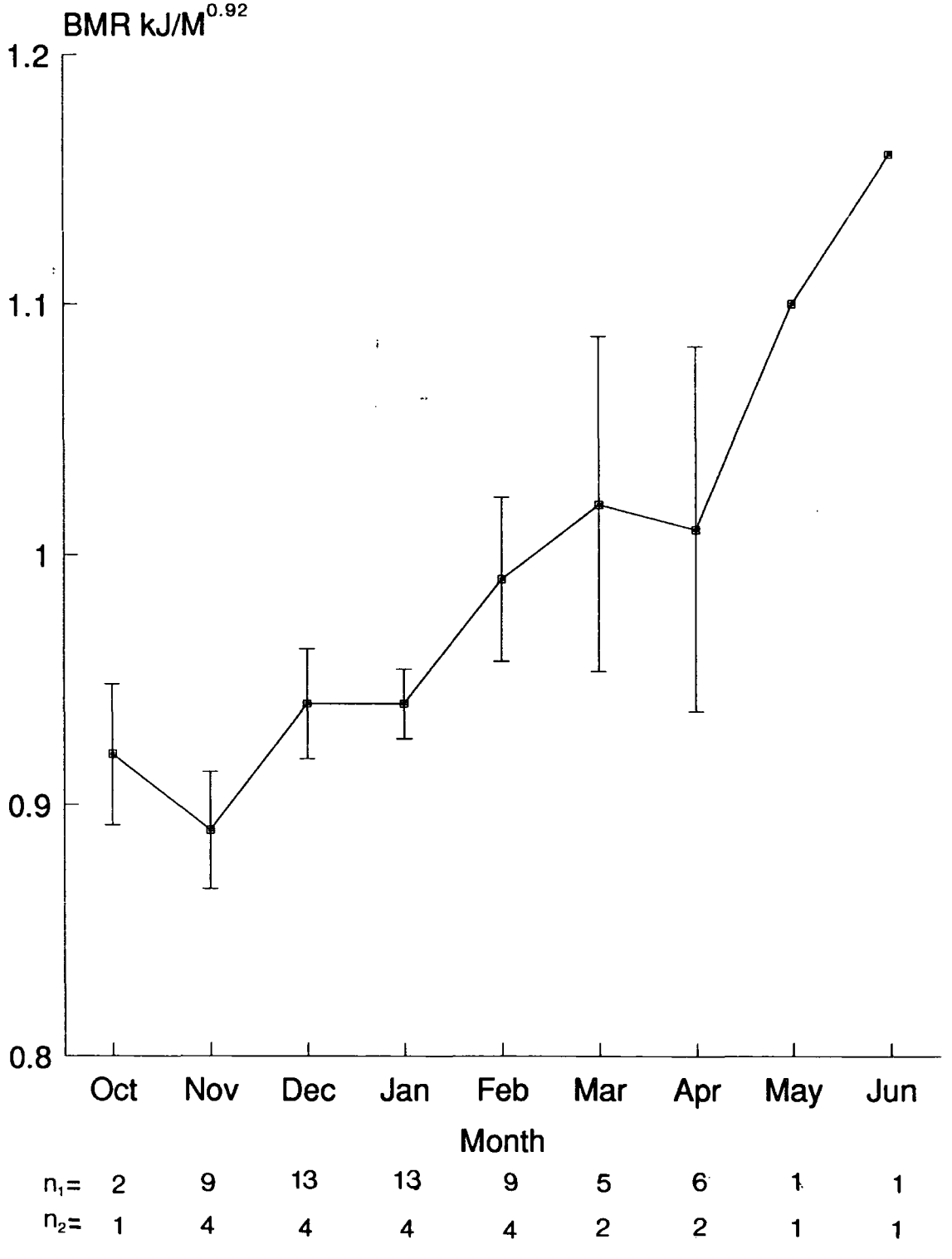
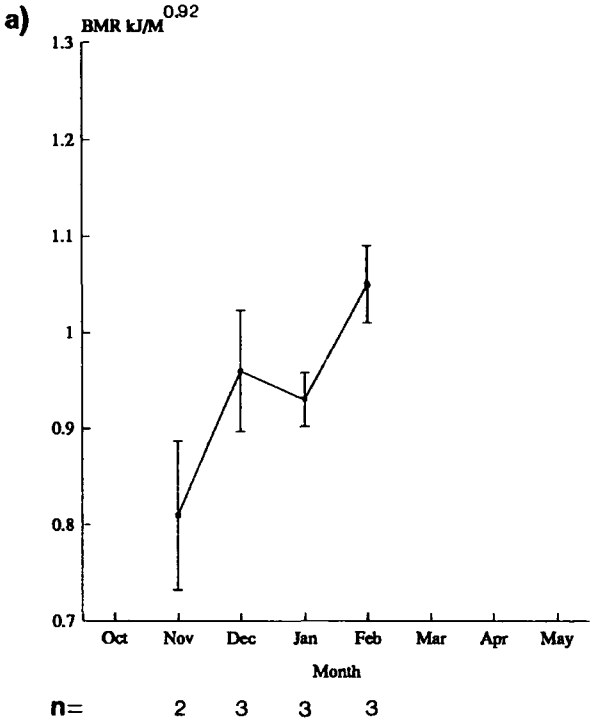
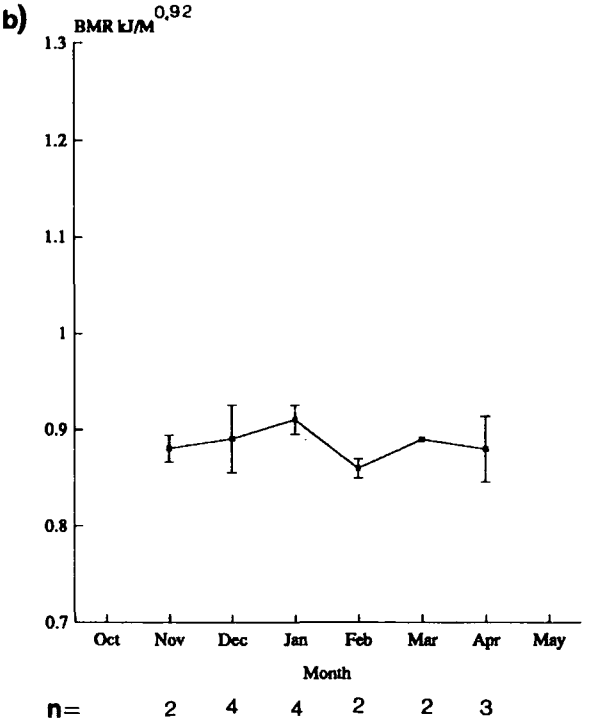
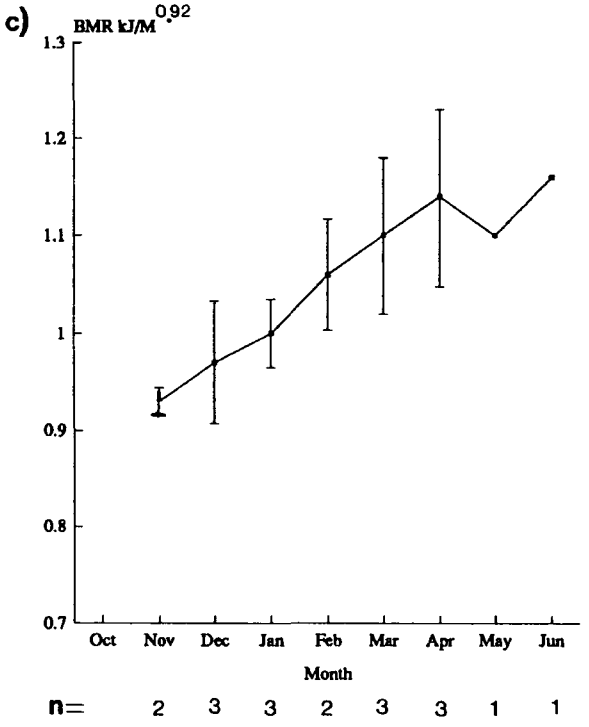


Fig 5.4a-d Seasonal variation in mass corrected BMR for individual Grey Plovers. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.5







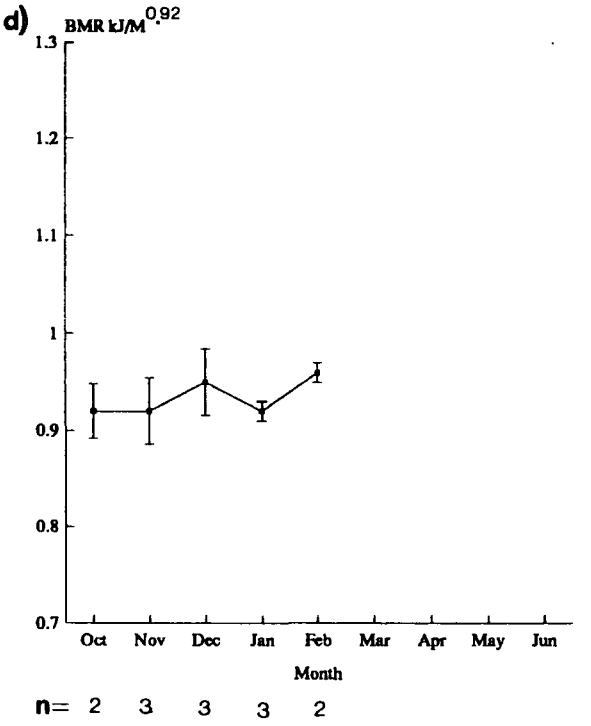


Table 5.2: ANOVA of Mass-corrected with individual, month and state of tide at time of test as independent variables for 4 Grey Plover held in captivity during the non-breeding season

Source of variation	Sums of squares	*d.f*	Mean squares	F	Signif. of F
Main effects	0.336	13	0.026	3.784	P<0.001
Individual	0.165	3	0.055	8.064	P<0.001
Month	0.093	8	0.012	1.712	P>0.05
State of tide	0.002	2	0.001	0.143	P>0.05
Explained	0.336	13	0.026	3.784	P<0.001
Residual	0.307	45	0.007		
Total	0.643	58	0.011		

Multiple stepwise linear regression analysis with day length, experimental day number, minimum temperature and maximum temperature on day of test as independent variables of mass-corrected indicate that for individuals 1 and 2 there is a significant relationship with minimum temperature on day of test, (r^2=0.47 $\underline{P}$<0.002 and r^2=0.29 $\underline{P}$<0.05 for individuals 1 and 2 respectively). For Grey plover No.3 there is a significant regression with duration of captivity r^2=0.42 $\underline{P}$<0.05). No significant regressions were detected for individual No.5.

When data are pooled by month, multiple regression analysis, using the variables detailed above, reveals duration of captivity as the only significant correlate (r^2=0.89 $\underline{P}$<0.001). This is also clearly shown by figure 5.3. Duration of captivity is also selected as the only significant correlate when all data points are regressed together (r^2=0.20 $\underline{P}$=0.001). Although, when variation due to mass is not taken into account, however, day length is selected as the most important variable (r^2=0.13 $\underline{P}$<0.01). The discrepancy in the above results is due to the co-variance of body mass with day length for the sample of Grey Plover (r=-0.63). Thus some of the variation which may be

accounted for by change in day length is removed when variation in BMR due to mass is taken into account.

5.3c Redshank

Figure 5.5 shows a plot of BMR/body mass$^{1.23}$ versus month for the sample of Redshank held in captivity during the duration of the study presented here. Figures 5.6a-j are similar plots for individual Redshank. Three ANOVAs of seasonal variation in mass-corrected BMR with (1) month and race, (2) month and captive group and (3) month, individual and state of tide at time of BMR determination, indicate that of these variables only individual variation is significant, explaining 29% of the total variation in mass-corrected BMR (Tables 5.3-5.5)

Table 5.3: ANOVA of mass-corrected with Race and Month as independent variables for 10 Redshank held in captivity during the non-breeding season

Source of variation	Sums of squares	*d.f*	Mean squares	F	Signif. of F
Main effects	0.003	7	0.0004	2.756	P<0.02
Month	0.003	6	0.0005	3.279	P<0.01
Race	0.0001	1	0.0001	0.341	P>0.05
2 way interacts.					
Month and Race	0.001	6	0.0002	1.326	P>0.05
Explained	0.004	13	0.0003	2.149	P<0.02
Residual	0.013	85	0.0002		
Total	0.017	98	0.0002		

Fig 5.5 Seasonal variation in mass corrected BMR for ten Redshank n_1= the number of BMR determinations in each month and n_2= the number of individuals. Horizontal bars represent 2x the standard error of the sample mean.

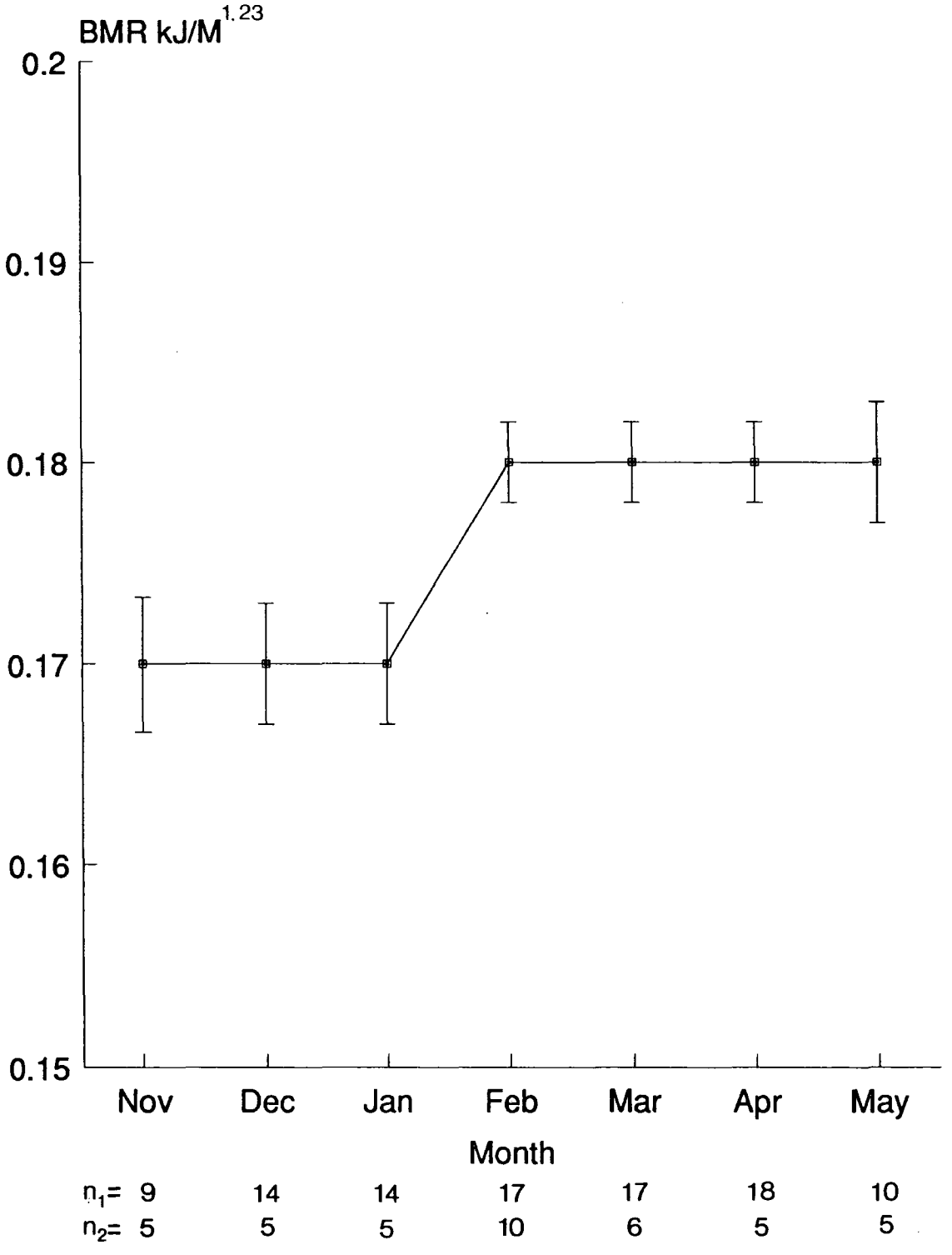
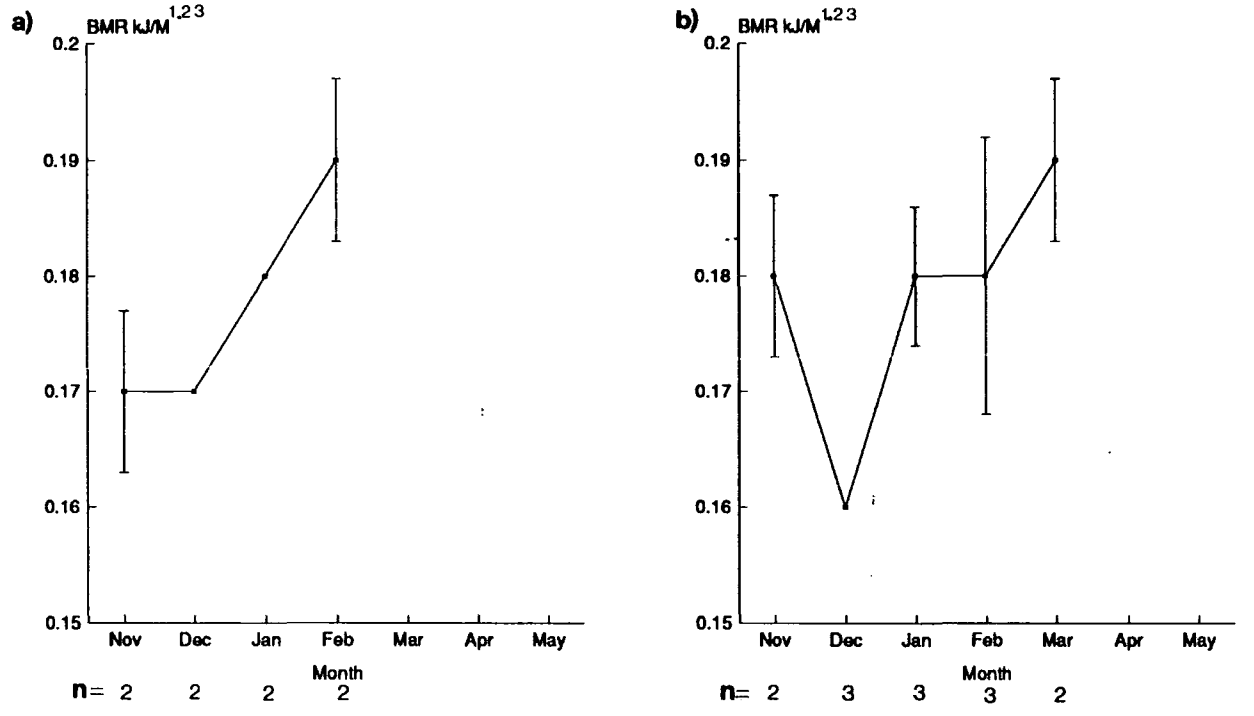


Fig 5.6a-j Seasonal variation in mass corrected BMR for ten individual Redshank. Horizontal bars represent 2x the standard error of the sample mean. a)=bird No.1 b)=bird No.2 c)=bird No.3 d)=bird No.4 e)=bird No.5 f)=bird No.6 g)=bird No.7 h)=bird No.8 i)=bird No.9 j)=bird No.10



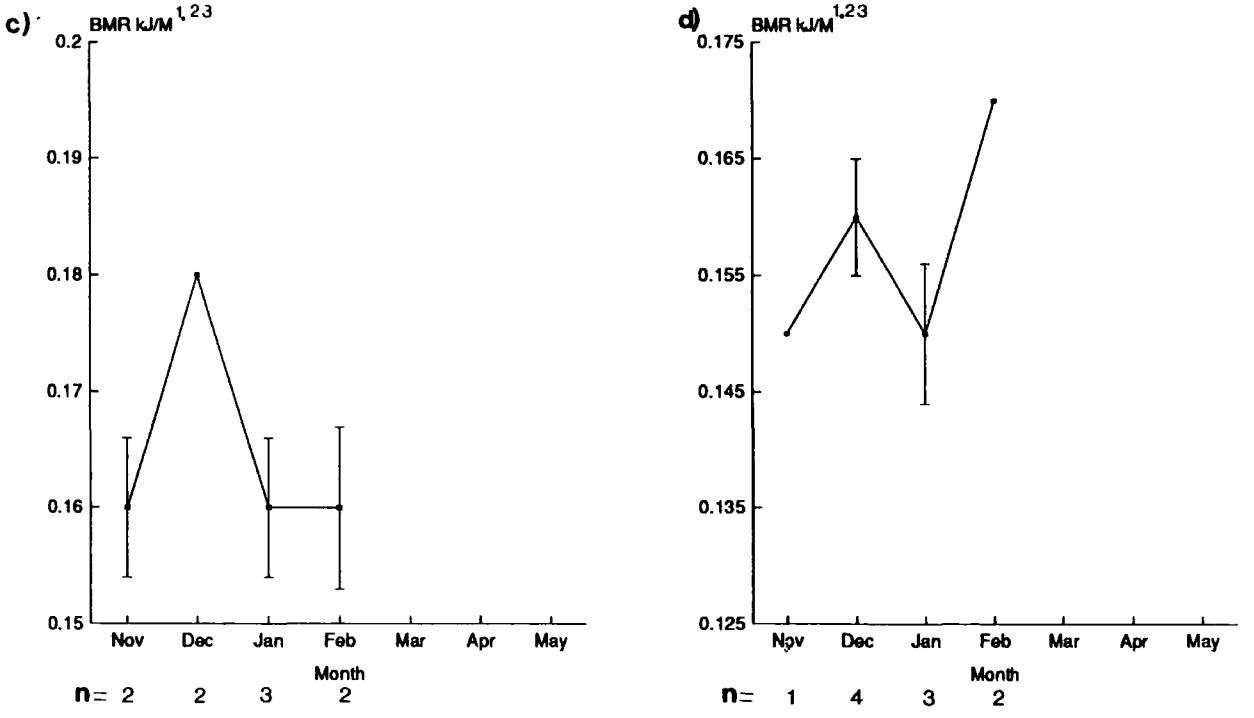
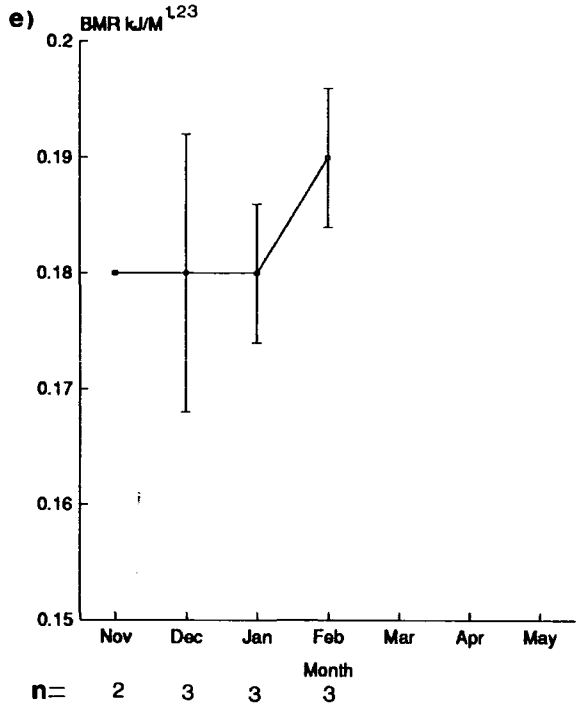


Fig 5.6 e-h


e)
BMR kJ/M
1,2 3
0.2
0.19
0.18
0.17
0.16
0.15
Nov
Dec
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Feb
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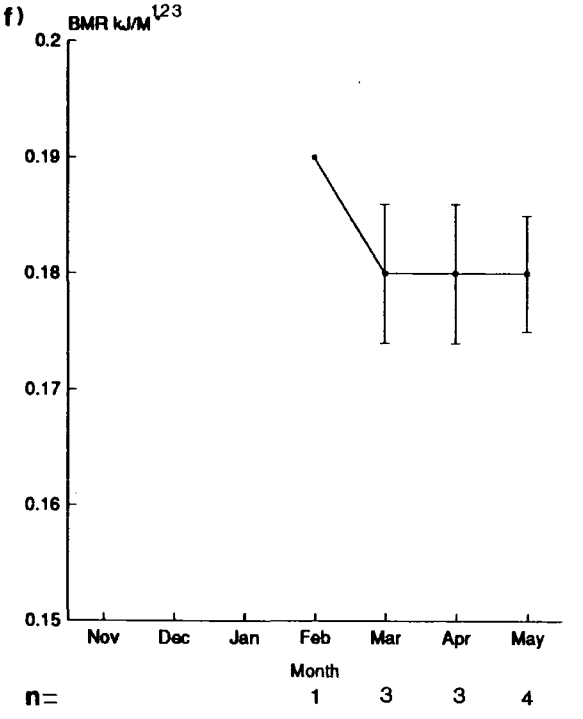
Apr
May
Month
n=
2
3
3
3


f)
BMR kJ/M
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0.2
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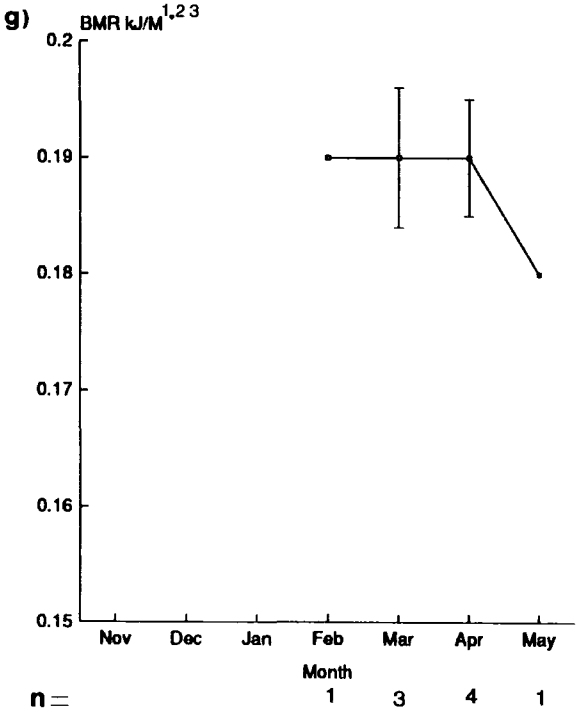
g)
BMR kJ/M
1,2 3
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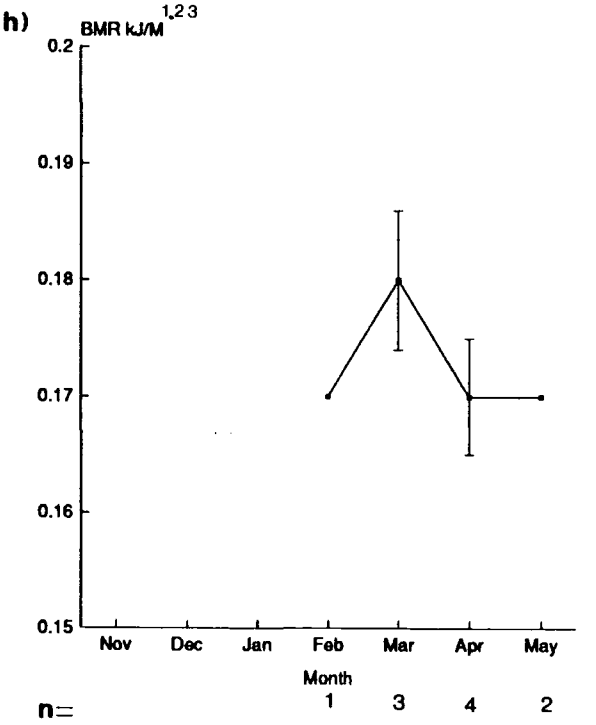
1


h)
BMR kJ/M
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0.18
0.17
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n=
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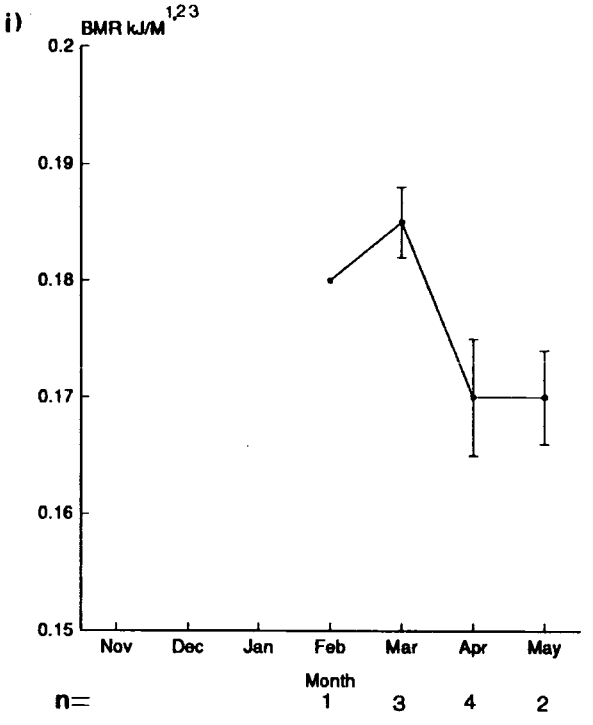
2

Fig 5.6 i & j


i)
BMR kJ/M 1,2,3
0.2
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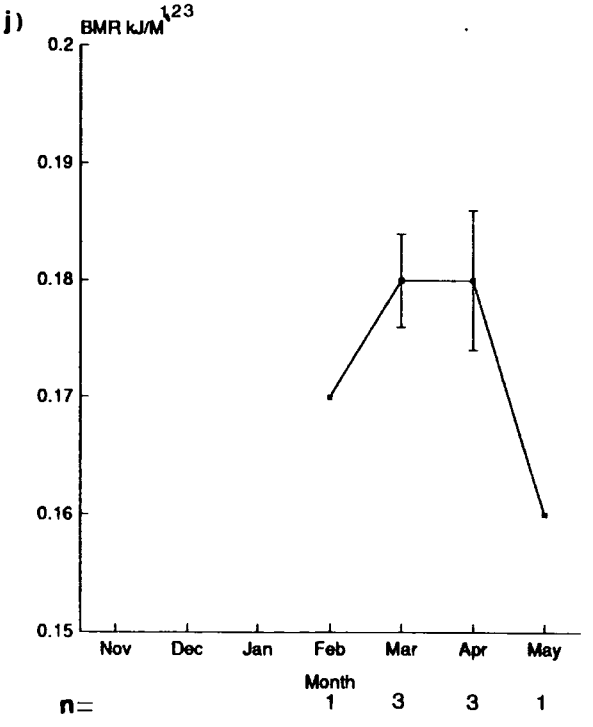
j)
BMR kJ/M 1,2,3
0.2
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0.18
0.17
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1

Table 5.4: ANOVA of mass-corrected with Capture group and Month as independent variables for 10 Redshank held in captivity during the non-breeding season

Source of variation	Sums of squares	*d.f*	Mean squares	F	Signif. of F
Main effect	0.003	7	0.0004	2.756	P<0.02
Month	0.002	6	0.0003	1.844	P>0.05
Group	0.0001	1	0.0001	0.018	P>0.05
2 way interacts.					
Month and Group	0.0001	1	0.0001	1.516	P>0.05
Explained	0.003	8	0.0004	2.601	P<0.02
Residual	0.013	90	0.0001		
Total	0.017	98	0.0002		

Table 5.5: ANOVA of mass-corrected with Month, individual and state of tide at time of test as independent variables for 10 Redshank held in captivity during the non-breeding season.

Source of variation	Sums of squares	*d.f*	Mean squares	F	Signif. of F
Main effects	0.009	17	0.001	0.285	P<0.001
Month	0.001	6	0.0002	1.978	P>0.05
Individual	0.005	9	0.0010	6.122	P<0.001
State of Tide	0.0001	2	0.00005	5.285	P>0.05
Explained	0.009	17	0.001	5.285	P<0.001
Residual	0.008	81	0.0001		
Total	0.017	98	0.0002		

Multiple stepwise regressions with day length, experimental day number, minimum temperature and maximum temperature on the day of test as independent variables of mass-corrected BMR indicate for individuals 1 and 2 that there is a significant linear relationship with day length (r^2=0.76 $\underline{P}$<0.005 and r^2=0.56 $\underline{P}$<0.05 for birds 1 and 2 respectively). No other significant regressions were found for any other individual. This was also true when data were pooled by month. When all data points were regressed together, duration of captivity was selected as the only significant variable (r^2=0.08 $\underline{P}$>0.05). It should be noted that there was also a high level of co-variance between duration of captivity and day length (r=0.95).

5.3d Effects of seasonal variation in body composition on seasonal variation in BMR

Multiple stepwise regression analysis has been performed with log TLM, log fat mass, day length, minimum temperature and maximum temperature on day of test as independent variables of BMR (uncorrected for body mass), for ten Redshank and one Grey Plover for which predictions of fat mass and TLM have been obtained using TOBEC. For all individuals except Redshank No.1 significant independent variables and thus regression equations are as those given in Table 4.10 of chapter 4. For redshank No.1 day length was selected as a significant variable, the regression equation for this individual is given below.

		Independents	
Dependant		**Log TLM**	**DL**
log BMR			
	Beta	0.944	0.206
	T	22.37	4.89
	P	<.0001	>0.01

Multiple r^2= 0.99

Significant equation= LogBMR= -0.505+0.245LogFat+0.0002DL
F=283.5 P<0.0001
where DL=day length

The above results are consistent with those given in sections 5.3a-c, where variation in BMR was body mass corrected. This is because as shown in chapter 1 & 2(Tobec) most variation in body-mass is due largely to variation in fat mass and as shown in chapter 4 within-individual variation in BMR with body mass correlates in most cases with variation in the fat mass carried. Thus, dividing BMR by the slope of the relationship of BMR/ body mass controls for variation in BMR due to fat mass.

5.4 DISCUSSION

The work presented in this chapter does not fully corroborate any one of the theories put forward in the introduction to explain seasonal variation in metabolic rate. On a month by month basis there is no evidence to suggest its occurrence. There is some evidence of correlation between seasonal variation in BMR and the variables suggested as being potentially important in the introduction to this chapter. When the data are pooled there is an indication that day length may be an important factor in determining seasonal variation in BMR. At an individual level, however, only the results from Redshanks 1 & 2 support this hypothesis and, as discussed previously, regressions which are based upon multiple measurements from a few individuals violate the assumption of independence of data points which underlie regression analysis (Sokal and Rohlf, 1982) The results must be treated with caution, therefore, particularly when there is a high level of co-variance between some of the independent variables. It could be that low numbers of BMR measurement for some individuals leads to results which are not significant and that the real relationship is portrayed by pooled data, but that the analytical result fails on a technicality. However the data presented graphically do not show an obvious trend in mass-corrected BMR for all individuals of any one species. It is more likely, therefore, that the results obtained from the pooled data do not reflect biological reality.

It is particularly difficult to interpret the results that two of the Grey Plover studied show a correlation between minimum

temperature on the day of test and mass-corrected BMR and another individual shows a correlation between mass corrected BMR and duration of captivity. This result may reflect genuine individual variation in response to environmental stimuli. Increased mass-corrected with duration of captivity may reflect increasing levels of stress displayed in individual No.3. Although I have no observational evidence to suggest that this bird was showing increased levels of stress, or more stress than any other Grey Plover. The correlations of mass-corrected BMR of individuals 1 & 2 with minimum temperature are weak particularly considering that variation due to body mass had been removed prior to analysis.

For the Redshank and Grey plover used during the study presented here variation in BMR with body mass accounts for, on average, 67% and 63% of the total variation in BMR respectively. Thus, any weak correlates or variables which show covariance with mass will not be shown by the analysis as being significant once variation due to mass has been taken into account.

The results obtained above are broadly consistent with the hypothesis of Biebach (1975), who suggested that in outdoor aviary studies where birds are exposed to both changing temperature and photoperiod, a seasonal variation in BMR is not apparent. He further suggested that this was due to short photoperiod causing depressed BMR at the same time as low ambient temperature elevates the BMR. During the study presented here some individuals appear to have responded to low ambient temperature, others to photoperiod and one to experimental day number, whilst the remainder show no obvious relationship between

BMR and external stimuli. Lack of correlation with external stimuli may reflect a high level of individual variability in response to external stimuli, and the increasing degree to which experimental error will be important in explaining variability, following the removal of a significant proportion of the variation which was attributed to body mass.

No variation in BMR with tidal cycle was found for any of the three species studied. This result may reflect the loss of the external stimuli (*ie.* tidal cycles) which may of given rise to the hypothesized tidal variation in BMR, rather than a result which would occur in the wild.

Those data presented in this chapter do not permit the dismissal of seasonal variation in BMR in shorebirds during the non-breeding season. It would appear, however, that variation among individual in mass independent BMR is more important in explaining overall variation in BMR than variation between months.

Verification of seasonality, or otherwise, in the BMR of shorebirds during the non-breeding season will require the use of larger sample sizes and as suggested by Biebach, (1975) the experimental control of environmental variables.

GENERAL DISCUSSION

During this study, for two species of shorebird Redshank and Grey Plover, seasonal body mass variations observed in the wild were similar to those observed in captivity, For Grey Plover the results were consistent with the hypothesis of internally regulated winter fat storage, those for Redshank were difficult to interpret, part because of a lack of data concerning the races of Redshank caught in the wild and also because of the escape of one group of captive birds. The captive Sanderling however displayed a pattern of seasonal mass variation which differed from that which Sanderling display in the wild. As the reaction of the birds to captivity may have been a reason for this difference, it is not possible to accept or reject the hypothesis of internally regulated fat storage for Sanderling. This problem with captive studies is discussed below.

Seasonal variation in body mass of Grey Plover and Sanderling in the wild was shown to be consistent with that for other shorebirds species, wintering in northern latitudes (Davidson, 1981a). The difficulties encountered in analysis of data from Redshank in the wild, however high-lights the need for amateur ornithologists to be made aware of the appropriate biometrics to record from birds they handle, as the inability to ascribe birds to a particular geographical race severely limited the interpretation of the data.

The majority of the seasonal variation in body mass of the three species studied could be attributed to variation in the

extent of the fat deposits, this as found by other workers (eg. Blem 1976). This explanation held for both wild birds, analysed destructively (Sanderling and Grey Plover) and captive birds analysed non-destructively by TOBEC.

A large difference was demonstrated between the oxygen uptake of adipose tissue, which was relatively low and that of liver and skeletal muscle *in vitro*. Despite the difference in metabolic output between lean and adipose tissues, increases in body mass in individual Redshank and Grey Plover generally were correlated with increases in BMR which were much larger than would be expected, based on *in vitro* determinations of the oxygen uptake of adipose tissue and given that most variation in body mass was attributable to variation in adipose tissue. Further interpretation of these results is hampered by inter-correlation between fat mass and lean mass and also by the lack of TOBEC measurements of TLM for the captive sample of Grey Plover, (these were the group of captives which showed the largest variation in total body mass). It had been hoped that in the final year of the study it would have been possible to obtain substantially more information from Grey Plover than was achieved. Unfortunately few juvenile Grey Plovers arrived at the Tees estuary and those that did took up roosting sites during the winter of 1989/90 where they were difficult to catch despite considerable efforts. The single Grey Plover which was caught was taken from its feeding territory. Intraspecifically BMR was shown to be highly variable, and the consequence of this, as regards the various theories put forward to explain the BMR/body mass relationship are discussed in Chapter 4.

Comparison of the BMR/body mass relationship and the fatmass/body mass relationship in mid-winter for *Charadrii* indicate that, as for mammals, larger species have a greater capacity for starvation endurance than smaller species, although the advantage gained by larger shorebirds is less than that gained by large mammals (see Chapter 4). The increased starvation endurance, taken with other advantages of being large, such as increased metabolic efficiency and a general ability to win competitive encounters (Branson, 1964 and Grant, 1969), would suggest that there should an evolutionary pressure towards increase in size. Stability in body mass must therefore be brought about by opposing benefits of being smaller, such as reduced absolute food demand and for shorebirds, increased maturation rate of young which may be of significance to the many arctic breeding shorebirds where the breeding season is very short.

No definite evidence was found of seasonal variation in mass independent metabolic rates, as indicated in Chapter 5. The results obtained were equivocal and as such the investigation would have been improved by increased frequency of testing of individuals throughout each season, This is particularly necessary given the high level of individual variability found in BMR. The investigation of seasonal variation in BMR would also have been enhanced by an increased level of experimental manipulation, such as controlling photoperiod and or temperature. However, this might have interfered with changes in body mass and

body composition and serves to high-light the difficulties of working with limited numbers of captive animals.

Studies carried out upon wild animals held in captivity are frequently criticized as producing results which do not appertain to the field situation. Reasons such as change in behaviour, diet and increased stress are given as untested suggestions why this may be so. It is probably more accurate to say that, in general, captive studies can only corroborate a hypothesis, as the experimental null hypothesis must invariably be weak due to the unknown effects of captivity. For example, during the study presented here, the "internally regulated hypothesis" of winter fat deposition was tested by comparing the pattern of body mass change observed in the wild with that in captivity, the null hypothesis being that "body mass is not internally regulated," and so in birds fed on an *ad libitum* diet, body mass should remain relatively stable. However, if the pattern of body mass change does not fit the proposed hypothesis, as was found for Sanderling, it is not possible to accept the null hypothesis because it is feasible to imply that the results were due to "effects of captivity." Where the results agree with the hypothesis (as was found for Grey Plover) they can be said to be supportive. Strong (1980) points out that in ecological studies the null-hypothesis is seldom explicitly stated and consequently erroneous conclusions are drawn from work which is corroborative and ignores the null-hypothesis.

In the work I have presented on captive individuals and seasonal variation in body mass/BMR, I have not ignored the null

hypothesis, but have recognised the weakness that is introduced by the "captivity factor." It may be necessary to recognise, in studies carried out upon captive wild animals where the "captivity effect" is unknown or cannot be controlled for, that hypothetico-deductive science (*sensu* Popper, 1968), which implies that falsification of scientific theories is always possible, cannot take place. Such studies can be used only to corroborate a hypothesis deduced from the wild situation.

To some extent the need to bring wild birds into captivity to investigate seasonal variation in metabolic rate may be avoided by the use of the doubly labelled water technique for determining daily energy expenditure in the field (for review see Bryant, 1988). However considerable difficulties still exist for the application of this technique to many species of bird during the non-breeding season, because each individual under test must be caught more than once and many species are adept at evading capture.

Questions concerning the applicability of
data from captive animals to the field situation do not apply to the investigation of the BMR/body mass relationship, because in most instances Basal conditions can only be secured when an animal is held in captivity. Measurements of average daily energy requirements, of birds, by the doubly labelled water technique however give a body mass exponent which is not significantly different from that of the BMR/mass relationship (Nagy, 1987), This lends support to the applicability of the laboratory

determined BMR/body mass relationship to explain field based observations and biological phenomena outside the laboratory.

The theoretical interpretation and understanding of the interspecific BMR/body mass relationship (Brody-Klieber "law"), has I believe, been misled by prior knowledge of the empirically-produced results by people developing hypotheses to interpret the results. If one knows that the mass exponent "should be" 0.75, then a theory is evolved in which the answer is 0.75; the theory is thus seemingly untestable, as it already fits the data. This method is a valid scientific procedure according, to Haila and Jarvinen, (1982) but only when the hypothesis generated becomes falsifiable when it is extended outside the domain of the original data. In the case of the Brody-Klieber relationship, most new interspecific BMR data that has been obtained recently lay within the confidence limits of the original data, and the new data were not collected with the aim of testing hypotheses put forward to explain the mass exponent. Outlying data points were classed as exceptions and so all hypotheses put forward to explain the mass exponent of 0.75 stood. It was not until 1982 that a rigourous attempt was made by Heusner to re-examine the data and to define the mechanisms and initial conditions that led to the assumption of regularity and thus a "biological law". It has become obvious that attempts to place functional meaning on the BMR/body mass relationship should have commenced at the intraspecific and not the interspecific level.

At an applied level, for the purposes of the preparation of energy budgets from allometric equations, it is apparent from the

relationship I found between BMR and body mass within an individual that, at any point during the non-breeding season, total body mass is the best predictor of BMR, not total lean mass. It should be emphasized, however that due to the high level of individual variation in BMR, and to some extent because of the variability around the interspecific regression line of the BMR/mass relationship, that values obtained from such allometric equations should be treated with great caution. The level of individual variation displayed by the animals I kept was higher than I had anticipated and, as pointed out by Bennett, (1987) care should be taken not to submit to the tyranny of the golden mean.

I would contend that the key to understanding the Brody-Klieber BMR/mass relationship lies with the source(s) of variation in the mass exponent at the intraspecific and within individual levels. Functional interpretation of BMR/mass equations should be performed at these levels taking into account within-individual variation in body composition. Thus further studies of BMR are required to investigate the causes of individual variability in both BMR and the BMR/body mass relationship. This could be achieved by intensive studies on a few individuals with monitoring of such factors as body temperature and blood hormone levels. The use of TOBEC or other non-invasive methods of assessing changes in body composition will also be useful. Further investigation into the causes of variation in the mass exponent, amongst differing species at the intraspecific level may prove profitable in elucidating fundamental principles of

biological design, particularly if a comparative approach is taken.

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