METABOLISM AND PERFORMANCE:

A STUDY OF PROVISIONING IN THE TREE SWALLOW,

Tachycineta bicolor

by

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ABSTRACT

One goal of evolutionary physiology is to relate phenotypic variation to Darwinian fitness via organismal performance. Within this framework, I used breeding tree swallows, (*Tachycineta bicolor*) to identify physiological correlates and potential fitness consequences of inter-individual variation in parental energy expenditure (sustained metabolic rate, SusMR).

I measured parental SusMR using the doubly labelled water technique and correlated it with variation in natural brood size and nestling growth rate and mass. SusMR was independent of natural brood size, although large broods had greater mass gain than small broods. I hypothesized that parental efficiency increases with brood size. Among adults rearing the same sized broods, SusMR increased with brood mass, and in one year, female SusMR and nestling growth rate were positively correlated. Natural selection is defined as correlation between variation in a phenotypic trait and variation in fitness. If nestling mass or growth rate are accurate indices of fitness, SusMR was under selection in this population.

Individuals with high SusMR had relatively large intestines; presumably increasing digestive capacity. This may result in an increased resting metabolic rate and identify a potential energetic trade-off. I determined the influence of body composition on resting oxygen consumption rate ($\dot{V}O_2$). The mass of most organs differed between breeding seasons, possibly due to environmental conditions. Individuals with high resting $\dot{V}O_2$ had large kidneys but relatively small intestines. The basis of a negative relationship is unclear because the intestine contributes positively to $\dot{V}O_2$ in other species.

A major determinant of parental life-time reproductive success is the survival of offspring to breeding. This is influenced by the quality of the rearing environment and its affect on offspring condition. Few studies have investigated what physiological and biochemical characters underlie variation in condition. I manipulated the number of nestlings in a brood and followed growth and resting $\dot{V}O_2$ until near fledging. Surprisingly, many

characters were insensitive to environmental variation. Nonetheless, nestlings in reduced broods had a greater mass of lipid, increased cardiac enzyme activity, and higher size-specific resting $\dot{V}O_2$ than individuals raised in enlarged broods. How these characters affect survival or the future adult phenotype remains unknown.

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PREFACE

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CHAPTER 1

GENERAL INTRODUCTION

Over 140 years ago, Darwin proposed that variation among species was the evolutionary result of natural selection acting on phenotypic variation among individuals. Despite the fact that variation is the cornerstone upon which the theory of evolution was built, it is only over the past 20 years or so that studies have investigated the extent of physiological variation that exists among members of the same population.

Strictly speaking, inter-individual variation in physiological characters has been recognized and studied for over 75 years (Prosser 1955). Such studies begin with the observation that populations of the same species experience different environmental conditions due to geographic location. The average value for a character of interest is reported for each population, with an associated standard deviation or error to indicate the degree of confidence in the values. Although these types of studies investigate members of a single species, biological differences they identify are really among populations rather than among individuals *per se* (Garland and Adolph 1991).

A description of central tendency is useful for addressing numerous questions concerning function. Such a design, however, ignores and indeed attempts to minimize an additional source of variation: that detectable among individuals within each population (Bennett 1987). The realization that repeatable and heritable variation is necessary for evolution via natural selection has resulted in increased interest in the causes and consequences of what has traditionally been viewed in many physiological studies as "noise" (Bennett 1987).

In this thesis, I focused on the variation that exists among individuals within a single population of birds. I looked for physiological correlates of sustained performance and attempted to link these via behavioural traits with Darwinian fitness. Accordingly, when

referring to intra-specific or inter-individual variation, I follow Darwin's definition, that of "...differences...observed in the individuals of the same species inhabiting the same confined locality" (Darwin 1888, p34).

In this introduction, I first review the organismal performance paradigm of Arnold (1983) and introduce approaches to studying inter-individual variation. As most work on performance to date has focused on non-sustainable measures of energy expenditure (e.g., sprinting or endurance), I review some of these studies before introducing the concept of sustained energy expenditure. I introduce breeding birds as a model system with which to study the evolution of sustained energy expenditure, and discuss the potential importance of the rearing environment in determining phenotypic variation. Finally, I introduce my study system and identify the aims of each research chapter.

Organismal performance paradigm

Over the past 20 years, the study of inter-individual variation has increased, particularly at the physiological level. Much of this is due to the influential papers of Lande and Arnold (1983), Bennett (1987) and the formulation of the "organismal performance paradigm" (Arnold 1983). This paradigm provided a method by which selection on morphological traits could be measured in natural populations. In its original formulation the paradigm focused on morphology, however, it can equally be applied to structure, physiology and behaviour. As outlined by Arnold (1983), the task of measuring selection on physiological traits can be broken into two parts: (1) the measurement of the effects of physiological variation on performance and (2) identifying relationships between performance on fitness. This paradigm was later modified by Garland and Carter (1994) to include additional terms within the causal pathway (e.g., behaviour).

Figure 1.1 outlines the organismal performance paradigm. In this version, an individual's genotype and its environment during development and ontogeny interact to

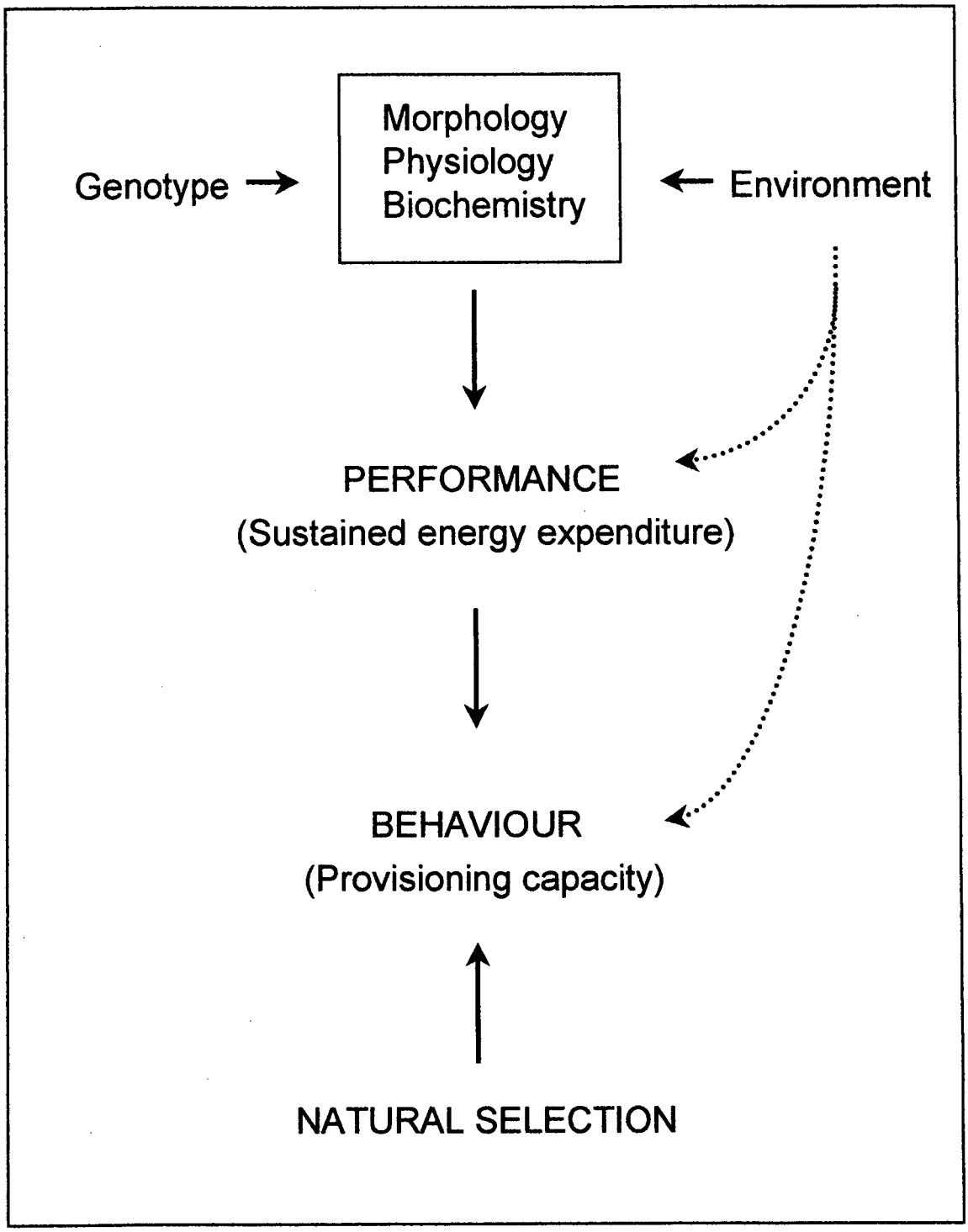


Figure 1.1 Organismal performance paradigm. The bracketed terms are examples of a performance and a behaviour found in my study (after Garland and Carter 1994).

determine its primary phenotypic characters. These characters are defined simply by the broad terms "morphology, physiology and biochemistry." These characters are assumed to act individually or together to set the upper limits on performance capacity. An example of a biochemical character would be the activity of hexokinase, an enzyme exerting considerable control over capacity for flux through glycolysis (e.g., Kashiwaya et al. 1994). Capacity for flux though a metabolic pathways likely limits an organism's performance abilities under various circumstances (e.g., Suarez 1996).

Organismal performance is an extremely broad concept representing numerous different activities. For example, in his review Pough (1989) identified over 120 studies falling into five categories of performance: (1) forced activity, (2) social behaviour and reproduction, (3) natural activity and foraging, (4) predatory activity and feeding, and (5) defensive behaviour. Within the evolutionary physiology literature, organismal performance has been most frequently used to describe the first two. However, regardless of the activity that it describes, organismal performance defines the limits of what an individual is capable of, while 'behaviour' defines what an individual actually does (Garland and Carter 1994). For example, although a female mouse may be physiologically capable of feeding 14 pups (Hammond and Diamond 1992), this may never occur in nature due to environmental constraints on foraging behaviour, such as the risk of predation (e.g., Lima and Dill 1990). Similarly, day length may place limits on parental energy expenditure through limiting available foraging time (Tinbergen and Verhulst 2000). In Fig 1.1, such constraints are indicated by the dashed lines which link the term Environment with Performance and Behaviour.

Natural selection acts on behaviours (Fig 1.1). If variation at the genotypic level affects behaviour via performance, there is potential for selection and evolutionary change. Studies of the physiological correlates of whole animal performance are useful as they can identify traits that may evolve under selection for increased whole organism performance. It

should be noted that selection is defined operationally as the correlation between Darwinian fitness and variation in a phenotypic trait (Garland and Carter 1994). This definition emphasizes that selection acts on phenotypes, irrespective of their genetic basis. Demonstration of selection, therefore, cannot be equated with evolutionary change.

Approaches to studying intra-specific variation

Within the organismal performance paradigm, there are currently two complimentary approaches in the study of intra-specific variation: (i) the gene-to-performance approach and (ii) the performance-to-gene approach (Pough 1989). In the first approach, a site of genetic variation (e.g., allelic isozymes) is identified and an attempt is made to trace the variation to higher levels of biological organization.

This first approach is exemplified by the studies of Powers and colleagues of latitudinal variation in the lactate dehydrogenase protein (*Ldh-B*) of killifish, *Fundulus heteroclitus* along the eastern seaboard of North America (reviewed in Powers and Schulte 1998). These studies have shown that variation in *Ldh-B* results in differences in physiological function, which in turn correlate with survival at high temperatures. Differences among populations in *Ldh-B* are found in terms of enzyme activity, mRNA levels and transcription rate. Recent work has shown that variation in transcription rates among populations is due to variation in regulatory sequences, suggesting the action of natural selection. Gene-to-performance studies are of inherent interest from the perspective of the evolution and adaptation of the products of a single locus. Unfortunately, they tell little about the evolution of the complex physiology in which the allozymes function (Mangum and Hochachka 1998).

The second approach to studying performance (performance-to-gene) begins by recognizing the existence of variation in performance at the whole animal level. Rather than being under the control of a single gene, whole animal performance is likely a quantitative trait under the control of numerous genes (e.g., Dohm et al. 1996, Swallow et al. 1998).

Investigation proceeds outward in two directions; one direction investigates how variation in performance affects fitness, while the other direction seeks to identify physiological or biochemical correlates of the observed differences in performance. To date, performance-to-gene studies have most frequently looked at forced activity, including burst sprinting, endurance and thermogenic capacity (e.g., Garland 1984, Garland and Else 1987, Bennett et al. 1989, Konarzewski and Diamond 1994, Hayes and O'Connor 1999). Other areas of study have included dominance (e.g., Røskaft et al. 1986), vocalization during courtship (e.g., Zimmitti 1999), lactational capacity (Hammond and Diamond 1992), and a combination of lactation and cold exposure (Hammond et al. 1994).

Regardless what the performance trait is, the first step in such studies is to assess the magnitude of inter-individual variation. With the exception of humans (e.g. Bouchard et al. 1992), by far the greatest amount of work on inter-individual variation in performance has been in ectotherms: fish (e.g., *Micropterus salmoides* Kolok 1992); lizards, (e.g., *Ctenosaura similis* Garland 1984; *Amphibolurus nuchalis* Garland and Else 1987; *Sceloporus merriami* Huey et al. 1990); salamanders (e.g., *Ambystoma tigrinum nebulosum* Bennett et al. 1989); snakes (e.g., *Thamnophis sirtalis* Jayne and Bennett 1990, Peterson et al. 1998); and anurans (e.g., *Hyla versicolor* Taigen et al. 1985., *Bufo woodhousei fowleri* Walton 1988, *Pseudacris crucifer* Zimmitti 1999). The study of inter-individual variation in small mammals and birds within an organismal performance paradigm is relatively recent, and increasing in frequency (e.g., Hayes et al. 1992, Dohm et al. 1996, Chappell et al. 1995, 1996, 1999).

Numerous studies have demonstrated that inter-individual variation in organismal performance is detectable, and often large. For example, the size corrected coefficient of variation (CV) for endurance was 63% in adult lizards (*C. similis*, Garland 1984). Among neonatal garter snakes, endurance has been shown to vary by up to 100-fold (Jayne and Bennett 1990). Variation among individuals can be even more extreme, and within a single population of canyon lizards (*S. merriami*), two females had endurance times in excess of six standard deviations of the mean (Huey et al. 1990). Although such aberrant values would

have traditionally been assumed to be 'outliers' due to measurement error, endurance shows high repeatability among trials (Van Berkum et al. 1989). Performance in mammals (other than humans), has been studied less frequently, however there is evidence of considerable phenotypic variation. In studies of individual house mice (*Mus domesticus*), some individuals run for many km per day, while others are virtually sedentary (Friedman et al. 1992, Dohm et al. 1994). In birds, inter-individual variation appears modest, but is detectable. In house sparrows, *Passer domesticus*, for example, the CV for $\dot{V}O_{2max}$ was approximately 16% (Chappell et al. 1999). This is comparable to that reported in reptiles (17%, Garland 1984).

Performance-to-fitness

Central to the study of organismal performance is the notion that those individuals with higher performance capacities will make greater contributions to the gene pool. Quantifying selection, however, requires measurement of individual differences in Darwinian fitness. As fitness is difficult to measure, studies frequently use correlates, for example, clutch size, seasonal reproductive success or survivorship. In the first study to demonstrate selection on performance traits in a natural population, Jayne and Bennett (1990) measured the locomotor capacity of newborn garter snakes in the laboratory and released them into their original environment. Variation in locomotor capacity as measured in the lab significantly predicted subsequent survivorship. As differential survivorship occurred prior to reproductive age, locomotor capacity was under natural selection in this population (Jayne and Bennett 1990).

Hayes and O'Connor (1999) recently demonstrated that thermogenic capacity was under significant directional selection in their study population of high altitude deer mice. Individual mice were captured in the field, had their thermogenic capacity measured ($\dot{V}O_{2max}$) via cold exposure, and were released into their original population. Attempts were made to capture individuals approximately 2 months later. In one of two years, individual

mice with relatively high thermogenic capacity had a greater probability of being recaptured, suggesting increased survivorship.

I am aware of only a single study of birds that has attempted to relate variation in lifetime reproductive success (LRS) with organismal performance. Bryant (1991) showed that in house martins, *Delichon urbica*, the relationship between daily energy expenditure and LRS followed a quadratic relationship; individuals with the highest LRS had intermediate expenditures. This suggests potential selection against individuals with high energy expenditures. The data set, however, included artificially enlarged broods, which in other studies has been shown to result in increased parental mortality rates (e.g., Daan et al. 1996). In contrast to house martins (Bryant 1991), other species show a positive relationship between energy expenditure and correlates of fitness (fledgling mass or condition; Merino et al. 1996, Moreno et al. 1997).

Physiology-to-performance

Studies such as those of Jayne and Bennett (1990) and Hayes and O'Connor (1999) are rare, and have successfully tackled one-half of the organismal performance paradigm: the relationship between performance and fitness. But what about the relationship between performance and its physiological or biochemical correlates? Inter-individual differences in performance have been studied primarily in ectotherms, and have been shown to correlate with various morphological and physiological variables. In one of the earliest intra-specific studies, Garland (1984) demonstrated that in the lizard, *C. similis*, ninety percent of the size-corrected inter-individual variation in endurance could be explained by four variables: $\dot{V}O_2max$, heart and thigh-muscle mass, and hepatic aerobic enzyme activity. Physiological predictors of performance are not universal across species. In *A. nuchalis*, another lizard, endurance was best predicted by heart lactate dehydrogenase and thigh pyruvate kinase and/or citrate synthase (Garland and Else 1987); none of which was a predictor in *C. similus*.

Recent work on vocalizations in male spring peepers, *P. crucifer*, has looked for physiological and biochemical correlates of calling rate (Zimmitti 1999). In this species, males with higher calling rates have increased mating success, while in a related species, *H. versicolor*, the offspring of males with long calls have higher growth rates and fitness (Welch et al. 1998). These studies provide a plausible link between calling performance and Darwinian fitness. Within a population of spring peepers, males with relatively high calling rates had significantly heavier ventricles, higher blood haemoglobin concentrations, and higher enzyme activities in the trunk muscles, than individuals that called less frequently (Zimmitti 1999). As some of these physiological and biochemical characters likely have a heritable basis (Garland et al. 1990), selection for increased calling frequency, may result in evolution of these characters.

While considerable work has been undertaken on inter-individual performance in ectotherms, relatively few studies have considered performance in homeotherms, particularly birds (but see Chappell et al. 1999). The evolution of flight likely places quite different selective pressures on locomotory behaviours. Although burst sprinting (e.g., take-off speed) has ecological relevance in terms of avoiding predators, in species with parental care an additional trait that is of likely selective importance is the capacity for long-term sustained energy expenditure (Peterson et al. 1990, Hammond and Diamond 1997).

Sustained energy expenditure

In studies of exercise performance in terrestrial vertebrates, three types of activity have been commonly examined: capacity for burst sprinting, maximal exertion and endurance (e.g., Bennett 1991). These three measures represent a broad spectrum of maximal activities and presumably influence how well an individual avoids predators, obtains mates or captures prey (Jayne and Bennett 1990). Although these measures of performance differ in terms of fuel utilization and time to fatigue (e.g., Roberts et al. 1996), one thing they have in common is that they rely on stored energy reserves.

There is a well recognized negative relationship between power output and duration of activity. Using an Olympic runner as an example (e.g., Peterson et al. 1990), the power output of an individual sprinting 100 m is considerably greater than that of a 1000 m runner, which is in turn greater than a 10,000 m runner. To supply adequate levels of ATP for muscle contraction, short, high intensity bouts of activity rely on phosphocreatine (PCr) hydrolysis and anaerobic glycolysis. In contrast, lower intensity exercise of higher duration (e.g., runs of 10,000 m) relies primarily on lipid oxidation or a mix of substrates (e.g., Roberts et al. 1996). In each of these cases individuals are relying on finite energy stores, and therefore performance cannot be sustained indefinitely.

It has been hypothesized that with increasing duration of activity, exercise intensity declines and approaches an asymptote (Peterson et al. 1990). This asymptotic level of energy expenditure has been called the sustained metabolic rate (SusMR). An individual's (or species') SusMR is its metabolic rate time-averaged over periods long enough that metabolism is fueled by food intake rather than depletion of energy reserves (Peterson et al. 1990). As it is a time-averaged measure, it includes the costs of resting metabolism, thermoregulation, feeding, and a variety of other expenses. Time averaged measures of energy expenditure have various names in the literature. As they are frequently measured in free living animals in the field, they are often referred to as field metabolic rate (FMR). Alternate names are daily energy expenditure (DEE) and average daily metabolic rate (ADMR, Speakman 1997).

Techniques for measuring sustained energy expenditure vary depending on the question asked and species studied (reviewed by Speakman 1997). If long-term energy turnover is to be measured in the field the most powerful technique to date involves the use of doubly labelled water (DLW, Lifson and McClintock 1966). This technique involves introduction of oxygen and hydrogen isotopes (^{18}O, and either ^{3}H or ^{2}H) in the form of enriched water. After an animal has been injected (or fed) with enriched water, the washout-rates of the isotopes can be measured by taking blood samples before and after an observation period. These washout-rates are then used to calculate respiratory CO_2 production, and

hence energy expenditure. Apart from the use of telemetry (e.g., Bevan et al. 1995), the DLW technique is the only method that allows for measurement of total rates of metabolism in free-ranging animals in their natural environment.

Physiological correlates of sustained energy expenditure

There is considerable inter- and intra-specific variation in levels of sustained energy expenditure (e.g., Konarzewski and Diamond 1994, Peterson et al. 1998, Nagy et al. 1999). But what are the proximate factors underlying differences among individuals and species? In the presence of excess food, a ceiling on SusMR is likely imposed either centrally or peripherally. A central limitation occurs if activity is limited by machinery shared by various energy consuming pathways. The most likely site of a central limitation would be in the capacity of the gut to digest and absorb food. Alternatively, a limitation to SusMR may reside in the energy consuming tissues themselves, for example, in the properties of skeletal muscle (reviewed by Hammond and Diamond 1997). To distinguish between these two hypotheses, experiments have pushed laboratory mice to their maximal SusMR using different modes of energy expenditure (e.g., cold exposure, lactation, running on treadmills). The level of metabolic ceilings differed between modes of energy expenditure, indicating that limitations were unlikely to reside in a shared pathway, but rather in peripheral tissues (Hammond and Diamond 1992, 1994, Hammond et al. 1994, Konarzewski and Diamond 1994).

In nature it is unlikely that individuals will ever be capable of obtaining unlimited food. However, despite this an important observation from laboratory studies was that mice forced to increase their energy expenditures displayed hypertrophy of the small intestine, kidneys, liver, and heart (Hammond and Diamond 1994, Hammond et al. 1994, Konarzewski and Diamond 1994). This supports previous suggestions from studies of shorebirds that a high SusMR requires a high level of support from the organs of the abdominal cavity (Kersten and Piersma 1987). As organs of the abdominal cavity have exceptionally high

mass-specific metabolic rates (Krebs 1950, Scott and Evans 1992), although they contribute only a fraction of an individual's total body mass, they contribute disproportionately to resting metabolic rate (e.g., Daan et al. 1989, 1990, Konarzewski and Diamond 1994, Meerlo et al. 1997).

If large internal organs are required to attain a high SusMR, and these organs contribute disproportionately to an individual's RMR, is there a correlation between SusMR and RMR? Evidence for this relationship is mixed (e.g., Hayes and Garland 1995). In laboratory mice there was a relationship between maximum daily energy intake (a surrogate of SusMR) and the masses of the small intestine and kidney, and between RMR and both heart and kidney mass. There was, however, no relationship between SusMR and RMR (Konarzewski and Diamond 1994). There has also been a failure to detect such a relationship in field caught voles (*Microtus agrestis*, Meerlo et al. 1997). An inability to detect a relationship between SusMR and RMR may be due to the relatively narrow range of energy expenditures found intra-specifically; the noise simply exceeds the signal. If the range of energy expenditures is enlarged through comparisons among species, mammals with a relatively high SusMR do have a relatively high RMR before and after correcting for the influence of phylogeny (Daan et al. 1991, Ricklefs et al. 1996). Although an inter-specific relationship between SusMR and RMR exists in birds (Daan et al. 1990), this relationship is reduced considerably after controlling for phylogeny (Ricklefs et al. 1996).

A failure to detect a relationship between SusMR and RMR in birds may be due to differences among species in the intensity of energy expenditures during parental care, and the existence of 'safety margins' (Toloza et al. 1990). If species with chronically high or low energy budgets are compared, there are apparent couplings among organ sizes, RMR and SusMR. Tropical birds and mammals tend to have smaller organs of the abdominal cavity and RMR than temperate species, and typically have a lower SusMR due to their decreased thermal requirements (Rensch and Rensch 1956, in Daan et al. 1990). Similarly, endotherms have SusMRs considerably higher than those of ectotherms (Nagy et al. 1999). The organs of

the abdominal cavity of endotherms are also considerably larger than those of ectotherms (Else and Hulbert 1981).

Organismal performance and breeding birds

As variation exists among individuals in burst sprinting or endurance exercise (Garland 1984, Garland and Else 1987, Bennett et al. 1989, Huey et al. 1990, Friedman et al. 1992, Dohm et al. 1994), it is not surprising that such variation also exists in sustained energy expenditures (e.g., Bryant and Westerterp 1982, Williams 1987, Moreno 1989, Konarzewski and Diamond 1994, Merino et al. 1996, Peterson et al. 1998, Potti et al. 1999).

Birds are excellent models for studies of the evolution of performance, in part because individuals within the same environment often display extensive variation in fitness related traits (e.g., Masman et al. 1989, Hochachka 1993, Pettifor 1993b, Blomqvist et al. 1996, Wendeln and Becker 1999). These traits include egg size (Blomqvist et al. 1996), clutch size (Pettifor 1993a), parental body condition (Wendeln and Becker 1999), and provisioning capacity (Wardrop 2000). Repeated measurements of the same adults have shown that in consecutive years individuals often lay similar sized clutches and have eggs of similar mass (Wiggins 1990, Pettifor 1993b). Two recent avian studies have raised the additional exciting possibility that measurements of resting and sustained energy expenditure are repeatable between breeding seasons (Bech et al. 1999, Potti et al. 1999). This suggests that individuals may differ consistently in their energy expenditure, and that traits associated with them may retain some genetic variance (Potti et al. 1999).

As energy requirements of offspring increase with increasing brood size, in theory so should parental energy expenditure. In fact, it has been hypothesized that the number of offspring that parents can raise may be limited by their physiological capacity to feed their young (Drent and Daan 1980). Because breeding adults remain in approximate energy balance, the most likely site of limitation on energy expenditure is in the rates of nutrient intake, digestion or assimilation (Kirkwood 1983, Masman et al. 1989, Peterson et al. 1990,

Weiner 1992). This hypothesis was tested by Dykstra and Karasov (1992, 1993). They compared near maximal rates of energy flow in house wrens, *Troglodytes aedon*, exposed to a combination of cold and exercise in the laboratory, with that of individuals of the same species rearing manipulated broods in the field. As the rate of parental energy expenditure in the field was considerably below that measured in the lab, they rejected the hypothesis that maximum rates of energy flow limit brood size (Dykstra and Karasov 1992, 1993).

Although an individual's brood size is probably not limited by the maximal rate of energy flow, this does not necessarily mean that brood size and parental daily energy expenditure are uncoupled. Support for such a relationship between SusMR and behavioural traits such as brood or clutch size is, however, equivocal. In their recent review, Williams and Vezina (2000) noted that only 6 of 20 avian studies (30%) could detect a relationship between the number of nestlings in a brood and parental SusMR. However, some of the studies in their review were of species that hold feeding territories (e.g., European kestrels, *Falco tinnunculus*, and wheatears, *Oenanthe oenanthe*). In these species clutch size may be adjusted to the quality of an individual's territory (Högstadt 1980). Individuals on low quality territories may have small clutches and expend the same amount of energy as individuals on high quality territories with larger clutches. A similar interpretation could explain (in part) why only 5 of 15 studies reviewed by Williams and Vezina (2000) could detect a relationship between SusMR and parental provisioning rate.

Although some studies fail to detect relationships between SusMR and surrogates of fitness, very convincing relationships are found in others. For example, when the brood size of pied flycatchers, *Ficedula hypoleuca*, was manipulated, the SusMR of males and females was positively correlated with nestling mass and tarsus length (Moreno et al. 1997). In addition, female SusMR was positively correlated with nestling quality, as indicated by *Trypanasoma* infection (Merino et al. 1996). These studies led Moreno et al. (1997) to suggest that parental energy expenditure (SusMR) was analogous to a performance trait,

constrained by "parental time-activity budgets or by condition-dependent physiological limits."

Adults from a number of species appear unable to respond to the energetic challenge of an increased brood size. For example, the recent work of Tinbergen and Verhulst (2000) showed that although female great tits, *Parus major*, reduced their energy expenditures when the number of nestlings was artificially reduced, under conditions of increased brood requirements they failed to elevate their SusMR. This supports the existence of an energetic ceiling set at their natural brood size. Tinbergen and Verhulst (2000) suggested that female energy expenditure is constrained by day length, which in turn limits available foraging time. Additional support for an energetic ceiling comes from studies that manipulated female energy expenditure independent of brood size. Moreno et al. (1999) clipped the primary feathers of female pied flycatchers (to increase wing loading). Females with clipped wings did not spend any more energy than controls (unclipped), and as a consequence their nestlings suffered a reduced weight gain. This again suggests that females were unable (or unwilling) to increase energy expenditure. Despite these studies, numerous others have shown that parents can increase the energy expenditures (Masman et al. 1989), but may suffer reduced condition or increased mortality as a consequence (Winkler and Alan 1995, Daan et al. 1996).

Dykstra and Karasov (1992, 1993) showed that maximum rates of energy flow likely do not limit brood size. However, if in order to attain a high SusMR hypertrophy of the organs of the abdominal cavity is necessary, individuals with relatively high SusMR may have to 'pay' in terms of increased energy expenditures while resting (Hammond and Diamond 1997). Consequently, the number and quality of nestlings that an adult produces may represent a balance between the fitness benefits derived from increased rates of recruitment, and the costs of maintaining the organs necessary to attain a high SusMR. To date, no study has investigated the physiological and fitness correlates of variation in SusMR; this is a major component of my thesis, and is presented in Chapters 2 and 3.

Environmental components of variation

Most of this thesis focuses on adults. However, as the fitness of adults depends on the number of their offspring recruited into the next generation, it is necessary to consider how variation in the quality of parental care may influence variation in the quality of nestlings. Next to successful mating, the most important determinant of lifetime reproductive success in many species is the survival and recruitment of offspring (Clutton-Brock 1988).

There is increasing evidence that variation in the quality of the rearing environment affects both nestling survival and subsequent future reproductive performance (e.g., Lindström 1997). Individuals that experience adverse conditions during early development are often smaller and lighter in mass at independence, and have decreased survival probabilities (Boag 1987, Richner 1989, Dijkstra et al. 1990, Korpimäki and Rita 1996, de Kogel 1997, Koskela 1998). Differences in body size that are established during the nestling phase and that are often maintained in adults, contribute to the non-heritable environmental component of variation (James 1983, Boag 1987, Richner 1989, Alatalo et al. 1990, de Kogel 1997). An elegant experiment demonstrated the importance of the rearing environment in determining adult structural size. James (1983) transplanted red-winged blackbirds from either end of a geographic cline in body size. Nestlings grew to resemble their foster parents rather than biological parents, demonstrating that much of the clinal variation in body size was due to environmental effects. A more common way to experimentally manipulate the environment during development is through increasing or decreasing the number of nestlings in a brood. Parents often fail to meet the energetic challenge of the increased brood size resulting in poor nestling growth. For example, in the laboratory, de Kogel (1997) produced morphologically stunted adult zebra finches by increasing the number of nestlings in the brood in which they were reared.

In addition to influencing skeletal morphology, conditions during early development can also affect future reproductive potential. Through presumed condition-mediated

mechanisms, female collard flycatchers reared in artificially enlarged broods laid fewer eggs in their first breeding attempt than individuals that were reared in control or reduced broods (Schluter and Gustafsson 1993). Increasing or decreasing brood size also affected subsequent sexual attractiveness of males (Gustafsson et al. 1995). During their first breeding season, males that were raised in artificially enlarged broods had a smaller forehead patch (a secondary sexual character) than individuals reared in reduced or control broods. This has reproductive consequences as males with large patches mate with more females (Gustafsson et al. 1995). Similarly, male zebra finches reared in small broods developed redder bills and were more attractive to females than their siblings reared in larger broods (de Kogel and Prijs 1996). Implicit in these studies is that variation in the environment experienced during early development affected an individual's 'condition' when an adult. Apart from recent work on immunosuppression (e.g. Merino et al. 1996), the physiological and biochemical traits defining condition remain relatively unexplored. Recent studies have shown that estimates of condition (residuals of mass on tarsus length) of nestlings are good predictors of their fat reserves up to 4 months later during migration (Merilä and Svensson 1997). This suggests that the window for selective events leading to a relationship between condition and survival maybe quite wide (Merilä and Svensson 1997).

There exists considerable variation among adult birds in body composition and resting metabolism (Chapter 3). Some of this variation likely correlates with inter-individual variation in aerobic capacity and perhaps dominance, as has been reported in other species (Røskaft et al. 1986, Bryant and Newton 1994, Chappell et al. 1999). Although there is a heritable basis to variation in physiological traits such as resting metabolism and the size of many internal organs (Schlager 1968, McKitrick 1990, Mahaney et al. 1993), the importance of early development in modifying this variation remains relatively unexplored (but see Dohm et al. 1996). Partitioning the relative influence of different sources of variation in these traits would be of interest, but would be a difficult task given the complexity of the experimental design (cross-fostering) and large sample sizes required (e.g., Garland et al. 1990). A useful

first step is to determine to what extent physiological and biochemical traits (e.g., heart mass or enzyme activity) are shaped by environment variation; this was performed in Chapter 4.

Study species and location

One of the best species in which to address questions of organismal performance is the tree swallow, *Tachycineta bicolor*. This species is an aerial insectivore that forages for up to 15 hours per day when feeding dependent young (Wiggins 1990). This results in adults having one of the highest SusMR ever measured, sometimes in excess of 6.0 X RMR (Williams 1988). As this value approaches the hypothesized ceiling of 7.0 X RMR for all species (Peterson et al. 1990, Hammond and Diamond 1997), it suggests a relatively small margin of safety (e.g., Toloza et al. 1990).

First time breeding female swallows are identifiable on the basis of plumage (1-year old, Hussell 1983), and were excluded from the present study. In females older than 2 years the confounding affects of age and breeding experience on fitness correlates such as clutch size are minimal (Strutchbury and Robertson 1988, Robertson et al. 1992). Incubation is performed exclusively by the female and lasts 14-15 days (Robertson et al. 1992). After the young hatch, both parents feed the nestlings at similar rates (Quinney 1986). Nestlings follow a sigmoidal growth curve, attain peak mass by approximately day 12 post hatch, and fledge at 18-22 days (Robertson et al. 1992).

During brood rearing, parents that feed their nestlings more frequently have higher energy expenditures (Williams 1988). Assuming that larger broods require more food (have increased energy requirements, Drent and Daan 1980), this suggests that parents feeding a larger number of nestlings will have a higher SusMR. As tree swallows do not hold feeding territories, I assumed that in the present study all individuals had similar access to food resources.

Data in this thesis were collected over 5 field seasons, 1994-1998, at the Creston Valley Wildlife Management Area, a 7000 hectare wetland area in southeastern British

Columbia. Throughout the Management Area there are a series of man-made dikes, upon which I placed between 140 and 200 nest boxes (depending on the year). Although tree swallows have been studied at this location since the early 1980's, the number of banded individuals represents <50% of the population.

Aims of thesis

To date, no study has looked for physiological correlates of sustained performance and attempted to link them via behavioural traits with fitness. In Chapters 2 and 3, I sought answers to three primary questions: (i) what are the physiological and biochemical correlates of one indicator of whole animal performance (SusMR); (ii) what is the relationship between performance (SusMR) and Darwinian fitness; (iii) what is the relationship between variation in organ size and resting rates of metabolism? In Chapter 4, I asked how variation in the environment during early development affects the physiology and biochemistry of individual nestlings near fledging.

CHAPTER 2

I measured the SusMR of adult tree swallows rearing different natural sized broods using the DLW technique. Correlations were sought among variation in performance (SusMR), natural brood size and both nestling mass and growth rates (as indices of fitness). Following measurement of their performance, I sacrificed a sample of adults to determine if variation in parental energy expenditure was associated with variation in physiological and biochemical characters.

The following hypotheses were tested:

1) There are positive relationships among parental SusMR, brood size, nestling mass and growth. That is, parents that can attain a high SusMR, will have larger broods and faster growing nestlings.

2) Individuals with a relatively high SusMR will have relatively large internal organs, and relatively high metabolic capacities in the pectoral muscle (as indicated by the activities of key enzyme activities from various metabolic pathways).

CHAPTER 3

There is intra-specific evidence that maintenance of relatively large organs in the abdominal cavity is metabolically costly for mammals, resulting in elevation of resting metabolic rate (Daan et al. 1991, Konarzewski and Diamond. 1994, 1995, Meerlo et al. 1997). Although this would be expected intra-specifically in birds, prior to the publication of Chapter 3 (Burness et al. 1998) no such data existed; nor had anyone reported inter-annual differences in the sizes of internal organs.

The following hypotheses were tested:

1) There will be a positive relationship between adult resting metabolic rate and the size of metabolically active organs of the abdominal cavity.

2) Resting metabolic rate and the size of internal organs will show inter-annual variation.

CHAPTER 4

I performed brood manipulations in the field to mimic environmental variation. I considered nestlings reared in enlarged broods to be in a "poor quality" environment, resulting from a dilution in parental care. For nestlings in reduced broods, the reverse was true. At 16 days of age, I measured the resting metabolic rate of nestlings representing the average individual for each brood. I then sacrificed these individuals to determine body composition and tissue biochemistry.

The following hypotheses were tested:

1) For a given structural size, nestlings in "good quality" environments will have larger hearts, liver and kidneys, greater mass of lipid and pectoral muscle, higher activities of oxidative and glycolytic enzymes, and higher resting metabolic rates, than individuals in "poor quality" environments.

2) Nestlings of a given structural size would differ between treatments in the size of their intestines and gizzards. I could not predict *a priori* whether there would be an increase or decrease in the size of these organs with increasing brood size (under food restriction either response is plausible, Galuso and Hayes 1998).

CHAPTER 2

PHYSIOLOGICAL CORRELATES OF PARENTAL QUALITY IN BREEDING TREE SWALLOWS, *TACHYCINETA BICOLOR*

INTRODUCTION

Many populations of passerine birds exhibit considerable variation in clutch size, even though individuals laying the largest numbers of eggs often raise the most recruits (e.g., Boyce and Perrins 1987). One hypothesis to explain this variation proposes that females adjust their clutch size to their own individual circumstances (individual optimization hypothesis, Perrins and Moss 1975, Pettifor et al 1988); for example, to the quality of their territory (Högstedt 1980) or to their own individual abilities. Although territory quality can be defined in terms of ecological variables such as predation risk or food resources, variables defining individual quality are less clear.

The provisioning of dependent nestlings requires an elevation of parental activity and consequently an elevation of metabolic rate (Drent and Daan 1980). One character that may differ among individuals within the same population is the level to which each individual can elevate its energy expenditure. This is an individual's sustained metabolic rate (SusMR), defined as the metabolic rate time-averaged over periods long enough that metabolism is fueled by food intake rather than depletion of energy reserves (Peterson et al. 1990). Because metabolism is fueled by food intake, individuals remain in energy balance. Provisioning of dependent young often lasts many weeks, and as there is a negative relationship between the intensity of activity and its duration, SusMRs during parental care are typically only a few times the basal or resting metabolic rate (Drent and Daan 1980, Peterson et al. 1990, Hammond and Diamond 1997).

In the same way that inter-individual variation exists in capacity for short term energy expenditure ($\dot{V}O_{2max}$, Chappell et. al 1999), variation in SusMR has also been reported (e.g., Konarzewski and Diamond 1994, Bryant 1991). A recent study of birds indicated that in

females (although not males) estimates of SusMR were repeatable between breeding seasons (Potti et al. 1999). The stability of such a trait over a period of a year suggests that it may have a genetic component. In this context, sustained energy expenditure may be viewed as an organismal performance trait with potential links to fitness (Garland and Carter 1994).

Presumably, individuals with high SusMR will display various physiological adaptations, including relatively large intestines (Konarzewski and Diamond 1994), hearts and kidneys (Daan et al. 1990), and the capacity for high flux rates through various metabolic pathways. Laboratory studies of small mammals have shown that inter-individual variation in maximum sustained energy expenditure does correlate with differences in body composition (Konarzewski and Diamond 1994, Koteja 1996). For example, individual mice with relatively high energy intake rates had relatively heavy kidneys and small intestines (Konarzewski and Diamond 1994). As the kidney accounts for a large fraction of resting metabolic rate, these individuals had an increased resting energy expenditure (Konarzewski and Diamond 1994). These data suggest the existence of a trade-off between the potential benefits of attaining an high SusMR and the costs of maintaining the organs necessary to do it (Kersten and Piersma 1987, Hammond and Diamond 1997).

Even though many physiological and biochemical characters display considerable plasticity, they likely still retain some genetic variance (Schlager 1968, Garland et al. 1990, McKitrick 1990, Mahaney et al 1993, Konarzewski and Diamond 1995). Identification of the physiological and biochemical correlates of SusMR may give insight into which characters would be subject to potential evolutionary change under selection for whole animal performance.

In an attempt to better understand the physiological causes and ecological consequences of variation in whole animal performance, I studied breeding tree swallows, *Tachycineta bicolor*. This species is an aerial insectivore and does not hold feeding territories (Robertson et al. 1992). Consequently, variance in nestling growth, a surrogate of fitness, is presumably due in large part to differences in individual parental quality (e.g., DeSteven

1980). I asked three primary questions: (1) Do parents rearing large natural broods trade-off nestling quality for quantity? (2) Does parental SusMR correlate with indices of fitness (brood size and nestling mass)? (3) What are the physiological and biochemical correlates of parental SusMR, and do these differ between adults rearing different sized broods?

MATERIALS AND METHODS:

Study site and choice of study nests

The field component of this study was performed in May-June 1996 and 1997 at the Creston Valley Wildlife Area, near Creston, British Columbia, Canada. Approximately 180 nest boxes were erected approximately 15-20 m apart, along man-made dikes within the Wildlife Area.

Beginning in the first week of May, boxes were checked daily for signs of breeding and the presence of eggs. Females lay a single egg per day, typically on consecutive days, until clutch completion. Clutch completion is followed by 14-15 days of incubation (Robertson et al. 1992). To minimize disturbance, no nest checks were conducted during incubation. Within 1-2 days of predicted hatch dates, nest checks were resumed to record dates of hatching (hatch = day 1). Within a clutch, hatching was relatively synchronous and was typically complete within 1-2 days.

In both 1996 and 1997, egg laying began during the first week of May and continued into early June. To minimize the possibility of including females laying replacement clutches, I only considered nests with clutches initiated in May. Study nests were chosen based on their original clutch sizes (5, 6 or 7 eggs). To minimize date as a correlate of clutch/brood size (Winkler and Allen 1996), I randomized the choice of study nests across each breeding season (i.e., not all 7 egg nests were selected early in the season).

Nestling mass and growth rate

On day 4, I weighed nestlings from each study nest using a spring-loaded scale (± 0.5 g) and banded them loosely. If a nestling was too small to be banded it was marked with indelible marker and banded within a few days. On day 8, nestlings were re-weighed and the bands tightened. If an egg failed to hatch by day 4, it was replaced by a 4 day old nestling (± 1 day) from another nest. Similarly, if a nestling died between days 4 and 8, to maintain the original brood size it was replaced by a nestling of similar age. However, no measure of growth for that brood was recorded.

Nestlings whose parents were involved in a study of energetics (below) were weighed a third time on day 9. This third weighing was used as an indirect measure of whether parents were behaving normally following injection and release on day 8 (i.e., did nestlings lose weight over the energetic study).

Doubly labeled water

I measured the sustained metabolic rate (SusMR) of adult tree swallows rearing natural broods of 5, 6 or 7 nestlings using the doubly labeled water technique (DLW; Lifson and McClintock 1966). To standardize for brood age (e.g., Sanz and Tinbergen 1999), all adults were captured at the nest box on day 8 of chick rearing. In broods of 5 and 7, attempts were made to capture both members of the pair, while in broods of 6, a single parent was captured.

I prepared the DLW injection solution by mixing 0.120 mL of 2.99 mCi $^{3}H_2O$ with 8.97 mL of 97 atom % $H_2{}^{18}O$. Using a calibrated glass syringe, I injected 0.10 mL of solution (ca. 33μCi tritium per individual) into the pectoral muscle of each adult. Each adult was then weighed using a spring-loaded scale (± 0.5 g), banded and held for 1 hr in an individual brown paper bag. This was sufficient time to allow for equilibration of the isotopes with the body water (e.g., Williams and Nagy 1984). Following equilibration, I collected approximately 0.150 mL of blood from the brachial vein into heparinized microcapillary tubes and then

released the bird. After approximately 24 hours, the bird was recaptured and a second set of blood samples was taken from the other wing. In each year, 2 non-experimental females were captured at the study site and a blood sample was taken to determine background levels of ^{18}O and ^{3}H.

In 1996, microcapillary tubes containing blood samples were immediately flame-sealed in the field using a butane torch. In 1997, tubes were first sealed with Critocaps, and then flame-sealed upon return to the lab at the end of the day. All blood samples were stored at 4°C until distillation and analysis by Dr. K. A. Nagy's Laboratory of Biomedical and Environmental Sciences, UCLA. ^{18}O concentration was measured in triplicate using cyclotron-generated proton activation analysis. ^{3}H activity was measured in duplicate using a liquid scintilation counter.

Adults rearing 6 nestlings were released following the second blood sample. To address questions of physiological and biochemical correlates of clutch size and energy expenditure, I sacrificed adults rearing either 5 or 7 nestlings (see below). Their nestlings were distributed among non-study nests in the population.

Environmental temperature

The daily maximum and minimum air temperatures during the study period were obtained from an Atmospheric Environment Service weather station, approximately 5 km from the study site. Most adults were captured and injected between 10:00 and 13:00. Consequently, the maximum temperature experienced during the DLW trial was assumed to occur on the day of capture (nestling day 8). The minimum temperature most likely occurred between approximately 00:00 and 0500, and was considered to be the lowest temperature recorded for the day of re-capture (nestling day 9).

Haematocrit and haemoglobin

I collected an additional 100-200 µl blood sample from adults rearing either 5 or 7 nestlings. To determine haematocrit (Hct, %), microcapillary tubes containing the samples were spun at maximum speed for 10 min. using an Adams micro-haematocrit bench top centrifuge. The percentage of the tube occupied by packed cells was then measured. Concentration of haemoglobin [Hb, g dL^{-1}] was determined using a portable HemoCue® B-Hemoglobin photometer (Ängelholm, Sweden). The number of replicates for each character was determined by the size of the blood sample and ranged from one to three (which were averaged).

For 5 individuals in which Hb but not Hct was measured, I estimated Hct from regressions of Hct on Hb for the 29 individuals in which both characters were measured. Separate predictive equations were necessary for each year because the slopes of the regression lines differed (ANCOVA, $P<0.15$). 1996: Hct = 13.83 + 1.80[Hb], $r^2=0.77$, $N=12$, $P<0.001$; 1997: Hct = 3.59 + 2.69[Hb], $r^2=0.83$, $N=19$, $P<0.001$). As Hct and Hb correlate strongly ($r=0.83$, $N=29$, $P<0.001$), only results for Hct are presented.

Body composition

During two breeding seasons, I sacrificed 49 adults (29 females and 20 males) rearing either 5 or 7 nestlings immediately following blood sampling (following the guidelines of the Canadian Committee on Animal Care). Within 1-2 min. of death, a sample (ca. 300 mg) of the right pectoralis major was removed from each individual and immediately frozen in a liquid N_2-charged dry shipper for enzyme assays. The remainder of the pectoralis and supracoracoideous (hereafter, "pectoralis") was then removed, followed by the heart, liver, small intestine, gizzard, and kidney. All tissues except the gizzard were stored in air-tight cryovials and frozen in the dry shipper. Each carcass (including the gizzard) was double bagged and stored at -20°C. Upon return from the field, I transferred the samples of

pectoralis to liquid N_2, and stored the remainder of the tissues and carcass at either -20°C or -80°C.

Wet weights were determined for all organs and tissues (± 0.0001g). The small intestine and gizzard were initially weighed full. The small intestine was cut into three sections of equal length. The gizzard, and each section of the small intestine were then cut longitudinally and the contents rinsed out with 0.9% NaCl. Each tissue was then blotted dry and re-weighed to determine empty mass.

Determination of lipid levels was restricted to the carcass and pectoralis. In preparation for fat extraction, carcasses were partially thawed, plucked of all feathers, and weighed (± 0.0001g). The carcass and pectoralis were dried to constant mass in a 70°C oven and freeze dryer respectively. These dried samples were then fat extracted for 7 hours in a Soxhlet apparatus containing petroleum ether as the solvent (Dobush et al. 1985). Following extraction, the carcass and pectoralis were placed in a fume hood to evaporate any remaining solvent, oven-dried overnight, and then re-weighed. The difference between the pre-extraction and post-extraction mass represented the mass of lipid.

Biochemical analyses

The subsamples (ca. 300 mg) of the pectoralis major were removed from liquid N_2, weighed frozen (± 0.0001g) and added to 9 volumes of 0°C homogenization buffer (20mM Na_2HPO_4, 0.2% BSA (defatted), 5mM ß-mercaptoethanol, 0.5mM EDTA, 100µg/mL aprotinin, glycerol 50% v/v, pH 7.4 at 21°C; Mommsen and Hochachka 1994). Each sample was minced on ice for 1 minute using scissors, followed by homogenization using a hand held Tissue Tearor (3 X 10 sec. bursts separated by 30 sec. breaks). Samples were further homogenized for 3 min. using a Lurex ground glass-on-glass homogenizor, and then sonicated for 3 X 10 sec bursts, separated by 30 sec breaks, using a Kontes Micro-ultrasonic cell disrupter. Homogenates were stored at -80°C until assaying (maximum 4 months). This

homogenization buffer allows samples to be frozen for extended periods with no loss of enzyme activity (Mommsen and Hochachka 1994).

As an index of maximum capacity for flux at specific steps through various metabolic pathways, I measured the maximum catalytic activity (V_{max}) of key metabolic enzymes under optimal conditions. All assays were performed on a temperature controlled 6 cuvette spectrophotometer (Perkin Elmer Lamda 2). Assay temperature was maintained at 42°C using a Lauda RM6 circulating water bath and water jacketed cuvette holders. In all assays, uncentrifuged homogenates were used to avoid potential loss of activity in the pellet. Each reaction was replicated in 3 cuvettes. The two cuvettes with the most similar activity were averaged; in cases where values from the three cuvettes were equidistant, all three were averaged. Preliminary experiments confirmed that all substrates and cofactors were saturating but not inhibitory. With the exception of citrate synthase, all assays were at pH 7.0 and 340 nm. Citrate synthase was assayed at pH 8.0, 412 nm. Enzyme activities are expressed as international units (μmoles substrate converted to product per minute) per gram wet weight of tissue.

Assays were performed as follows. Lactate dehydrogenase (EC 1.1.1.27; LDH): 50mM Imidazole, 0.15mM NADH, 10mM ß-mercaptoethanol, 1.0 mM NaCN, 1.0 mM Pyruvate. 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35; HOAD): 50mM Imidazole, 0.15mM NADH, 10mM ß-mercaptoethanol, 1.0 mM NaCN, 0.05 mM acetoacetyl CoA. Citrate synthase (EC 4.1.3.7; CS): 50mM Tris buffer, 0.05% Triton X-100, 0.2mM DTNB, 0.2mM acetyl CoA, 0.5mM oxaloacetate (omitted from the control cuvette). Pyruvate kinase (EC 2.7.1.40; PK): 50mM Imidazole, 0.15mM NADH, 10mM ß-mercaptoethanol, 1.0 mM NaCN, 100mM KCl, 10mM $MgCl_2$, 10μM fructose 1-6 bisphosphate, 5.0 mM ADP, 5mM phospho (enol) pyruvate, excess LDH (ca 5U/mL). To account for PK contamination in the coupling enzyme (LDH), I ran 6 additional control reactions containing no homogenate. The rate of change in absorbance over time was calculated for each of these 6 control reactions and averaged. The average control rate was subtracted from all PK reaction rates before

calculating enzyme activity. Additional control reactions (containing no substrate) were initially run for each enzyme. The rates of control reactions were low for all enzymes except CS and were subsequently omitted.

Calculations of sustained metabolic rate

An individual's initial total body water pool (TBWi, in mL) was estimated using the ^{18}O dilution space, calculated according to Appendix I of Nagy (1983). On occasion a small amount of water remained on the skin following an injection. Due to the occasional uncertainty concerning the exact amount of water that was injected, instead of individual values, a mean percentage body water of 66.2% was assumed in all cases. This percentage estimate was based on the mean dilution space calculated for 43 individuals with injections of known volume. The final body water pool (TBWf, in mL), was estimated from TBWi, assuming both a linear change in pool size and that TBWf occupies the same percentage of the body mass as TBWi .

Rates of CO_2 production (rCO2) were calculated using equation (1) of Nagy (1983), re-expressed in mL d^{-1}:

$$rCO_2 = \frac{622.32\ (TBWf - TBWi)\ \ln[(Oi - Ob)(Hf - Hb) / (Of - Ob)(Hi - Hb)]}{\ln[(TBWf / TWBi)]\ (t)} \qquad (1)$$

Where: Oi, Of, Hi and Hf are an individual's initial and final concentrations of ^{18}O and ^{3}H, Ob and Hb are the background levels of ^{18}O and ^{3}H measured in 2 non-experimental animals, t refers to the time between release and recapture (in days), and ln is natural logarithm.

During the experiment, adult swallows lost an average of 0.91 ± 2.85% of their mass per 24 hours (Range: -7.27% to + 4.17%, N=43). To allow for conversion of CO_2 production to energy expenditure, it was necessary to assume that the majority of individuals

were in approximate energy balance. I converted the rate of CO_2 production to J d^{-1} assuming 26.2 J mL^{-1} CO_2 for insectivorous food (Weathers and Sullivan 1989). For birds that lost >4% of their initial body mass (N=6), I followed Weathers and Sullivan (1989) and included the heat produced from the oxidation of fat in estimates of SusMR. This resulted in a marginal increase in my estimates of SusMR (0.84 ± 0.16%, N=6). One swallow increased in mass by 0.78 g, representing a gain of 4.2%. As changes in body composition between release and recapture were unknown, I made no attempt to correct my estimates of SusMR for mass gain.

One individual in 1996 could not be re-captured within 24 hr and was captured the following morning (total elapsed time = 39 hr). This individual spent more time at rest than the other birds, which would lead to an underestimate of its SusMR if expressed simply per 24 hours. In order to estimate the volume of CO_2 that this individual would have produced in a 24 hour day (15 hours of daylight, 9 hours of darkness), I needed to calculate the daytime and nighttime rates of CO_2 production ($\dot{V}CO_2$). I calculated the nighttime $\dot{V}CO_2$ from the estimated nighttime rate of oxygen consumption ($\dot{V}O_2$), assuming a respiratory exchange ratio of 0.75. The average nighttime $\dot{V}O_2$ was estimated as 1.9 X basal $\dot{V}O_2$ (Tinbergen and Dietz 1994), and I assumed the basal $\dot{V}O_2$ to be 75% of the daytime resting $\dot{V}O_2$ (Aschoff and Pohl, 1970). I calculated daytime resting $\dot{V}O_2$ from a species-specific allometric equation generated for adults (Burness et al. 1998, Chapter 3). Based on the time of initial capture and subsequent recapture, I estimated that this bird spent 18 hours at a nighttime $\dot{V}CO_2$ and the remaining 21 hours at a daytime "active" $\dot{V}CO_2$. To calculate the volume of CO_2 produced during the daytime (in 21 hours), I subtracted the volume produced during the 18 hours of darkness from the total CO_2 produced in 39 hours (from DLW). By knowing the daytime and nighttime $\dot{V}CO_2$, I could calculate the average $\dot{V}CO_2$ over 24 hours.

Sixty-two adult tree swallows were successfully recaptured, and of these, 47 yielded reliable estimates of SusMR during provisioning. Estimates of SusMR were considered to be unreliable, and were excluded from analyses if either the final ^{3}H or ^{18}O values had decayed

to background (N=4), or the estimated SusMR was less than the allometrically predicted BMR (N=7). Four additional adults were omitted because either their SusMR was < 1.5 X BMR (considered unlikely in an aerial insectivore provisioning young, Williams 1988), and/or their nestlings displayed large weight loss during the trial, suggesting potential negative effects of the injection on the parents.

Statistical analyses

Data were transformed as necessary to meet assumptions of multivariate statistical tests (e.g., normality of residuals). The influence of potential covariates (e.g., time, date, body mass), main effects (e.g., year, sex or brood size) and interaction terms on dependent variables were first explored using either a forward or backward stepwise regression. Probabilities for inclusion and exclusion were set at 0.05 and 0.10 respectively. Terms significant in the stepwise regression were then included in 1 or 2-way analysis of variance (ANOVA), analysis of covariance (ANCOVA), or multiple regressions. Interaction terms were excluded from models when $P>0.15$. In order for some results to be viewed graphically, on occasion I analyzed residuals. In these cases P-values were corrected to account for the degrees of freedom lost in generation of the residuals (Hayes and Shonkwiler 1996).

Whenever possible year and sex of parents were pooled (with either year or sex included as a main effect). However, as both the male and female were often captured from the same nest, in analyses involving brood size or nestling mass and growth, sexes were considered separately.

To avoid the possibility of spurious autocorrelation in analyses of body composition, the mass of each organ was subtracted from total body mass before each computation (Christians 1999). Unless otherwise noted, data are reported as least squares means ± 1 S.E.M. (standard error of the mean) and probabilities are 2-tailed. Statistical significance was claimed at $P<0.05$. Analyses were performed using JMP statistical software. I performed power analyses using PASS 6.0.

RESULTS:

Brood size and nestling growth rate

Growth was followed in 52 unmanipulated nests between days 4 and 8 (22 in 1996; 30 in 1997). The total mass gain of broods per day (brood growth rate) increased with increasing natural brood size ($F_{2,49}$=41.479, P<0.001; all brood sizes significantly different, Tukey HSD P<0.05; Fig 2.1A). The growth rate of individual nestlings was independent of brood size (P=0.054, Fig 2.1B). Growth rates did not differ between years (P>0.10).

Potential correlates of SusMR

Various factors can influence estimates of SusMR (Speakman 1997). These need to be identified before relationships among SusMR, brood size and nestling growth can be determined.

Year, sex and body mass

The SusMR of adult tree swallows ranged from 56.1 - 136.3 kJ d^{-1}, with an average value of 101.5 kJ d^{-1} (S.D.=18.9, N=47); males and females did not differ (P>0.15). The average SusMR in 1996 was less than in 1997 (Wilcoxon test, Z = -2.198, P<0.05). 1996: 89.4 kJ d^{-1} (S.D.=25.5, N=14), 1997: 106.6 kJ d^{-1} (S.D.=12.6, N=33). There was a weak but significant increase in SusMR with increasing body mass ($F_{1,44}$=4.759, P<0.05, Fig 2.2), after controlling for year effects. When each year was considered separately, mass explained at most 11% of the variance in SusMR (1996: r^2=0.11, N=14, P=0.242; 1997: r^2=0.09, N=33, P=0.084); neither regression was significant.

Environmental temperature

Daily minimum temperature was not correlated with SusMR in either 1996 or 1997 (P>0.15). In 1997 only, there was a marginally significant decrease in SusMR with increasing daily maximum temperature (r=-0.31, N=33, P=0.079).

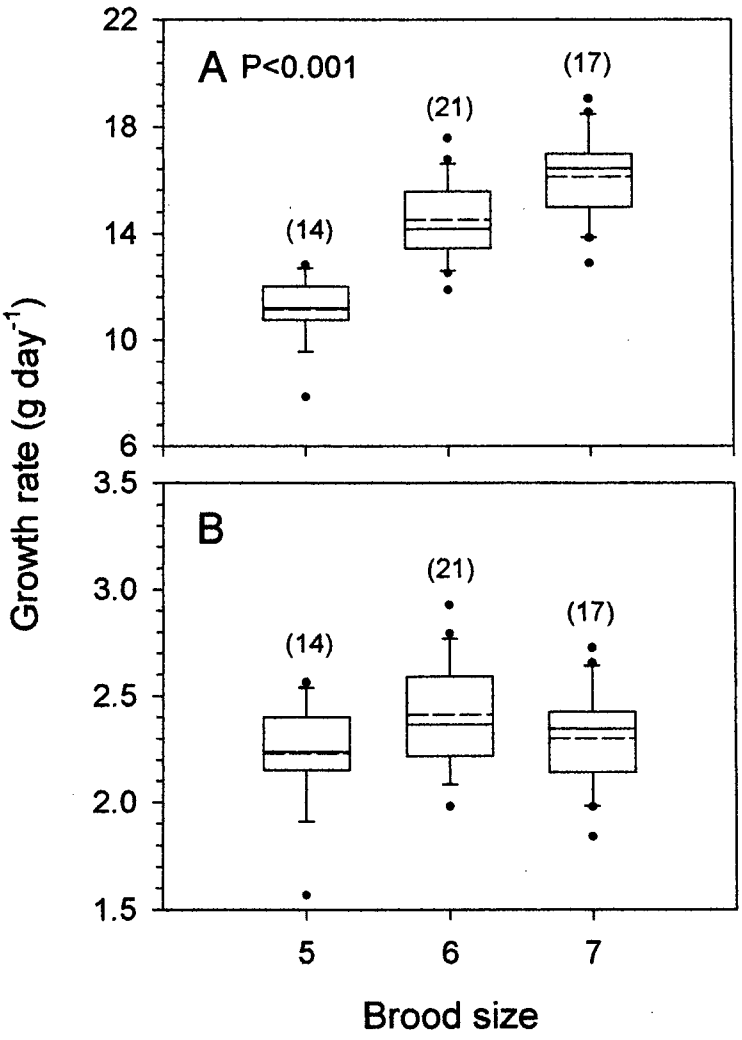


Figure 2.1 Box plots of growth rates between days 4 and 8 of (A) entire tree swallow broods and (B) individual nestlings. The solid horizontal line in the middle of each box is the median, the dashed line is the mean. The bottom and top of the boxes are the 25th and 75th percentiles. The vertical lines above and below the boxes are the 10th and 90th percentiles; data points falling outside this range are indicated by solid circles. Sample sizes are in brackets.

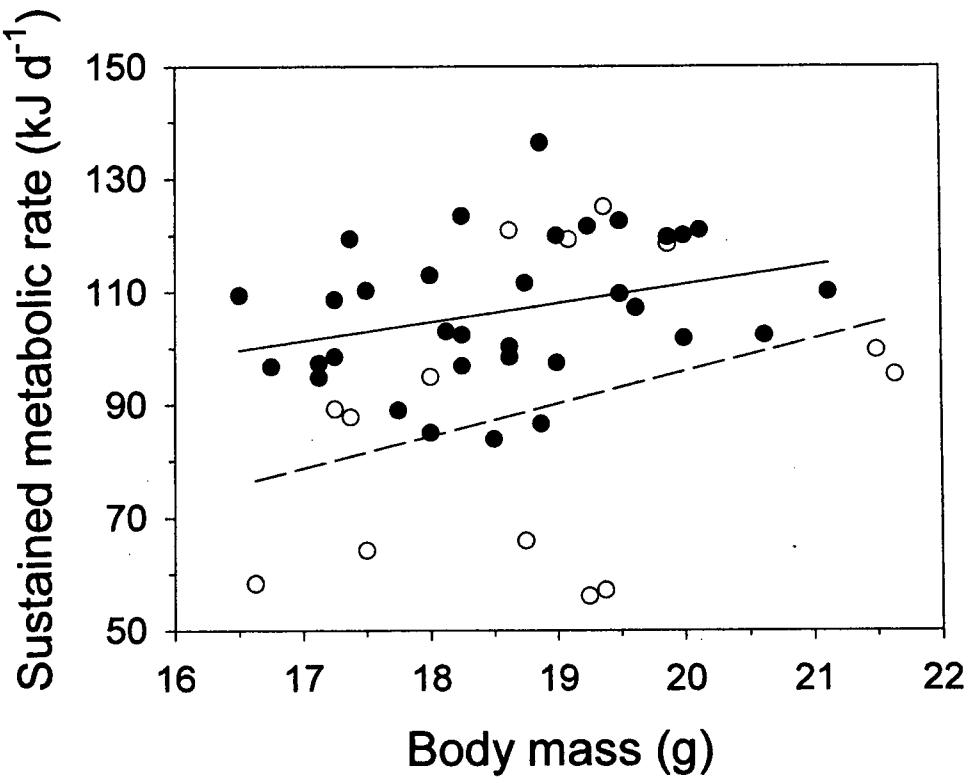


Figure 2.2 Allometric scaling of parental sustained metabolic rate and body mass in breeding tree swallows. (o) 1996; (•) 1997. When considered independently, neither year was significant.

Mass change and recapture interval

Adults lost on average 0.17 g d^{-1} (S.D.=0.529, N=43, Appendix 1). Change in body mass was not a significant predictor of SusMR (P>0.50; both body mass and year were significant in the model, P<0.05).

The mean elapsed time between release and recapture was 24 hr 41 min (S.D.=2 hr 10 min, N=47; Range: 18 hr 45 min to 28 hr 49 min). After controlling for body mass and year of study, there was no relationship between SusMR and the deviation of the recapture from 24 hrs (recapture interval - 24 hr; $F_{1,43}$=0.083, P>0.50).

Parental SusMR and correlates of fitness

Brood size

Parental SusMR was independent of brood size (P>0.50, Fig 2.3). A lack of statistical significance was probably not due to insufficient power. From Fig 2.1A, I estimated that broods of 7 nestlings had ~ 30% greater mass gain per day than broods of 5 nestlings. Consequently, I predicted *a priori* that the SusMR of individuals rearing 7 nestlings would be ~30% higher than those rearing 5 nestlings. I had a power of 0.80 to detect a 25% difference in energy expenditure among females rearing each of the 3 brood sizes, and the ability to detect a 35% difference among males. Finally, if the sexes were pooled, parental SusMR remained independent of brood size (P>0.90) despite the ability to detect a 21% difference among means (at a power of 0.80).

When parental SusMR was expressed per nestling rather than per brood, females rearing broods of 5 expended more energy per nestling than females rearing broods of 6 or 7 ($F_{2,26}$ = 14.393, P<0.001; Tukey HSD P<0.05; Fig 2.3). In males, SusMR per nestling was independent of brood size (P=0.066, Fig 2.3). If years were considered separately, in 1997 males rearing broods of 5 or 6 nestlings expended significantly more energy per nestling than males rearing broods of 7 ($F_{2,11}$ = 7.134, P<0.05; Tukey HSD P<0.05). SusMR in 1996 was measured in only 3 males, precluding a separate analysis.

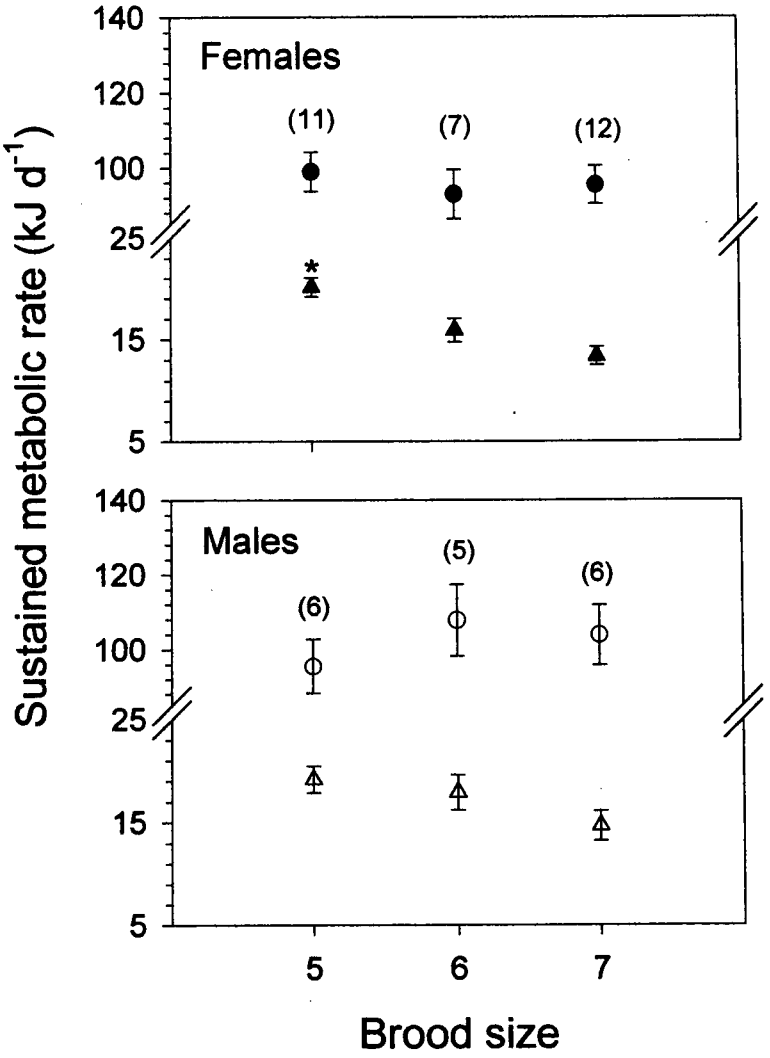


Figure 2.3 Sustained metabolic rate of adult tree swallows rearing natural sized broods. Circles represent the total parental energy expenditure, triangles are the energy expenditure per nestling. Least squares means ± 1 S.E.M., *P<0.05. Sample sizes are in brackets.

Nestling mass and growth rate

Parental SusMR was analyzed with respect to brood mass on day 8, and the previous mass gain of the brood between days 4 and 8. Years and sexes were analyzed separately due to significant interaction terms. As I predicted positive relationships among variables *a priori*, P-values are 1-tailed.

In 1997, females with an high SusMR were rearing broods that had previously displayed a high growth rate between days 4 and 8 ($F_{1,13}$=10.832, P<0.01; Fig. 2.4A). A single nest with a high studentized residual (-2.891) was omitted from analysis; the nestlings from this nest had the highest body mass on day 4 (>2.5 S.D. from the mean), and had little mass gain between days 4 and 8. Male SusMR was independent of the previous mass gain of his brood (P=0.122). There was a positive correlation between female and male SusMR and the total mass of their broods on day 8 (Females: $F_{1,14}$=21.400, P<0.001; Males: $F_{1,9}$=4.571, P<0.05; Figs. 2.4B).

In 1996, there was no relationship between female SusMR and the mass gain of her nestlings between days 4 and 8 (P>0.20). The correlation between female SusMR and brood mass on day 8 was more complex than in 1997, and varied between brood sizes (SusMR*brood size interaction, P<0.01). Females rearing broods of 5 or 6 nestlings increased their SusMR with increasing brood mass on day 8 ($F_{1,3}$=7.771, P=0.034, N=6). In contrast, females rearing 7 nestlings showed a significant negative correlation (r = -0.98, P<0.05, two-tailed test, N=4). As only 3 males were labeled in 1996, analysis was not possible.

Relationships among growth, brood mass and parental SusMR were not driven by covariation with temperature (*sensu*, Dykstra and Karasov 1993). There was no relationship between temperature on day 8 (maximum or minimum) and either mass gain of the brood between days 4 and 8, or total brood mass on day 8 in either year (P>0.05).

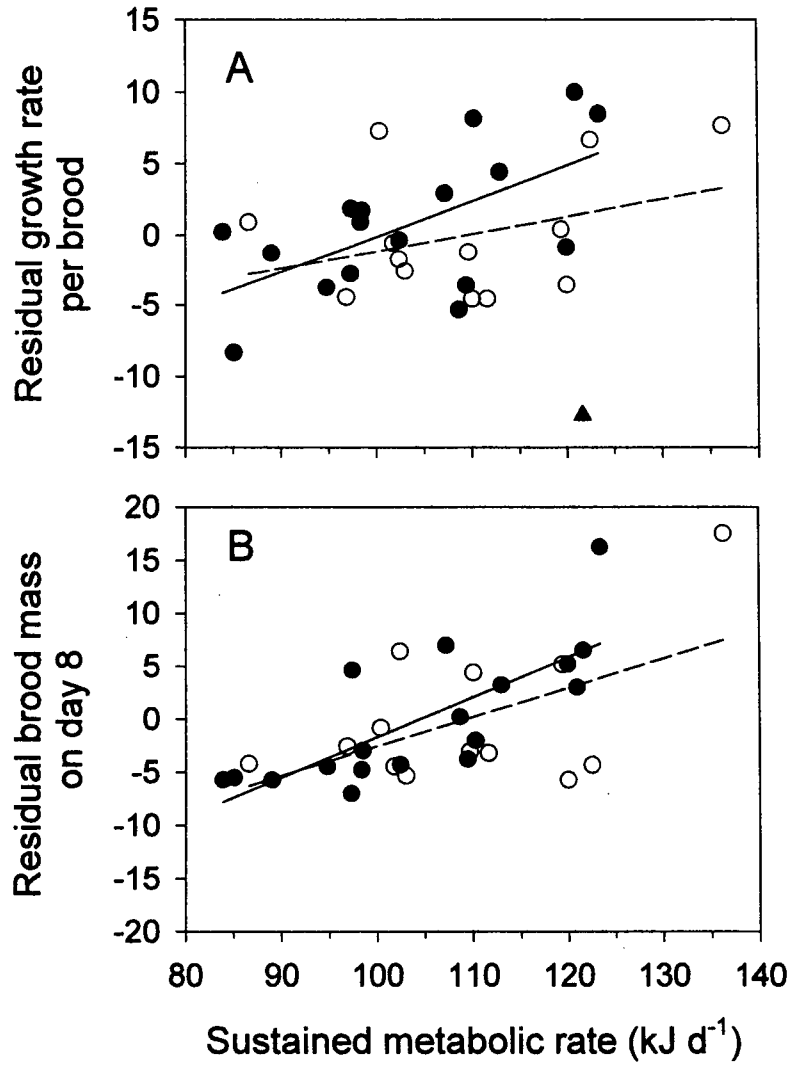


Figure 2.4 Sustained metabolic rate of adult tree swallows and (A) the residual growth rate of their broods between days 4 and 8 and (B) the residual mass of their broods on day 8. Residuals controlled for the effect of brood size on the dependent variable. Females: solid circles, solid line; Males: open circles, dashed line Solid triangle was an outlier (see text) and was omitted from analyses. All correlations significant ($P<0.05$), except for that of males in panel A ($P=0.12$).

Physiological correlates of brood size

The mean Hct was 43.4 % (S.D.=5.1, N=46), and ranged from 33.2 - 54.3%. Mean Hct did not vary between brood size or year of study for either sex (P>0.30, Table 2.1). Females captured later in the season had significantly lower Hct than those captured early ($F_{1,23}$=6.068, P<0.05); this relationship was not seen for males (P>0.40).

Adult organ and tissue masses were unrelated to brood size (P>0.10), but differed between years (Tables 2.2, 2.3). In females, the mean mass of the pectoralis and kidney were respectively, 8% and 15% greater in 1997 than in 1996 (Pectoralis: $F_{1,24}$=7.303, P<0.05; Kidney: $F_{1,23}$=8.776). In males, mean liver mass was 35% greater in 1997 than 1996 ($F_{1,14}$=5.864, P<0.05).

The mass of stored lipid did not differ between size of brood or years (Tables 2.4, 2.5). In females, a significant interaction term was due to a single point with a relatively large studentized residual (-2.487). Excluding this point, the only significant predictor of lipid mass was lean body mass ($F_{1,23}$=5.622, P<0.05). In males, total lipid mass was correlated with lean body mass (P<0.05) and both the time and date on which individuals were captured (P<0.05).

Physiological correlates of parental SusMR

Sexes were pooled but data from the two years were considered separately. In 1997, the only organ to correlate with SusMR was the small intestine. After controlling for both recapture date and body mass, individuals with relatively heavy intestines had a relatively high SusMR ($F_{1,20}$=8.088. P=0.01; Fig. 2.5). This relationship was dependent on inclusion of recapture date as a significant covariate. In 1996, an individual's SusMR could not be predicted by the mass of any of its organs (P>0.05). In neither year was there a relationship between an individual's Hct and its SusMR (P>0.30).

Table 2.1 Variation in the haematocrit of adult tree swallows rearing different sized broods.

		Brood size		P-value	
Sex	N	5	7	Brood size	Year
Males	(10, 9)	43.19 ± 1.98	41.72 ± 1.84	0.525	0.594
Females	(15, 12)	44.56 ± 1.32	42.78 ± 1.47	0.375	0.592

Values (%) are least squares means ± S.E.M. (standard error of mean) from ANCOVA. Sample sizes are in brackets.

Table 2.2. Physiological correlates of brood size in female tree swallows.

		Brood size		P-value	
Character	N	5	7	Brood size	Year[a]
Pectoralis	(14, 14)	2.62 ± 0.05	2.62 ± 0.05	0.979	<u>0.012</u>
Heart	(15, 14)	0.23 ± 0.01	0.23 ± 0.01	0.643	0.562
Kidney	(14, 14)	0.25 ± 0.01	0.25 ± 0.01	0.811	<u>0.007</u>
Liver	(15, 13)	0.60 ± 0.02	0.64 ± 0.02	0.153	0.057[b]
Intestine	(15, 13)	0.68 ± 0.02	0.69 ± 0.03	0.865	0.162
Gizzard	(14, 14)	0.46 ± 0.02	0.43 ± 0.02	0.302	0.224

Values are least squares means ± S.E.M. (standard error of mean) from ANCOVA. Masses are in grams. Sample sizes (bracketed) varied across organs due to missing data. The pectoral muscle, kidney, and gizzard each had a single outlier with a large studentized residual (>3.0) omitted. [a]When significant difference occurred between years, 1997>1996. [b]Significant year*time of capture interaction. Significant P-values are underlined.

Table 2.3 Physiological correlates of brood size in male tree swallows.

		Brood size		P-value	
Character	N	5	7	Brood size	Year[a]
Pectoralis	(11, 9)	2.71 ± 0.12	2.77 ± 0.12	0.722	0.084
Heart	(11, 9)	0.25 ± 0.01	0.24 ± 0.01	0.527	0.850
Kidney	(11, 9)	0.25 ± 0.01	0.26 ± 0.01	0.671	0.203
Liver	(10, 9)	0.65 ± 0.04	0.58 ± 0.04	0.249	0.030
Intestine	(11, 9)	0.75 ± 0.03	0.71 ± 0.03	0.350	0.118
Gizzard	(10, 8)	0.45 ± 0.03	0.42 ± 0.03	0.605	0.090

Values are least squares means ± S.E.M.(standard error of mean) from ANCOVA. Masses are in grams. Sample sizes (bracketed) varied across organs due to missing data. [a]When significant difference occurred between years, 1997>1996. Significant P-values are underlined.

Table 2.4 Correlates of total lipid mass in female tree swallows.

	Lipid mass			
Source	df	SS[a]	F	P-value
Brood size	1	0.010	0.490	0.491
Year	1	0.032	1.533	0.229
LBM[b]	1	0.106	5.117	0.034
Capture date	1	0.125	6.023	0.023
LBM*capture date	1	0.122	5.897	0.024
Error	22	0.455		

[a]Partial sum of squares. [b]LBM (Lean body mass). Significant P-values are underlined. N=28.

Table 2.5 Correlates of total lipid mass in male tree swallows.

	Lipid mass			
Source	df	SS[a]	F	P-value
Brood size	1	0.006	0.473	0.504
Year	1	<0.001	0.001	0.977
Lean body mass	1	0.077	6.353	0.026
Capture time	1	0.104	8.620	0.012
Capture date	1	0.279	23.122	<0.001
Error	13	0.157		

[a]Partial sum of squares. Significant P-values are underlined. N=19.

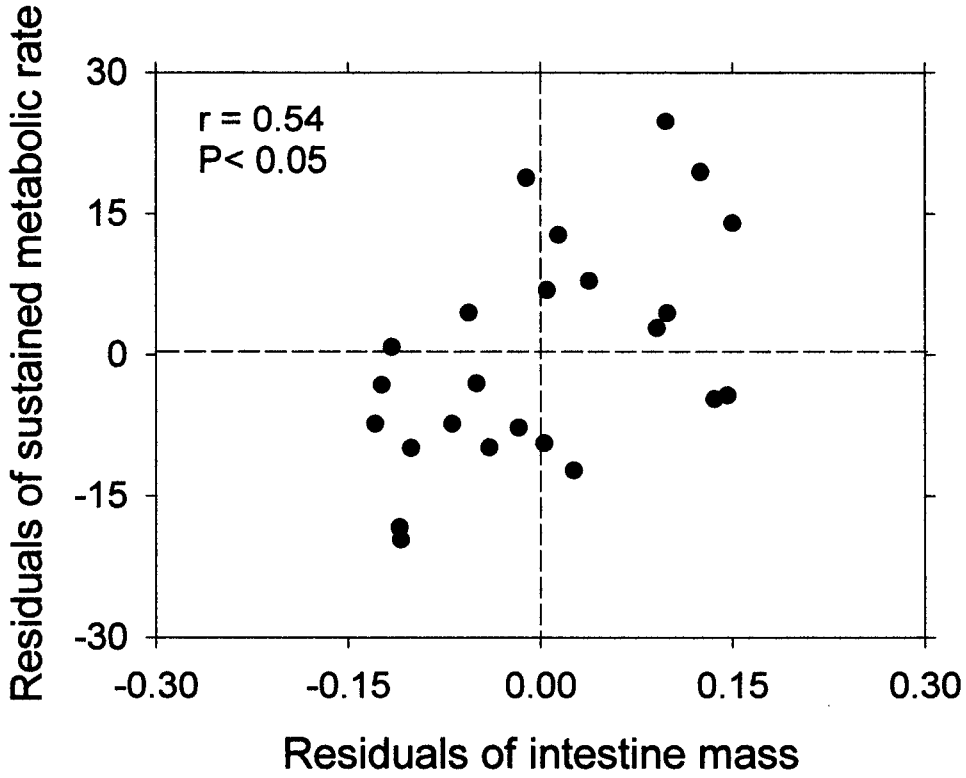


Figure 2.5 Parental sustained metabolic rate and the wet mass of the small intestine. Residuals controlled for the effects of body mass and date.

Table 2.6 Enzyme activity in the pectoralis of adult tree swallows.

	Brood size		Statistics	
Character	5	7	t	P-value
Females				
PK	761.7 ± 69.84	736.4 ± 77.00	0.706	0.491
CS	277.6 ± 31.62	284.5 ± 39.59	0.391	0.701
HOAD	115.1 ± 18.80	117.8 ± 23.76	0.255	0.802
LDH	349.4 ± 41.44	326.2 ± 31.90	1.300	0.213
Males				
PK	696.7 ± 80.82	669.5 ± 23.59	0.859	0.405
CS	288.6 ± 39.84	276.1 ± 22.38	0.737	0.473
HOAD	117.2 ± 18.7	131.3 ± 27.39	1.220	0.243
LDH	350.3 ± 77.7	364.0 ± 49.79	0.407	0.690

Enzyme activities are in U/g tissue, expressed as means ± 1SD. Sample sizes: Females, 5 chicks (N=9), 7 chicks (N=8); Males, 5 chicks (N=9), 7 chicks (N=7).

Biochemical correlates of brood size and parental SusMR

The only enzyme that demonstrated a significant allometric scaling with mass was pyruvate kinase ($\log_{10}PK = 3.54 + -0.54 \log_{10}Mass$; $r^2=0.15$, $N=33$, $P<0.05$). In contrast to previous studies of other taxa (e.g., mammals, Emmett and Hochachka 1981), the slope of the allometric relationship was negative rather than positive. Despite considerable variance in pectoral muscle enzyme activity, activity and brood size were unrelated ($P>0.25$; Table 2.6). Enzyme activity was also unrelated to parental SusMR ($P>0.30$).

DISCUSSION

This study of natural brood size variation in tree swallows demonstrated that: (i) adults did not trade-off nestling quality for quantity; (ii) SusMR and brood size were unrelated; (iii) parental SusMR and brood mass were related; (iv) adult body composition and muscle biochemistry were unrelated to brood size; and (v) parental SusMR was correlated with the mass of the small intestine.

Parental effort: brood size and SusMR

The growth rate of nestlings in large natural broods was the same as in small natural broods, indicating that parents did not trade-off nestling quality for their quantity. Although energetic savings due to decreased heat loss per nestling in large broods may play a role in the observed patterns (Royama 1966), variance in parental ability is probably more important. Indirect evidence for this comes from brood enlargements in this same population. Nestlings in artificially enlarged broods gained less mass than those in control broods (GPB unpublished data, see also Chapter 4), which is inconsistent with energetic savings in large broods.

Despite differences in the total mass gain among broods of different sizes, I could detect no relationship between either male or female SusMR and the number of nestlings

reared. Adults rearing broods of 7 spent significantly less energy, per nestling, than parents rearing broods of 5. One possible explanation is that parents rearing 7 nestlings were energetically more efficient than those rearing 5. The reason for this is unknown, but may be related to differences in experience or skill levels. For example, individual European kestrels (*Falco tinnunculus*) with differing natural brood sizes had the same estimated SusMR (based on time-activity budgets), spent the same amount of time in flight and delivered the same amount of food per nestling (Masman et al. 1989). In that study, males with larger natural broods had better territories and a greater rate of prey capture per unit time.

In an aerial insectivore such as the tree swallow, variance among individuals in foraging ability is likely important. In addition to increasing their energy expenditure, provisioning parents have many ways to deal with variation in brood demand. For example, they may increase their load size or change the type of prey delivered to nestlings (Wright et al. 1998). Whether adult swallows forage in different locations is unknown.

A lack of relationship between natural or manipulated brood size and SusMR is not unusual. Williams and Vezina (2000) reviewed 20 studies that attempted to correlate SusMR with brood size. Of these, 14 (70%) failed to detect a significant correlation. Even when a significant correlation was reported, it was not necessarily consistent between sexes, populations or nestling ages. These previous studies, and the present one, support the hypothesis that parents adjust their brood size to their own feeding capacity. This may allow all adults to work at similar levels of energy expenditure irrespective of the number of nestlings (Drent and Daan 1980).

Parental effort: SusMR and brood mass

Despite a lack of difference in SusMR between brood sizes, among broods of a given size parental SusMR was correlated with nestling mass. Moreno et al. (1997) found a similar correlation in the pied flycatcher (*Ficedula hypoleuca*). In that species, there was a strong positive correlation between female SusMR and both nestling mass and tarsus length. In

males, nestling tarsus length could be predicted from SusMR, but only after controlling for the effect of treatment (brood enlargement).

The data in the present study and that of Moreno et al. (1997) are correlational. Consequently, a relationship between parental SusMR and brood mass can be interpreted in two ways: (1) parents were adjusting their SusMR to the food requirements of their nestlings, with heavier nestlings requiring more food, (2) parental effort, as reflected by SusMR, determines nestling growth rates and mass. Traditionally, it has been assumed that nestling food requirements determine feeding rate, resulting in variation in parental SusMR (e.g., Tinbergen and Dietz 1994). In contrast to this, Moreno et al. (1997) argued that an individual's SusMR is relatively constrained, and should therefore be viewed as the independent rather than dependent variable. As parental SusMR rarely responds to changes in brood demand, they argued that levels of activity are constrained by either a parental time-activity budget or a physiological limit to energy expenditure (Drent and Daan 1980, Weiner 1992).

Additional support for a constraint on energy expenditure comes from the recent studies of Moreno et al. (1999). They increased flight costs of female pied flycatchers directly by clipping two primary flight feathers from each wing. Females with clipped feathers maintained the same body mass and did not change their SusMR relative to controls. The nestlings of females with clipped feathers had lower body mass and increased mortality rates. These data suggested that females were unable or unwilling to increase their energy expenditure.

Wardrop (2000) has shown that individual tree swallows vary in their capacity to respond to an energetic challenge. She enlarged broods by 2 nestlings, and followed nestling mass gain (as an index of parental provisioning capacity) over the next two days. The entire brood was then replaced with nestlings from elsewhere in the population (to avoid autocorrelation), and the growth of the replacement nestlings was followed until day 15. Parents who had a relatively high provisioning capacity early in the breeding season produced

relatively heavy replacement fledglings. These results demonstrated that parents differed in their abilities to respond to an energetic challenge, that these differences were maintained throughout the breeding season, and that they likely had fitness consequences.

The recent finding that estimates of SusMR in female pied flycatchers are repeatable between years (Potti et al. 1999) suggests that energy expenditure may retain a genetic component of variation. In light of this finding, and the work of Wardrop (2000), the variation in nestling body mass and growth rates that I detected likely reflect differences among parents in their capacity to attain a high SusMR.

Physiological correlates of SusMR

In this study, I viewed parental SusMR as a performance trait, much like $\dot{V}O_{2max}$. In the same way that correlates of $\dot{V}O_{2max}$ have been identified previously (e.g., Garland 1984, Taylor et al. 1987, Chappell et al. 1999), I sought physiological correlates of an individual's SusMR. These would define a 'high quality' individual physiologically, and identify characters that may be subject to evolutionary change under selection for whole animal performance.

In contrast to short term measures of activity such as $\dot{V}O_{2max}$, sustained energy expenditures require that an individual remain in approximate energy balance, i.e., energy intake must equal energy output. At a mechanistic level, sustained energy expenditures are likely constrained by either: (1) an individual's investment in nutrient acquisition (including foraging, digestion, absorption; Weiner 1992) or (2) at the site of energy expenditure (e.g., contractile properties of skeletal muscle). Alternatively, there may be no single site of limitation, and the capacities of various steps may be approximately equal (symmorphosis, Taylor and Weibel 1981). There is increasing laboratory evidence that in the presence of excess food, maximum SusMR is more likely limited by peripheral tissues (e.g., working muscles) than centrally (e.g., by digestion; Hammond and Diamond 1997). Although individual tree swallows varied considerably in pectoral muscle mass and enzyme activity,

this variance was unrelated to SusMR. This suggests that the upper limit to an individual tree swallow's SusMR in the field was unlikely to be limited peripherally.

There was, however, a positive correlation between an individual's SusMR and the mass of its small intestine. A similar correlation has been found in laboratory studies of small mammals (Konarzewski and Diamond 1994). To my knowledge, this is the first demonstration of such a relationship under field conditions. Although my study suggests that attainment of a relatively high SusMR requires a relatively large gastro-intestinal system, a simple correlation is not sufficient to identify the intestine as a bottleneck. Only through a comparison of energy expenditure during provisioning with that during other activities (e.g., shivering thermogenesis) could the proximate factors imposing a ceiling be identified (Peterson et al. 1990, Hammond and Diamond 1997). For example, if the SusMR during shivering was greater than during provisioning, a ceiling on provisioning could not be determined by shared machinery (e.g., intestine).

It is well recognized that individuals will undergo intestinal hypertrophy under conditions of chronically high energy expenditure (e.g., Dykstra and Karasov 1992, Hammond and Diamond 1997). It is unknown whether inter-individual variation in intestine mass allowed some swallows to attain a high SusMR, or whether individuals underwent intestinal hypertrophy in response to a high SusMR. Use of non-invasive techniques that allow repeated sampling of the same individual (e.g., ultrasonography, Dietz et al 1999) may allow for these two possibilities to be disentangled.

Dykstra and Karasov (1992, 1993) argued that provisioning effort in the field is unlikely to be constrained physiologically. They showed that the SusMR of house wrens feeding nestlings was considerably below that measured in the lab under conditions of cold and exercise. In support of these findings, Tinbergen and Verhulst (2000) recently showed that although an energetic ceiling is apparently set for female great tits (*Parus major*) at the level of their unmanipulated brood size, the ceiling varied between years. Although this does not negate the need for an increased digestive capacity under conditions of high energy

expenditure, it does suggest that a ceiling on SusMR in the field is more likely set by ecological factors (e.g., day length) rather than physiological ones (e.g., digestive capacity).

Inter-annual variation SusMR and body composition

There was a significant inter-annual difference in SusMR. This difference was due in part to 5 of 14 individuals, each with a particularly low SusMR in 1996 (Fig 2.2). As I performed no behavioural observations I cannot tell whether these individuals were behaving normally during the 24 hour trial. If the low values were a consequence of handling stress, low SusMRs would have also been expected in 1997, yet these were not observed.

In addition to low values for SusMR, individuals sacrificed in 1996 had on average smaller internal organs than in 1997. Whether interannual differences in body composition were related to differences in SusMR is unknown, although relatively large internal organs may be a prerequisite for an elevated SusMR (e.g., Kersten and Piersma 1987, Daan et al. 1990). Inter-annual differences in both SusMR and organ size may be related to variation in food availability. It is unclear, however, whether an increase or decrease in the size of internal organs would be predicted in response to increased food abundance (e.g., Daan et al. 1989, Geluso and Hayes 1999). Regardless, inter-annual differences in organ mass have been found for this same population in other years (Chapter 3), suggesting that phenotypic flexibility is a general phenomenon (Piersma and Lindström 1997).

SusMR and fitness.

There is increasing evidence that rates of energy expenditure in birds are stable over relatively long time periods (Chappell et al. 1996, Potti et al. 1999, Bech et al. 1999). But why do only some individuals attain a high SusMR if it is potentially linked with Darwinian fitness? One possibility is that in order to attain a high SusMR it is necessary to maintain a relatively large gut (e.g., Konarzewski and Diamond 1994, Dykstra and Karasov 1992). This may result in an elevation of an individual's energy expenditure while resting (Piersma et al.

1996, Chappell et al. 1999, but see Chapter 3), which likely carries associated costs. Correlations between SusMR and intestinal mass would presumably result in covariation between resting and sustained metabolic rates (aerobic capacity model, Bennett and Ruben 1979). Support for such covariation remains equivocal (Hayes and Garland 1995, Ricklefs et al. 1996).

Conclusion

Energy allocation by parents to their to offspring is predicted to increase with increasing brood size. Despite this, I could not detect a relationship between brood size and parental energy expenditure. One explanation is that there exists inter-individual variation in parental foraging efficiency. This supports a previous suggestion in the literature that clutch size is adjusted to the amount of food that can be delivered to nestlings for the same parental energy expenditure (Masman et al. 1989). In one of two years, there was a positive relationship between parental SusMR and brood mass, suggesting potential reproductive benefits. Individuals with relatively high SusMR had relatively large intestines, which presumably allowed for an increased digestive capacity. This suggests the existence of a trade-off between the reproductive benefits of attaining a high sustained energy expenditure, and the costs associated with maintaining expensive metabolic machinery.

CHAPTER 3

INTER-INDIVIDUAL VARIABILITY IN BODY COMPOSITION AND RESTING OXYGEN CONSUMPTION RATE IN BREEDING TREE SWALLOWS

PREFACE

This chapter is adapted from a paper published by G. P. Burness, R. C. Ydenberg and P. W. Hochachka (Physiol. Zool. 71: 247-256). I was responsible for all data collection, analysis and presentation. My co-authors provided guidance and editorial advice.

INTRODUCTION

Basal metabolic rate (BMR) is defined as the minimum rate of energy expenditure in a non-growing, post-absorptive organism, at rest in its thermoneutral zone (Brody 1945), during its period of daily inactivity (Aschoff and Pohl 1970). A large comparative data set exists relating BMR to body mass across a wide variety of taxa. Studies investigating BMR have often been concerned with accurate determination of the slopes of these allometric relationships (e.g., mammals , Elgar and Harvey 1987; birds, Bennett and Harvey 1987). These studies and others (e.g., Koteja and Weiner 1993) have also been interested in species that deviate from the regression lines. For a given body mass, two species can vary considerably in their BMRs. As an example, the Virginia opossum (*Didelphis virginiana*) has a BMR 30% lower than predicted for a similarly sized eutherian mammal (Fournier and Weber 1994). Such deviations also exist in birds. For example, island species have much lower BMRs than mainland species of the same body size (McNab 1994).

The mechanistic basis underlying variability in BMR among similarly sized species is gradually being determined. McNab (1994) found a positive correlation between BMR and pectoral muscle mass in many flightless birds. Kersten and Piersma (1987) speculated that inter-specific differences in BMRs among birds reflected differences in the size of a species'

"metabolic machinery". Daan et al. (1990) subsequently demonstrated that those species of birds with relatively high BMRs for their body size have relatively large masses of hearts and kidneys. In fact, in an analysis of 22 avian species, these two organs, which contribute only 0.61% of body mass, explained 50% of the variation in BMR (Daan et al. 1990). Both of these organs have exceptionally high oxygen consumption rates in tissue slice preparations (Krebs 1950, Scott and Evans 1992).

Within a species the relationships between BMR and organ masses are unclear. As the slopes from regressions of BMR on mass vary depending on the taxonomic level studied (e.g., Bennett and Harvey 1987) different mechanisms may be acting within species from those acting between species. To date most research on this question has considered small mammals (Konarzewski and Diamond 1994, 1995, Koteja 1996, Speakman and McQueenie 1996, Meerlo et al. 1997) and lizards (Garland 1984, Garland and Else 1987).

As an estimate of BMR many studies measure resting metabolic rate (RMR), which does not assume that individuals are post-absorptive or in their period of daily inactivity. In comparisons among and within strains of inbred mice, Konarzewski and Diamond (1994, 1995) demonstrated that individuals with high RMRs have relatively large kidneys, livers, hearts, and intestines. In contrast, relationships between organ mass and BMR are weak or absent in *Peromyscus maniculatus* (Koteja 1996). Meerlo et al. (1997) demonstrated a significant positive relationship between mass-independent residuals of BMR and heart mass in the field vole (*Microtus agrestis*). Finally, using principal component analysis, Speakman and McQueenie (1996) suggested a relationship between BMR and organs of the digestive system in mice.

As variability is necessary for the evolution of a trait through natural selection, assessment of trait variability is important. This was the first field study of any avian species to relate inter-individual variability in RMR with body composition (but see Chappell et al. 1999, Bech et al. 1999). In addition, as food resources likely vary across years, there may be considerable variability in RMR and the masses of energetically

expensive organs between breeding seasons. Although individual body composition changes cannot be followed practically across more than one breeding season, annual averages are informative.

I present data from tree swallows (*Tachycineta bicolor*) collected over two breeding seasons. This study had two aims: (1) to determine the extent of variation in RMR (as an approximation of BMR) and body composition in a wild population of birds, and (2) to address the question of relationships between RMR and organ and tissue masses.

MATERIALS AND METHODS

This study took place during May-June, 1994 and 1995, in Creston, British Columbia, Canada. Tree swallows in this population typically lay between one and eight eggs in nest boxes, with a modal clutch of six. Only those pairs that laid 6 eggs were chosen for study. First-time breeding females can be distinguished on the basis of plumage (Hussell 1983) and were excluded from this study. In 1994, tree swallows were captured at the nest box 8-9 days after hatch of chicks and transported to a field lab for resting oxygen consumption rate measurements ($\dot{V}O_2$). In 1995, the experimental protocol was modified slightly. First, to increase parental effort, when chicks were 4 d old one additional nestling was added to each brood. This increased brood sizes from six to seven. Second, as part of another study on foraging energetics, all experimental adults were captured and injected intramuscularly on day 8 with 100 μL of doubly labeled water ($^3H_2{}^{18}O$; see Chapter 2 for methodology). Birds were held for 1 h, a sample of blood (150μl) was taken, and the bird was released. Upon recapture after 24 h, a second blood sample was taken, and the birds were transported to the field lab as in 1994. Although there was a difference in field protocols between 1995 and 1994 , the gross morphological measurements I performed on adults were unlikely to have been affected by such protocol changes.

Resting oxygen consumption

As an approximation of BMR, I measured daytime resting $\dot{V}O_2$. In both 1994 and 1995, VO_2 was determined using closed system manometry (e.g., Williams and Prints 1986, Obst et al. 1987).

After capture, birds were immediately transported to a field lab for $\dot{V}O_2$ measurements. Adult swallows were weighed on an electronic pan balance (to the nearest 0.2 g). Flattened wing chord (measured from the wrist to the distal tip of the ninth primary feather, ±0.5 mm) was measured using a ruler with a stop at one end. Keel length was measured using dial calipers (±0.05 mm). Birds were then placed in a 1,000-mL black Plexiglas metabolic chamber. The floor of the chamber contained soda lime and Drierite to absorb CO_2 and H_2O, respectively. A steel mesh covered with a sheet of tissue paper on which the bird stood was placed over the chemicals. After an equilibration time of 1.5-2.5 h, a V-shaped Plexiglas manometer filled with Krebs manometer fluid was attached to the chamber. Five milliliters of oxygen was injected from a calibrated glass syringe, causing an increase in chamber pressure. The time taken for the bird to consume the 5 mL of O_2 was indicated by movement of the manometer fluid. This procedure was repeated over the next 45-60 min, and the values were averaged. The minimum acceptable number of replicates for a successful trial was 7 with a maximum of 12. The mode was 10.

If the bird was active in the chamber, the manometer fluid would 'jump'. Only measurements in which the level of the manometer fluid decreased linearly and smoothly were used, indicating quiescence. Similar manometric systems to the one in this study have been in good agreement when compared against flow through systems (Obst et al. 1986, Williams and Prints 1986). In addition, the coefficients of variation for $\dot{V}O_2$ for birds measured in the present study fell well within the range calculated for similarly sized passerines (e.g., Dutenhoffer and Swanson 1996).

The thermoneutral zone for tree swallows is between approximately 30° and 35°C (Williams 1988). Thermoneutrality was maintained by submerging the chambers in a Lauda

RM6 temperature-controlled water bath. Chamber temperature was monitored (±0.1°C) using a thermocouple inserted approximately 4 cm down into the sealed chamber. Daily changes in room temperature and consequently water bath settings resulted in slightly different chamber temperatures between individuals. Such temperatures were, however, always within the thermoneutral zone. The thermocouple also recorded rapid temperature fluctuations coincident with 'jumping' of the manometer fluid. This indicated activity in the chamber, and recordings were not attempted during such periods.

To minimize variability due to circadian rhythms (Aschoff and Pohl 1970), all measurements were performed between 1000 and 1800 hours. Forty-eight of 51 birds captured had their $\dot{V}O_2$ measured on the same day as capture. Three in 1995 were captured late in the day, and to avoid variability due to circadian rhythms were held overnight until measurement the following day.

Birds were left in the chamber for between 1.5 and 2.5 h before beginning measurements, so it is unknown if they were all post-absorptive. To assess this, small intestine contents were measured and correlated against mass independent $\dot{V}O_2$ to determine if there was an apparent heat increment of feeding. During trials, room temperature and barometric pressure were recorded and all values of $\dot{V}O_2$ were corrected to STPD.

Haematocrit

Following measurement of resting $\dot{V}O_2$, birds were removed from the chamber and re-weighed. The before and after weights were averaged. To address variability in Haematocrit (Hct) in 1995, blood samples were collected into heparinized microcapillary tubes. These tubes were immediately spun at maximum speed for 10 min using an Adams micro-haematocrit centrifuge. The percentage of the tubes occupied packed cells was measured. The number of replicates was determined by the size of the blood sample and ranged from one to three replicates.

Tissue masses

In both years birds were killed by cervical dislocation immediately following measurement of $\dot{V}O_2$. Both the left and right pectoralis major and supracoracoideous were removed together (referred to as pectoralis), followed by the heart, liver, small intestine, and kidney. All tissues were stored in air-tight cryovials, frozen in a portable dry shipper charged with liquid N_2, and transported back to Vancouver, where they were transferred to a -80°C freezer until analysis.

Wet weights were determined for all organs and tissues (± 0.0001 g). Two individuals from 1994 did not have their left pectoralis weighed. These were estimated from a regression of left pectoral muscle mass on right pectoral muscle mass generated from 18 other individuals (r^2=0.70, P<0.001). The small intestine was initially weighed full and empty mass was determined as in Chapter 2. The difference between full and empty intestine mass was assumed to be gut contents. The intestine was then dried to a constant mass for 36 h at 75°C. I report dry tissue mass only, as the correlation between wet and dry mass is high (r = 0.93, P < 0.001). No other tissues were dried.

Statistical analyses

All variables measured are likely to scale allometrically with body mass. All data with the exception of small intestine contents were $\log_{10}$ - transformed and regressed on mass using simple linear regression. To improve normality, intestine contents were square-root transformed. The effect of year and sex on the dependent variables (masses of heart, kidney, pectoralis, liver, small intestine, and intestine contents) were explored using an ANCOVA. I initially included the interaction terms of year and mass, and sex and mass as additional covariates. As no significant interactions were found, all further multiple regressions were performed including only the covariate (mass) and main effects (year or sex). Residuals were then generated from either ANCOVAs or, when the effect was not significant, from simple

linear regressions. Residual analysis removes the effect of the covariate (mass) from the dependent variable. I report model and error degrees of freedom following the F-Value.

To test for outliers, the studentized residuals for the dependent variable were generated from the multiple regressions on mass. These residuals follow a t-distribution (Wilkinson et al. 1992). I felt that characters such as resting $\dot{V}O_2$ were more likely to be influenced by measurement error (e.g., stress of birds) than were tissue masses. Consequently, for resting $\dot{V}O_2$ data, studentized residuals significant at $P < 0.05$ were excluded; for all other variables, $P < 0.001$.

To examine the variability of each dependent variable, I followed Garland (1984) and compared the standard deviation of the residuals generated from the simple linear regressions on body mass. The standard deviation of residuals from a $\log_e$ transformed data set is approximately equal to the coefficient of variation of the untransformed data set after removal of the effect of body mass. As residuals in the present study were generated from $\log_{10}$-$\log_{10}$ regressions on mass, and not $\log_e$, data were converted from $\log_{10}$ to $\log_e$. To convert from $\log_{10}$ to $\log_e$ the standard deviation of the residuals was multiplied by 2.3026. When a significant regression could not be generated, the standard deviation of the residuals is simply equal to the coefficient of variation of the data set before log transformation. Conversion of the standard deviation of the residuals to coefficient of variation allows comparison with literature values.

To determine the extent to which variation in resting $\dot{V}O_2$ reflected variation in organ and tissue mass, residuals from multiple regressions were included in correlations and a stepwise multiple regression. Residuals of resting $\dot{V}O_2$ were first correlated against residuals of resting organ and tissue masses using a Pearson-product moment correlation or, in the case of the non-normally distributed Hct data, a Spearman-rank correlation. A predictive equation for residual resting $\dot{V}O_2$ was generated using a forward stepping multiple regression.

Residuals of Hct and organ masses were correlated. To control the probability of a Type I error (rejecting the null hypothesis when it should not be rejected), a sequential

Bonferroni correction (Rice 1989) was applied to the data to correct for the number of tests. Correlations within this matrix were considered significant at $P < 0.002$ (alpha = 0.05). All other comparisons were considered significant at $P < 0.05$.

Most analyses utilize mass corrected residuals. To account for the degrees of freedom lost in generating the residuals, N-3 degrees of freedom were used when testing significance of Pearson correlations (Hayes and Shonkwiler 1996), and N-1 for Spearman-rank correlations. As significant P-values were very small in both the correlation matrix (Table 3.3) and stepwise regressions, the degrees of freedom were not adjusted. Analyses were performed using Systat 5.2.1 (Wilkinson et al. 1991). 95% confidence intervals for slopes and intercepts of regressions were generated using SAS (SAS Institute Inc. 1988).

RESULTS

Allometric relationships

I measured $\dot{V}O_2$ and body composition for a total of 51 individuals. When $\dot{V}O_2$ was plotted against mass, three points had exceptionally high studentized residuals (3.033; 2.312; 2.036 ; all $P < 0.05$). Similarly, when heart, pectoralis, and intestine mass were when plotted against body mass each had a single point identified as an outlier (studentized residuals: 3.672; 3.606; 6.528 , $P < 0.001$). These points were included in the figures although not in the analyses.

Males were significantly heavier than females (Males, 18.44 ± 0.97 g; Females, 16.95 ± 0.92 g: $t = 5.610$, $df = 49$, $P < 0.001$). Resting $\dot{V}O_2$, and most tissue and organ masses showed no significant sex differences when body mass was included as a covariate: $\dot{V}O_2$ ($P = 0.886$), heart ($P = 0.057$), kidney ($P = 0.895$), liver ($P = 0.675$), small intestine ($P = 0.240$). A significant difference was found between the sexes for pectoralis ($F_{1,48} = 7.811$, $P = 0.007$). This was probably attributable to the terms sex and mass sharing considerable variance. For the pectoralis, residuals were generated from regressions with and without the term 'sex' included. In subsequent analyses of these residuals, inclusion of 'sex' did not change any

trends or conclusions. As the pectoralis was the only tissue showing significant sex differences, I pooled the sexes for all variables and report results of pooled analyses only.

To generate allometric equations and assess the amount of variance attributable to body mass alone, data were pooled across the two years. The mean body mass was 17.7± 1.2 g, and ranged from 15.3 g - 20.6 g. All variables except Hct ($P = 0.939$) and gut contents ($P = 0.156$) scaled significantly with mass (Table 3.1).

Using ANCOVA with body mass as a covariate, I found no difference between years in the slopes of regressions for heart ($P = 0.223$; Fig. 3.1B); kidney ($P = 0.377$; Fig. 3.1C), small intestine ($P = 0.699$; Fig. 1D) and liver ($P = 0.800$; Fig. 1E). For a given body mass these organs were, however, heavier in 1994 than in 1995: heart ($F_{1,47} = 19.967$, $P < 0.001$), kidney ($F_{1,48} = 45.760$, $P < 0.001$), small intestine ($F_{1,47} = 12.457$, $P = 0.001$), and liver ($F_{1,48} = 17.780$, $P < 0.001$). No significant difference was found for resting VO_2 (Fig. 3.1A) in either slope ($P = 0.878$) or intercept ($P = 0.839$). Similarly, no significant differences were found between years for pectoral muscle either in slope ($P = 0.935$) or intercept ($P = 0.493$, Fig. 3.1F). In all cases where there were differences in intercept of the regression lines over the range of values measured, the lines for 1994 fell above those of 1995.

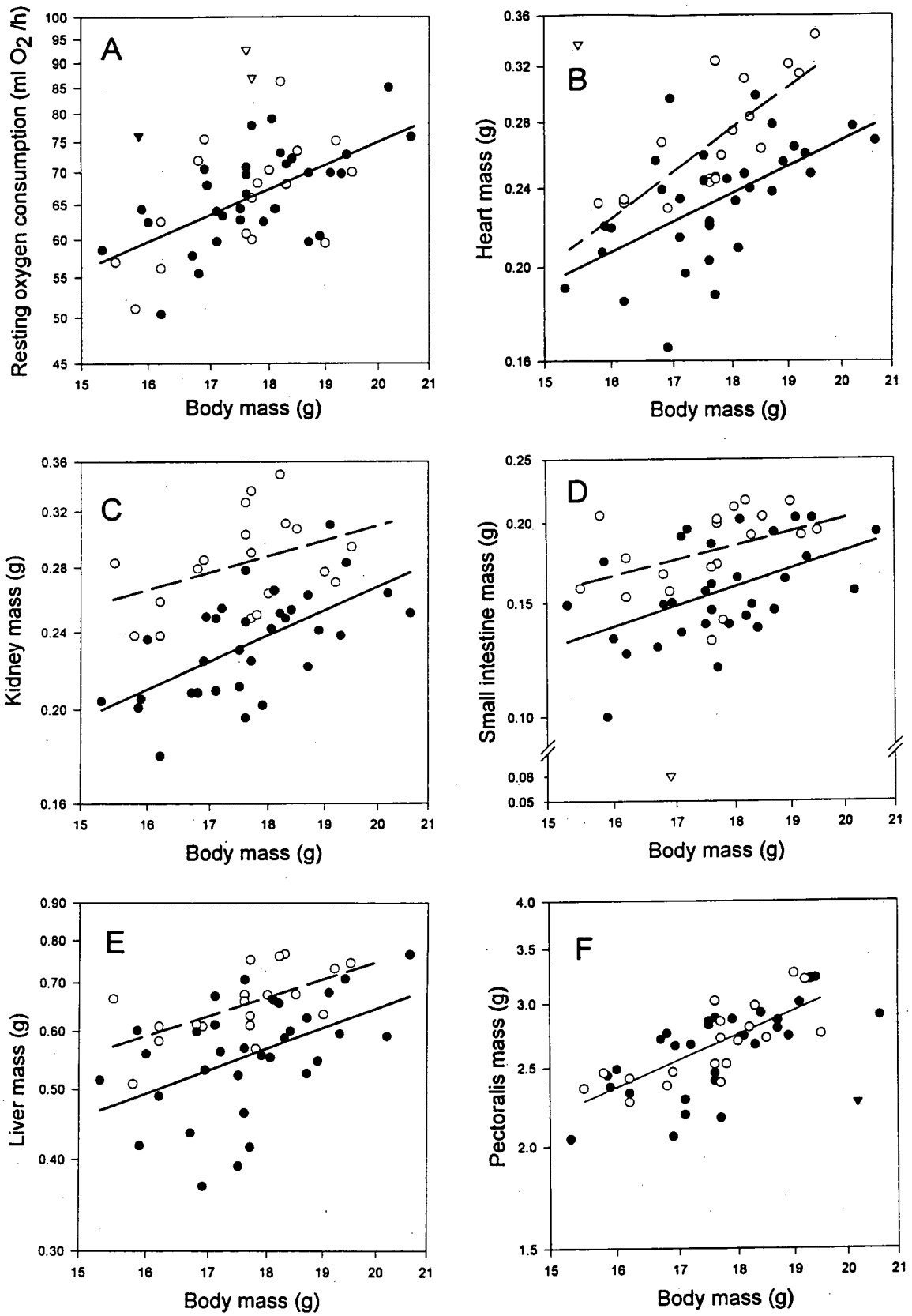
To explore the inter-annual variation further, I generated an index of structural size using principal component analyses (e.g., Cooch et al. 1991). I extracted the first principal component (PC1) from the correlation of wing (mm) and keel length (mm) for the 45 individuals for which I had both measures. PC1 explained 79% of the variation in these characters. I then included PC1 as a covariate and regressed mass against it and included year as the main effect. The interaction term was not significant ($P = 0.511$) and was excluded from the model. For a given body size, birds in 1994 were not significantly heavier than those in 1995 ($P = 0.285$).

Table 3.1 Allometric relationships and descriptive statistics for variables measured in tree swallows

Character	N	Mean	Range	Slope±95%CI	Intercept±95%CI	r^2	SEE[a]	CV%[b]
$\dot{V}O_2$ (mL O_2h^{-1})	48	66.77	50.40 - 86.26	1.03 ±0.413	0.54 ± 0.516	0.35	0.042	9.4
Heart (g)	50	0.24	0.17 - 0.34	1.33 ±0.559	-2.27 ± 0.697	0.32	0.056	12.7
Liver (g)	51	0.60	0.37 - 0.77	1.09 ±0.668	-1.59 ± 0.833	0.18	0.069	15.7
Kidney (g)	51	0.25	0.18 - 0.35	0.88 ±0.584	-1.69 ± 0.728	0.16	0.060	13.8
Pectoralis (g)	50	2.66	2.04 - 3.26	1.25 ± 0.351	-1.13 ± 0.437	0.52	0.035	7.8
Intestine (g)	50	0.17	0.10 - 0.22	1.04 ±0.698	-2.08 ± 0.870	0.16	0.072	16.3
Hct (%)	30	41.71	25.70 -53.30			18.4		

Note. [a]SEE is Standard error of estimate from regression equation. [b]CV% calculated as the standard deviation of residuals generated from allometric regressions, multiplied by 2.3026 to convert from $\log_{10}$ to $\log_e$ (see text for details).

Figure 3.1 Allometric scaling of body mass and (A) resting oxygen consumption rate, (B) heart mass, (C) kidney mass, (D) small intestine mass, (E) liver mass, and (F) pectoralis mass. Open circles are birds measured in 1994; filled circles are for 1995. Triangles are outliers not included in analyses (see text). Dashed line is regression for 1994; solid line for 1995. If only one line present, there was no difference between years. Significance of regressions is given in text. Axes are $\log_{10}$ transformed.


A
Resting oxygen consumption (ml O_2 /h)
Body mass (g)
B
Heart mass (g)
Body mass (g)
C
Kidney mass (g)
Body mass (g)
D
Small intestine mass (g)
Body mass (g)
E
Liver mass (g)
Body mass (g)
F
Pectoralis mass (g)
Body mass (g)

In addition, apart from male wing length, there were no other differences between years for the other external characters that I measured (body mass and keel length; Table 3.2) .

Variability among individuals

The coefficient of variation for each character was calculated as the standard deviation of the residuals generated from the regressions of Table 3.1; this demonstrates variation after the effects of body mass have been removed. The coefficients of variation ranged from a low of 7.8 for pectoral muscle mass to a high of 18.4 for Hct. Resting $\dot{V}O_2$ showed moderate variability with a coefficient of variation of 9.4. The small intestine, a relatively plastic organ (e.g., Secor and Diamond 1995), not surprisingly had a high coefficient of variation (16.3%).

Correlations among characters

Residuals generated from either ANCOVAs or linear regressions were correlated against each other to address two questions: (1) Do individuals with a relatively high resting $\dot{V}O_2$ for their body mass have relatively large organs and tissues? and (2) Do individuals with a relatively large mass of one organ or tissue for their body mass have other relatively large organs and tissues?

To assess if all birds were post-absorptive, I first correlated residual intestine contents against residual $\dot{V}O_2$. Intestine contents were obtained for 43 birds. As there was no significant relationship between the residuals ($r = 0.114$, $P > 0.40$), I concluded that any differences in $\dot{V}O_2$ among individuals were unlikely to be due to a heat increment of feeding.

I correlated residual $\dot{V}O_2$ against the residuals of different tissues and organs. As hypothesized, those individuals with a relatively high $\dot{V}O_2$ had relatively large kidneys ($r = 0.29$, $N = 48$, $P < 0.05$. Fig 3.2). No other morphological variable correlated with residual $\dot{V}O_2$: (heart, $r = -0.15$, $N = 47$, $P > 0.30$; intestine, $r = -0.19$, $N = 47$, $P > 0.20$; liver, $r = -0.056$, $N = 48$, $P > 0.70$; pectoral muscle, $r = -0.23$, $N = 47$, $P > 0.10$).

Table 3.2 External morphometrics between years for male and female tree swallows.

	Year		Statistic	
	1994	1995	t	P
Body mass (g)				
Male	18.4 ± 0.70 (9)	18.4 ± 1.11 (16)	0.049	0.961
Female	16.9 ± 0.90 (10)	17.0 ± 0.96 (16)	0.427	0.673
Wing length (mm)				
Male	119.1 ± 2.80 (9)	122.7 ± 2.61 (15)	3.190	0.004
Female	114.6 ± 3.21 (9)	116.9 ± 2.38 (15)	2.049	0.053
Keel length (mm)				
Male	21.26 ± 0.74 (8)	21.41 ± 0.72 (14)	0.449	0.658
Female	19.74 ± 0.33 (9)	20.19 ± 0.63 (16)	1.973	0.061

Note. Values are means ± 1 S.D. Significance assessed using t-tests on log-transformed data. Sample size is in parentheses.

Values for Hct were non-normally distributed (Lillifore's test, $P = 0.011$) and were compared to residual $\dot{V}O_2$ using a Spearman-rank correlation (the degrees of freedom were reduced by 1). Residual Hct correlated positively with residual $\dot{V}O_2$ ($r = 0.38$, $N = 29$, $P < 0.05$).

I performed a stepwise multiple regression with residual $\dot{V}O_2$ as the dependent variable and the residual of each organ or tissue as a potential predictor variable. The residuals of kidney ($t = 3.112$, $P = 0.003$), and intestine mass ($t = -2.616$, $P = 0.012$) were the only significant predictors of residual $\dot{V}O_2$. Although residual kidney mass loaded positively, residual intestine mass loaded negatively. Together, kidney and intestine explained 21% of the variation in residual $\dot{V}O_2$ ($N = 47$, $F_{2,44} = 5.855$, $P = 0.006$). A second stepwise regression was performed, including residual Hct as an additional predictor. Residual kidney mass ($t = 3.628$, $P = 0.001$), and residual intestine mass ($t = -3.267$, $P=0.003$), were still significant. Residual Hct approached significance ($P = 0.053$).

Table 3.3 is a correlation matrix showing all possible correlations among characters. All significant correlations were positive. Without correcting for body mass, numerous correlations existed among organs and tissues. The number was reduced when body mass independent residuals were correlated. Individual swallows with relatively large livers for their body mass also had relatively large kidneys ($r = 0.52$, $P < 0.001$), and intestines ($r = 0.50$, $P < 0.001$). Individuals with relatively large hearts also had relatively large pectoral muscles ($r = 0.49$, $P < 0.001$). No other correlations were significant.

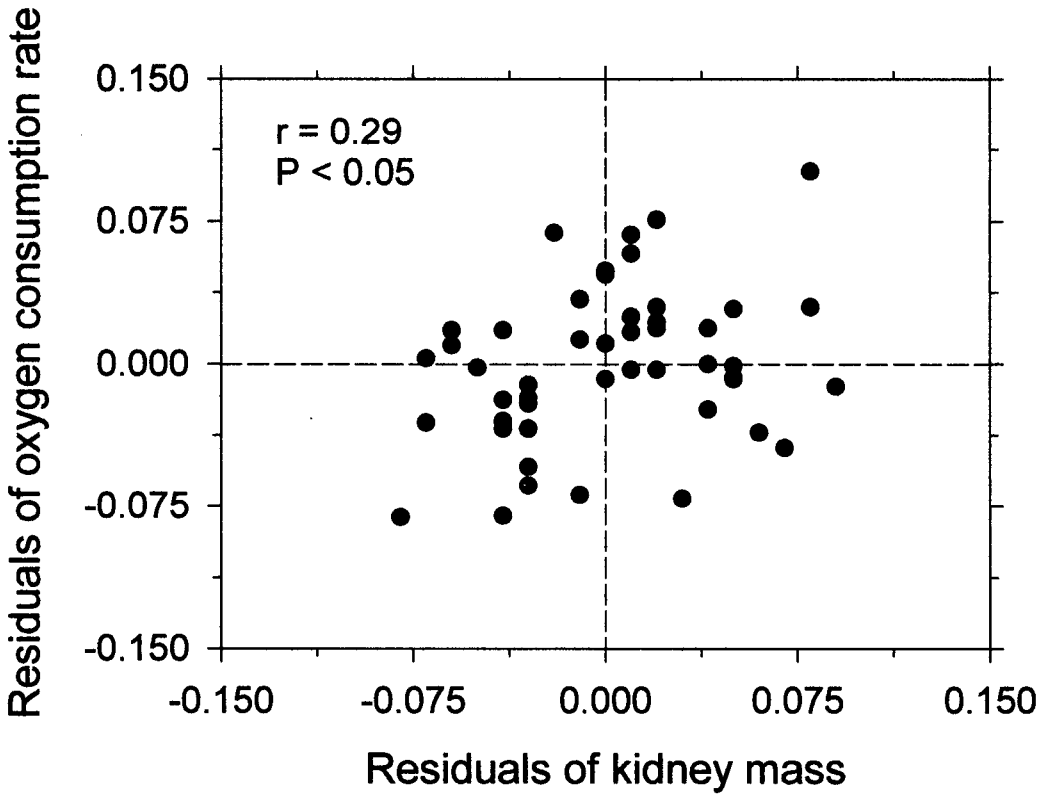


Fig. 3.2 Correlation between resting oxygen consumption rate and kidney mass. Residuals controlled for the effect of body mass on both variables.

Table 3.3 Inter-individual Pearson correlations among tissue and organ masses.

	Heart	Liver	Kidney	Pectoralis	Intestine	Hct
Heart		0.221	0.114	0.489	-0.036	-0.218
		(0.122)	(0.432)	(<0.001)	(0.805)	(>0.20)
Liver	0.583		0.516	0.237	0.502	0.090
	(<0.001)		(<0.001)	(0.098)	(<0.001)	(>0.50)
Kidney	0.580	0.733		0.117	0.317	-0.021
	(<0.001)	(<0.001)		(0.418)	(0.025)	(>0.50)
Pectoralis	0.627	0.466	0.369		0.101	-0.273
	(<0.001)	(0.001)	(0.008)		(0.492)	(>0.10)
Intestine	0.399	0.678	0.595	0.387		-0.005
	(0.005)	(<0.001)	(<0.001)	(0.006)		(>0.50)
Hct	-0.225	-0.011	-0.016	-0.338	-0.061	
	(>0.20)	(>0.50)	(>0.50)	(<0.10)	(>0.50)	

Note. Values below diagonal are correlations among raw values; above diagonal are correlations among body mass-independent residuals generated from regressions. Correlations with non-normally distributed Hct were assessed with Spearman-rank correlation. P-values are bracketed. A sequential Bonferroni correction set the initial critical P <0.002. Correlations significant at P<0.002 are underlined.

Analyses to avoid potential part-whole correlation.

A potential spurious correlation can result if the dependent variable is included in the independent variable (part-whole correlation; Sokal and Rohlf 1995, Christians 1999). The degree of importance varies with the magnitude of the dependent variable relative to the independent. To assess the potential for spurious correlations, I performed a second set of analyses in which the mass of each organ or tissue was first subtracted from the body mass of the animal before an allometric equation was generated. For no tissue or organ other than pectoralis muscle was there any change in significance. The pectoralis contributes approximately 10% of the mass of the bird. Removal of the mass of the pectoralis from body mass reduced the coefficient of determination from 0.52 (Table 3.1) to 0.19. The regression was still significant ($F_{1,49} = 11.41$, $P < 0.001$). A significant negative relationship was now found between residual pectoral muscle mass and residual $\dot{V}O_2$ ($r = -0.29$, $N = 48$, $P < 0.05$). The results from the stepwise regression did not change.

DISCUSSION

Allometric relationships.

Resting $\dot{V}O_2$ scaled intra-specifically as $mass^{1.03}$ in tree swallows. The 95% confidence intervals are fairly large (Table 3.1); consequently this intra-specific slope is not significantly different from the phylogenetically corrected inter-specific slope of $mass^{0.63}$ found for birds (Reynolds and Lee 1996). Similarly, the slopes of all regressions for body components on mass were close to $mass^{1.0}$ (Table 3.1). Again, considering confidence intervals, these are close to the avian inter-specific values, which range from $mass^{0.95}$ for breast muscle to $mass^{1.04}$ for liver (Daan et al. 1990).

Relative to inter-specific studies, the fraction of the variance in resting $\dot{V}O_2$ explained by body mass in this study was low. For example, body mass explained between 95%-97% of the inter-specific variance in BMR (measured as $\dot{V}O_2$) in birds (Daan et al. 1990). In

contrast, body mass explained only 35% of the variance in this character within tree swallows (Table 3.1). This is likely due to the narrow range of body masses occurring within a single population when compared to that occurring between species. In inter-specific studies to date, the heaviest species is 200 times the lightest (Daan et al. 1990). In contrast, in this study, the heaviest tree swallow (20.6 g) was only 1.3 times the lightest (15.3 g). The correlation coefficients for resting $\dot{V}O_2$ in this study are, however, comparable to those from studies of random-bred domestic mice (*Mus domesticus*) which cover a similarly narrow range of body mass (Hayes et al. 1992).

Inter-annual variability in tissue mass.

For most organs and tissues there were significant inter-year differences. Animals captured in 1994 had larger tissues for a given body mass than those from 1995. I feel this was unlikely due to a systematic error between years; for example, the same pan balances were used in both years. The capacity for individuals to demonstrate inter-annual up- and down-regulation of organ sizes is not surprising. In birds, large scale changes in pectoral muscle mass occur during pre-migration (e.g., Marsh 1984) and moulting (Gaunt et al. 1990), while in snakes there are rapid changes in gut mass during feeding (Secor and Diamond 1995). To my knowledge, however, such changes have never been demonstrated between consecutive breeding seasons.

As outlined in Chapter 2, I feel differences between years may be driven at least partly by the level of food resources available to the breeding birds. Daan et al. (1989) demonstrated that adult kestrels kept on restricted diets have decreased masses of hearts and kidneys. In the present study, both of these tissues showed significant inter-annual differences. Differences between years may also be analogous to a training effect. In 1995, in order to increase the provisioning parents' work load, an additional chick was added to their nests; which may result in an increased parental visitation rate. Although small scale physiological changes in response to training can be seen over a period of a week (e.g.,

increases in Na^+/K^+ ATPase; Green et al. 1993), it is unknown if the large scale adjustments in organ sizes I saw can be made over a period of 5 d (but see Stark 1999, Battley et al. 2000).

Individuals with smaller organs for a given body mass were apparently not in poorer condition than those individuals measured the previous year. They were not lighter in body mass for a given body size (PC1) nor in mass, independent of size (Table 3.2). A change in organ mass with little change in body mass has been found previously in house wrens challenged with increased exercise and cold (Dykstra and Karasov 1992). The study on wrens and this study suggest that use of relationships between size and body mass as a condition index should be used with caution.

Variability among individuals

For there to be evolution of a trait, there must be variability in the population, and the trait must be heritable. One major aim of this study was to assess this first point by determining the extent of physiological variability among individuals within a population. After effects of body mass had been removed (except for Hct, which did not scale allometrically), the coefficients of variation (Table 3.1) ranged from a low value of 7.8 for pectoral muscle mass to a high of 18.4 for Hct.

Resting $\dot{V}O_2$ showed moderate variability (CV = 9.4%) when compared with other characters measured. As resting $\dot{V}O_2$ is probably the most widely assessed trait in avian energetics (Daan et al. 1990), there is an enormous comparative data base on which to compare variability in $\dot{V}O_2$. As an example, I calculated approximate coefficients of variation for 10 species of passerines whose BMRs were measured by Dutenhoffer and Swanson (1996). Using their reported mean masses, BMRs, and standard deviations, I calculated the range of coefficients of variation assuming a mass exponent of 0.76 (their inter-specific exponent). The coefficients of variation of BMR ranged from a low of 4.5% for the house sparrows (*Passer domesticus*; N = 6) to 21% for the Eastern wood-pewee (*Contopus virens*; N = 5), with the average coefficient of variation being 11.4%. The coefficient of variation for

tree swallows measured in the present study fell well within the range calculated for these other species.

Few studies have considered the inter-individual variability in organ and tissue mass. The coefficients of variation for organ and tissue masses in this study (Table 3.1) are comparable to those of one lizard (*C. similus*; Garland 1984), but lower than those of a second species (*A. nuchalis*; Garland and Else 1987).

Correlations among characters.

The second principal aim of this study was to establish potential morphological and physiological correlates for the reported variability in resting $\dot{V}O_2$ in a wild population of birds. I addressed the question: do individuals with relatively high resting $\dot{V}O_2$s for their body mass have relatively large metabolically active internal organs? Such a relationship was demonstrated only for the kidney, where a positive relationship was found between kidney mass and $\dot{V}O_2$ (Fig 3.2). This has been shown previously, inter-specifically in birds (Daan et al. 1990) and intra-specifically in mice (e.g., Konarzewski and Diamond 1994, 1995). The kidney has one of the highest $\dot{V}O_2$s of any organ (Krebs 1950). Surprisingly, the heart, which is another metabolically active organ (Krebs 1950) and a significant predictor of resting $\dot{V}O_2$ in other inter- and intra-specific studies (e.g., Daan et al. 1990; Konarzewski and Diamond 1994, 1995; Meerlo et al. 1997, Chappell et. al 1999), showed no relationship with resting $\dot{V}O_2$ in tree swallows.

Those individuals with relatively high $\dot{V}O_2$s had relatively small pectoral muscles (when pectoral muscle mass was first subtracted from the dependent variable). The basis behind this relationship is unclear, but one possibility is that individuals with the smallest pectoral muscles had the greatest mitochondrial volume densities. This would result in an increased $\dot{V}O_2$ per gram of tissue. The activity of citrate synthase, a mitochondrial marker enzyme, does not, however, support this contention (GPB unpublished data).

No other tissues correlated with residual $\dot{V}O_2$. In a stepwise regression, kidney mass was a significant positive predictor, and surprisingly, small intestine mass was a significant negative predictor. In tree swallows, after controlling for body mass and kidney mass, birds with higher resting $\dot{V}O_2$s have smaller intestines. This is the reverse of previous studies that have found relationships between resting $\dot{V}O_2$ and small intestine mass (Konarzewski and Diamond 1994, 1995, Koteja 1996).

I correlated the masses of internal organs and tissues (Table 3.3). Individuals with relatively large hearts also had relatively large pectoral muscles, suggesting a functional matching of the two organs. This same correlation was recently reported by Chappell et al. (1999) in house sparrows. In tree swallows, individuals with relatively heavy livers for their body mass had relatively heavy kidneys and intestines. This agrees with the findings of Konarzewski and Diamond (1995) who found positive correlations among internal organs in mice. Surprisingly, Chappell et al. (1999), did not find a correlation among organs of the abdominal cavity in sparrows.

Kidney mass and intestine mass (wet) when combined account for about 5% of an adult swallow's body mass. Kidney mass and dry intestine mass explain 21 % of the variation in residual $\dot{V}O_2$. Konarzewski and Diamond (1994) found that kidney and heart when combined explained 12% of the variation in RMR in mice. Both of these estimates are considerably below the 52% of variation in RMR explained by heart, liver, intestine, and kidney masses of among inbred strains of mice (Konarzewski and Diamond 1995). However, such mice strains have been selectively bred to maximize differences.

Individual swallows with a relatively high resting $\dot{V}O_2$ had a relatively high Hct. Although avian red blood cells are nucleated and undergo oxidative metabolism, I feel the relationship between Hct and $\dot{V}O_2$ is not causal.

Costs and benefits of a high RMR

Traditionally, physiologists have considered much of the variation surrounding intra-specific regression lines as noise (Bennett 1987). Such variation can, however, be acted on by natural selection, and can potentially impact on fitness (Jayne and Bennett 1990). For example, in pied flycatchers (*Ficedula hypoleuca*), great tits (*Parus major*; Røskaft et al. 1986) and willow tits (*P. montanus*; Høgstad 1987) the most dominant individuals also have the highest resting metabolic rates (but see Vezina and Thomas 2000). Similar relationships have also been demonstrated for BMR in dippers (*Cinclus cinclus*; Bryant and Newton 1994).

If a relatively high resting $\dot{V}O_2$ is associated with increased fitness (through for example, increased dominance), it is unclear how such physiological variability is maintained in the population. One possibility is that it is energetically expensive to maintain the organs (e.g., kidney) associated with a high $\dot{V}O_2$ (Konarzewski and Diamond 1994; 1995). Although individuals with relatively high RMRs may have potential fitness benefits, during extended food shortage high levels of maintenance metabolism likely carry associated costs.

Conclusion

Within a single population of tree swallows I detected considerable inter-individual variation in resting $\dot{V}O_2$ and in the size of various internal organs and tissues. Inter-individual differences in the masses of the kidney and small intestine explained 21% of the variation in resting $\dot{V}O_2$. Although individuals with relatively high $\dot{V}O_2$s had relatively large, metabolically active kidneys, they had relatively small intestines. Mechanistically, a negative relationship with intestine mass is difficult to interpret. Significant inter-annual differences were found for most tissues, although not for resting $\dot{V}O_2$. The cause of inter-annual variation in organ size is unknown, although it is hypothesized that it is a response to variation in food availability.

CHAPTER 4

EFFECT OF BROOD SIZE MANIPULATION ON OFFSPRING PHYSIOLOGY: AN EXPERIMENT WITH PASSERINE BIRDS

PREFACE

This chapter is adapted from a paper by G.P. Burness, G.B. McClelland, S.L. Wardrop, and P.W. Hochachka, which has been accepted for publication in the Journal of Experimental Biology. Measurements of resting metabolic rate were performed with the assistance of G.B. McClelland; S.L. Wardrop assisted with field work. I generated the research question, collected the majority of the data, and was responsible for all aspects of data analyses and presentation.

INTRODUCTION

The environment experienced during avian and mammalian ontogeny can have important morphological, behavioural and life history consequences. Individuals raised under poor conditions often exhibit smaller structural size, are lighter in mass at independence, and have decreased over winter survival and recruitment rates (e.g., Perrins 1965, Boag 1987, Richner 1989, Dijkstra et al. 1990, Koskela, 1998). As breeding adults, these individuals may have reduced fecundity (e.g., smaller clutches) or decreased attractiveness of secondary sexual characters (Haywood and Perrins 1992, Schluter and Gustaffson 1993, Gustaffson et al. 1995, de Kogel and Prijs 1996).

Implicit in the above studies is that variation in the quality of the rearing environment has an effect on the "physiological condition" of individuals reared in that environment. Differences in condition at fledging are then manifested through survival probabilities and variation in the adult phenotype (Perrins 1965, Haywood and Perrins 1992, Schluter and Gustaffson 1993, de Kogel and Prijs 1996). Despite the prominent role that the concept of

condition plays in many evolutionary studies (e.g., McNamara and Houston 1992), the physiological and biochemical characters that define it remain relatively unexplored.

I used the tree swallow (*Tachycineta bicolor*) to investigate whether the brood size experienced during ontogeny would affect the physiology and metabolism of nestlings shortly before fledging. Brood size was experimentally manipulated and the resting metabolic rate of individuals reared in each brood was determined. To look for potential trade-offs in energy allocation near fledging, total lipid mass, and the masses of skeletal muscle and internal organs were measured. As I hypothesized *a priori* that differences in condition may also be linked to differences in blood oxygen carrying capacity, I measured blood haemoglobin concentration and haematocrit. Finally, the activities of the following key metabolic enzymes were measured in various tissues: (i) citrate synthase (an index of aerobic capacity), (ii) pyruvate kinase (an index of glycolytic capacity), (iii) 3-hydroxyacyl CoA dehydrogenase (an index of capacity for fatty acid catabolism), (iv) lactate dehydrogenase (an index of capacity for anaerobic glycolysis). Although some characters responded to brood manipulation, overall, the rearing environment appears to play a relatively minor role in determining the physiological and biochemical phenotype of individuals near fledging.

MATERIALS AND METHODS

Study site and species

The field component of this study was performed in May-June 1996 and 1998 at the Creston Valley Wildlife Area, near Creston, British Columbia, Canada. Beginning in early May checks of nest boxes began in search for signs of breeding by tree swallows. Females in this population lay between 1 and 8 eggs with a modal clutch of 6 (Chapter 3). Clutch completion is followed by 12-14 days of incubation. After hatching, nestlings follow a sigmoidal growth curve, reaching maximum mass at ca. day 12 (hatch day = day 1). This is followed by a weight recession which continues until fledging at 18-22 days of age (for a

review, see Robertson et al. 1992). Nestlings cannot be handled beyond day 16 due to risk of premature fledging (De Steven 1980).

Manipulation of nestling environment

In both years of study, manipulations consisted of either increasing or decreasing the number of nestlings in a brood. One nestling was either added to or removed from a nest on day 4 (1996) or day 6 (1998). All nestlings were banded, and the growth of members of the brood was followed until day 16. I did not use a control group as I was interested only in demonstrating an effect of manipulation and not in predicting a directionality of the response (i.e., an increase versus a decrease in a given character). In 1996 I used only nests in which females had laid 6 eggs; due to a shortage of suitable nests in 1998, I used both 5- and 6-egg nests. It is unknown if differences in protocol between years will affect the measurements, consequently, the term "year" was included in all statistical analyses.

Morphometrics

All nestlings in experimental broods were weighed (± 0.5 g) on either day 4 (1996) or day 6 (1998) and then again on days 8, 12, and 16. At day 16 the nestling with the mass closest to the average for a given brood had the following additional measurements taken: tarsus length, total body length, middle toe and keel lengths, bill length, depth and width. In addition, the length of the ninth primary feather (plucked) was measured, as its length at 16 days of age correlates with age of nest departure (De Steven 1980). To minimize inter-observer variability, the same individual performed all measurements of a given character.

Resting $\dot{V}O_2$ and $\dot{V}CO_2$

In 1998, on day 6, 8, 12, and 16 the nestling of average mass from each brood was transported to the field lab for determination of resting metabolic rate (RMR). As the same individual was never used on two consecutive days, a repeated measures experimental design

was not possible. Rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were obtained using a flow-through respirometry system (Sable Systems TR-3, Henderson, NV), consisting of an Ametek S-3A oxygen analyzer and a Li-Cor LI-6251 carbon dioxide analyzer.

Within approximately 30 minutes of being removed from the nest, nestlings were placed in a black Plexiglas metabolic chamber in the field lab. The volume of the chamber was either 500 mL or 1000 mL depending on the size/age of the nestling. Air inlet and outlet of the metabolic chamber consisted of brass tubes, extending from the top to the bottom of the chamber, and perforated along their length to maximize mixing of air within the chamber. The chamber was placed in a temperature controlled cabinet. The temperature inside the chamber was maintained at 32.1-33.0°C, and was continuously monitored using a thermocouple placed in the air outlet of the metabolism chamber.

Water- and carbon dioxide-free air was drawn through the metabolic chamber at 200 - 500 mL/min. using a combination pump/mass flow meter (Sable Systems TR-SS1). A sub-sample of out-flowing air was drawn through the analyzers at 150-200 mL/min after being dried with magnesium perchlorate ($Mg(ClO_4)_2$). Measurements were taken for 60 minutes and the lowest 5 minutes of recording in the last 30 minutes was used in calculations of resting oxygen consumption rate. The system was found to be accurate to ± 1% (N=3) by burning methanol.

Frequently, two nestlings were brought to the lab simultaneously. In these cases, to minimize metabolic variation due to differences in the degree of post-absorptiveness, one individual was placed in a metabolic chamber for 60 minutes (as above), and the other was fed ca. 0.4 g of moistened cat food (Vineland, Abottsford, BC). The fed individual was then placed in a duplicate chamber, and both chambers were then placed in the temperature controlled cabinet. For the fasted individual, the time elapsed between last possible parental feeding and first measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ was 60 minutes. For the individual fed in

the lab, measurement was 90 minutes post feeding. There was no systematic bias between the two treatments.

Following metabolic trials, birds were removed from the chamber, re-weighed and if 6, 8, or 12 days old, returned to their nest. Nestlings that were 16 days old were retained for additional measurements.

Calculations

Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated by Datacan 5.1 using the following equations:

$$\dot{V}CO_2 = \dot{V}E\,(FECO_2 - FICO_2) \quad (1)$$

$$\dot{V}O_2 = \frac{\dot{V}E\,(FIO_2 - FEO_2) - \dot{V}CO_2\,(FIO_2)}{1-FIO_2} \quad (2)$$

Where $\dot{V}E$ is the flow rate of air leaving the metabolic chamber corrected for standard temperature and pressure. $FICO_2$ and FIO_2 are the fractional concentrations of carbon dioxide and oxygen entering the chamber. $FECO_2$ and FEO_2 are the fractional concentrations of carbon dioxide and oxygen leaving the chamber.

Blood parameters

Following metabolism trials, a 100-200 μL blood sample was collected from each 16 day old nestling into heparinized microcapillary tubes. Haematocrit and haemoglobin were determined as reported previously (Chapter 2). The number of replicates for each character was determined by the size of the blood sample and ranged from one to three (which were averaged).

Carcass analyses

I sacrificed Day 16 nestlings immediately after blood sampling (following the guidelines of the Canadian Committee on Animal Care). A sample (ca. 150 mg) of the right pectoralis major and liver were removed from each bird (within 1-2 min. of death) and immediately frozen in a liquid N_2 charged dry shipper. These samples were later transferred to liquid N_2 for 3 month storage. Nestling were dissected, and the organs and carcass were stored as described previously for adults (Chapter 2).

Carcasses were weighed (±0.0001g) upon removal from the freezer and plucked of all feathers. All muscles on the tibiotarsus and femur were then removed from one side of the bird, rinsed with 0.9% NaCl, blotted dry and weighed (± 0.0001g). To calculate total leg muscle mass, values were multiplied by 2. Wet weights were determined for other organs and tissues. The empty mass of the small intestine and gizzard were determined as described previously (Chapter 2).

In preparation for fat extraction, all organs and tissues (with the exception of the heart, liver and kidney) were freeze dried to constant mass. Carcasses were dried to constant mass in a 70°C oven. All dried samples were then fat extracted as in Chapter 2.

Enzyme assays

Sub-samples (ca. 150 mg) of the pectoralis major and liver, and the ventricles of the heart were prepared for enzyme assays following the protocol in Chapter 2. Homogenates were stored at -80°C until assays were conducted (maximum 1 month).

As an index of capacity for flux through various metabolic pathways I measured the maximum catalytic activity (V_{max}) of key metabolic enzymes under optimal conditions. All assays were performed on a 96-well Thermomax microplate reader (Molecular Devices Corp., Sunnyvale, CA). In all assays, un-centrifuged homogenates were used to avoid potential loss in the pellet. Each reaction was replicated in 5 wells. The wells with the highest and lowest activity were omitted and the remaining 3 values were averaged. Preliminary experiments

confirmed that all substrates and cofactors were saturating but not inhibitory. Initially, control wells (containing no substrate) were run simultaneously with all reactions. The control rates for pyruvate kinase and lactate dehydrogenase represented <2% of total activity and in subsequent assays were omitted. Control wells were included for all 3-hydroxyacyl-CoA dehydrogenase and citrate synthase assays. With the exception of citrate synthase, all assays were at pH 7.0 and 340 nm. Citrate synthase was assayed at pH 8.0, 412 nm. All reactions were at 40°C. Activities are expressed as international units (μmoles substrate converted to product per minute) per gram wet weight of tissue. Although the enzymes assayed were the same as described previously (Chapter 2), some of the assay conditions differed.

Assays were performed as follows. Citrate synthase (EC 4.1.3.7; CS): 50mM Tris buffer, 0.05% Triton X-100, 0.2mM DTNB, 0.12mM acetyl CoA, 0.5mM oxaloacetate (omitted from the control well). 3-hydroxyacyl CoA dehydrogenase (EC 1.1.1.35; HOAD): 50mM Imidazole, 0.15mM NADH, 10mM ß-mercaptoethanol, 1.0 mM NaCN, acetoacetyl CoA (0.1mM for the pectoralis and ventricle, 0.05mM for the liver; omitted from the control well). Pyruvate kinase (EC 2.7.1.40; PK): 50mM Imidazole, 0.15mM NADH, 10mM ß-mercaptoethanol, 1.0mM NaCN, 5.0mM ADP, 100mM KCl, 10mM $MgCl_2$, 5mM PEP, 10μM fructose 1,6-bisphosphate, excess lactate dehydrogenase (5U/mL); assayed in the ventricles and pectoralis only. Lactate dehydrogenase (EC 1.1.1.27; LDH): 20mM Imidazole, 0.15mM NADH, 2mM Pyruvate, 10mM ß-mercaptoethanol, 1.0 mM NaCN; assayed in the ventricles and pectoralis only.

Statistical analyses

Many physiological variables scale allometrically with the mass of an animal. In my study, the effect of brood manipulation on body mass was of interest. Consequently, rather than controlling for body mass, in most analyses I instead controlled for structural size. To generate an index of size, I performed a principal component analysis (PCA) on the

correlation matrix of 7 external morphological variables (tarsus length, total body length, middle toe and keel lengths, bill length, depth and width). Loadings were positive for all variables and ranged from 0.24-0.47, with a corresponding eigenvalue of 3.13. The first principal component (PC1) accounted for 44.7% of the total original variance. I used the scores along PC1 as a measure of body size (e.g., Alisaukas and Ankney 1987) with positive values representing individuals that were larger than average body size and negative values representing individuals that were smaller than average.

The effects of treatment (brood manipulation) and year on phenotypic variation of 16 day old nestlings were explored using a 2-way analysis of covariance (ANCOVA) with body size included as a covariate. Initially, all interaction terms were included as additional covariates and if not significant were excluded. Further analyses were then performed including only the covariate and main effects (treatment and year). I used a liberal $P<0.15$ for inclusion of interaction terms; for main effects significance was claimed at $P<0.05$. Unless otherwise noted, all means are least squares means ± 1 S.E.M. All analyses were performed using JMP statistical software (SAS Institute Inc.).

RESULTS

Growth and metabolism of nestlings

To determine the impact of brood manipulation on nestling growth I averaged the mass of all individuals within a brood. On the day of manipulation (Day 4 or 6) there was no difference between treatments in the mass of the average nestling ($P>0.50$, Fig 4.1). By 12 days of age, however, nestlings in the Reduced broods were significantly heavier than those in Enlarged broods ($F_{1,29}=7.963$, $P=0.009$); this difference was maintained at 16 days ($F_{1,29}=8.857$, $P=0.006$, Fig. 4.1).

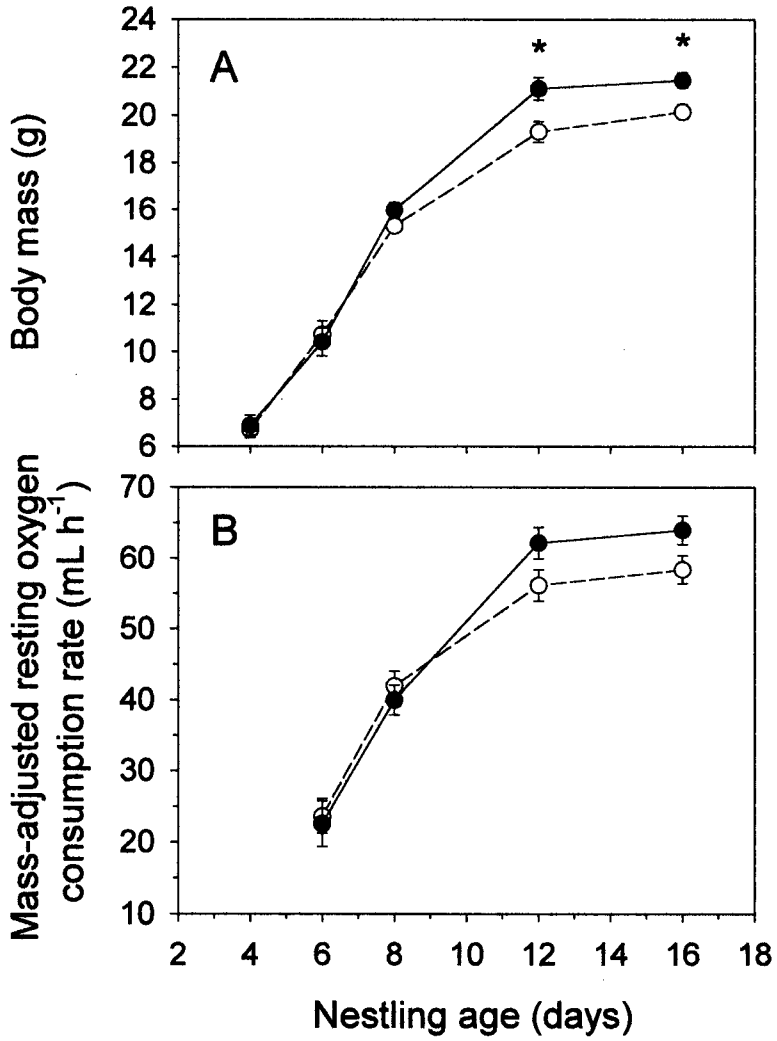


Fig. 4.1 (A) Body mass, and (B) body mass adjusted-resting oxygen consumption rate of tree swallow nestlings, as a function of age and treatment. Enlarged broods (o), Reduced broods (•). Sample sizes (Enlarged, Reduced), Panel A: Day 4 (9, 6), Day 6 (8, 8), Days 8, 12, and 16 (17, 15). Panel B: Day 6 (6, 4), Day 8, (8, 8), Day 12 (8, 8), Day 16 (7, 7). Values are least squares means ± 1 S.E.M. Asterisks indicate that treatments differed significantly from each other ($P<0.01$).

I measured the resting $\dot{V}O_2$ and $\dot{V}CO_2$ of the nestling of average mass from each brood at 6, 8, 12 and 16 days of age. Different nestlings were used each day. There was no effect of manipulation on the RER ($P>0.15$, Table 4.1). I could not generate an index of structural size for nestlings less than 16 days old and instead adjusted resting $\dot{V}O_2$ for body mass. Body mass-adjusted $\dot{V}O_2$ at 6, 8, and 12 days of age did not differ between treatments ($P>0.10$, Fig. 4.1). By 16 days of age, nestlings in reduced broods had marginally higher mass-adjusted $\dot{V}O_2$ ($F_{1,11}=3.548$, $P=0.086$). I re-analyzed the $\dot{V}O_2$ of the 16 day old nestlings with PC1 (rather than mass) included as a covariate. Individuals in Reduced broods had a 15% greater body-size adjusted $\dot{V}O_2$ than those in Enlarged broods ($F_{1,11}=6.108$, $P=0.031$).

Morphology and physiology of 16 day old nestlings

Brood manipulation had no effect on nestling structural size (PC1, $P=0.500$), nor on the length of the ninth primary feather ($P=0.681$) at 16 days of age. Individuals from the Reduced broods were heavier than those from Enlarged broods after controlling for size ($F_{1,28}=8.450$, $P=0.007$). In 1998, 16-day nestlings were structurally larger ($F_{1,29}=64.517$, $P<0.001$) and had longer primaries ($F_{1,29}=9.018$, $P=0.006$) than in 1996, but were no heavier ($P=0.484$).

To explore the basis of body mass differences resulting from brood manipulation (above), I measured total lipid mass. PC1 was included as a covariate, with year and treatment as main effects. Nestlings from Reduced broods had 19% greater lipid mass at day 16 than those from Enlarged broods ($F_{1,28}=5.623$, $P=0.025$, Fig. 4.2). There was no difference between years ($P=0.486$).

Table 4.1 Rates of resting oxygen consumption and carbon dioxide production of tree swallow nestlings.

Age	N	Mass (g)	$\dot{V}O_2$ (mL h^{-1})	$\dot{V}CO_2$ (mL h^{-1})	RER
6 Days					
R	(4)	11.40 ± 0.32	26.89 ± 3.97	19.82 ± 2.31	0.75 ± 0.03
E	(5)	10.21 ± 0.34	20.39 ± 2.37	16.34 ± 1.24	0.80 ± 0.04
8 Days					
R	(8)	16.40 ± 0.40	41.46 ± 2.73	31.72 ± 2.19	0.77 ± 0.01
E	(8)	14.84 ± 1.10	38.19 ± 4.63	28.49 ± 3.30	0.76 ± 0.02
12 Days					
R	(8)	21.36 ± 0.31	65.61 ± 2.63	47.05 ± 2.12	0.72 ± 0.01
E	(8)	19.79 ± 0.34	52.64 ± 1.93	39.48 ± 1.88	0.75 ± 0.04
16 Days					
R	(7)	21.19 ± 0.61	65.81 ± 2.73	45.97 ± 1.56	0.70 ± 0.02
E	(7)	20.05 ± 0.31	56.52 ± 2.01	39.68 ± 1.27	0.71 ± 0.03

Values are means ± 1 S.E.M. R, broods reduced by a single individual; E, broods enlarged by a single individual. RER, respiratory exchange ratio

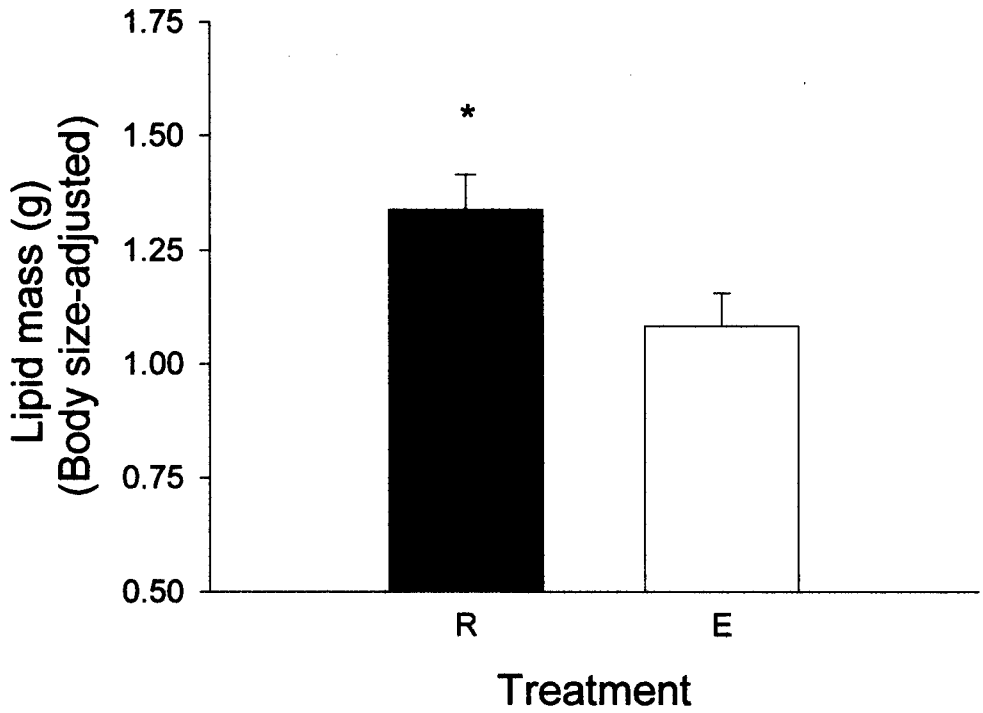


Fig. 4.2 Lipid mass of 16 day old tree swallow nestlings, adjusted for body size (PC1; see text), as a function of brood manipulation. Reduced broods (black bar), Enlarged broods (white bar). Least squares means + 1 S.E.M. Enlarged, N=17; Reduced N=15. Asterisks indicate that treatments differed significantly from each other ($P<0.05$).

After controlling for structural size, individuals in the Reduced treatment had heavier organs than those in the Enlarged treatment. However, only the mass of the gizzard showed a significant difference ($P<0.05$, Table 4.2). There was no effect of year on the wet mass of any organ except the intestine ($P<0.01$).

There was no effect of brood manipulation on the wet masses of either the pectoral or leg muscles ($P>0.05$, Table 4.3). The water content of a muscle (total water/lipid-free wet mass of tissue) decreases with chronological age (Konarzewski 1988), and is a useful index of muscle maturation (Ricklefs and Webb 1985). There was no significant effect of treatment on water fraction of either the pectoral or leg muscles ($P>0.10$, Table 4.3), suggesting that at 16 days of age individuals from the two treatments were of similar degrees of functional maturity. Significant year effects (Table 4.3) may be due to desiccation in the freezer, consequently I assign them no particular functional significance.

As an index of blood oxygen carrying capacity I measured the Hct and Hb content of the blood. The average Hct was 41.7 % (S.D. = 4.50, N=32) and ranged from 27.3 - 51.0 %; the average Hb concentration was 13.51 g dL^{-1} (S.D.=2.063, N=31) and ranged from 6.7 -16.3 g dL^{-1}. There were no significant effect of treatment or year on either character ($P>0.25$).

Enzyme activities

There was little effect of brood manipulation on maximum enzyme activities. Individuals from Reduced broods had significantly higher HOAD activity in the heart ($P<0.01$, Table 4.4), suggesting an increased capacity for fatty acid oxidation. No other enzyme changed significantly between treatments.

Table 4.2 Effect of brood manipulation on organ masses of 16 day old tree swallows.

	Treatment		P-value	
	Enlarged	Reduced	Treatment	Year
Heart (g)	0.25 ± 0.008	0.26 ± 0.008	0.476	0.498
Liver (g)	0.90 ± 0.030	0.98 ± 0.032	0.074	0.990
Kidney (g)	0.25 ± 0.011	0.27 ± 0.012	0.149	0.927
Gizzard (g)	0.57 ± 0.017	0.62 ± 0.018	<u>0.043</u>	0.472
Intestine (g)	0.75 ± 0.028	0.83 ± 0.030	0.079	<u>0.006</u>

Values are least squares means ± 1 S.E.M. from two-way ANCOVA with body size as a covariate (PC1; see text), interaction terms were not significant (P>0.15). Samples sizes: Enlarged, N=17; Reduced, N=15. Significant P-values are underlined.

Table 4.3 Effect of brood manipulation on muscle mass and water fraction of 16 day old tree swallows.

	Treatment		P-value	
Variable	Enlarged	Reduced	Treatment	Year
Pectoralis (g)	1.98 ± 0.065	2.15 ± 0.069	0.092	0.315
Water (g/g)	0.78 ± 0.003	0.78 ± 0.003	0.907	0.007
Leg (g)	0.66 ± 0.020	0.62 ± 0.021	0.181	0.946
Water (g/g)	0.78 ± 0.006	0.77 ± 0.007	0.154	<0.001

Values for pectoral and leg muscle are least squares means ± 1 S.E.M. from two way ANCOVA with body size as a covariate (PC1; see text). Values for water fraction are least squares means ± 1 S.E.M. from two-way ANOVA. All interactions were non-significant (P>0.15). Sample sizes: Enlarged, N=17; Reduced, N=15. Significant P-values are underlined.

Table 4.4 Maximum enzyme activities from tissues of 16 day old tree swallows.

	Treatment		Statistic	
	Enlarged	Reduced	F	P-value
Heart				
CS	132.9 ± 11.6	131.4 ± 11.6	0.008	0.930
PK	146.8 ± 13.6	173.9 ± 13.6	1.937	0.187
LDH	61.5 ± 5.4	74.9 ± 5.4	3.019	0.106
HOAD	14.3 ± 1.1	19.1 ± 1.1	9.502	<u>0.009</u>
Pectoralis				
CS	136.3 ± 12.6	137.6 ± 12.6	0.006	0.941
PK	405.7 ± 20.8	387.1 ± 20.8	0.394	0.541
LDH	406.7 ± 24.6	447.0 ± 24.6	1.304	0.274
HOAD	10.3 ± 1.0	9.1 ± 1.0	0.867	0.369
Liver				
CS	11.3 ± 0.7	9.7 ± 0.7	2.757	0.121
HOAD	36.1 ± 1.3	35.5 ± 1.4	1.209	0.293[a]

Values are least squares means ± 1 S.E.M. from one-way ANCOVA with body size as a covariate (PC1; see text).For enzyme names, see text. [a]Significant body size*treatment interaction ($P<0.15$). Enzyme activity is in U/gram tissue (μmoles substrate converted to product per minute). N=8 for each treatment. Significant P-values are underlined.

DISCUSSION

The brood size of tree swallows was manipulated to determine if the environment experienced during ontogeny would affect the physiology and biochemistry of nestlings shortly before they were to fledge. As skeletal characters are known to respond to environmental variation during development (Lindström, 1999, and references within), a minimal response at the physiological and biochemical level was surprising. A lack of variation in basal measures suggests that physiological and biochemical development may be relatively invariant except perhaps, under extreme conditions (e.g., Schew and Ricklefs 1998). Nonetheless, variation in the rearing environment did affect some characters. A decrease in the number of nestlings in a brood resulted in increased body mass, total lipid mass, gizzard mass, body size-adjusted $\dot{V}O_2$, and the activity of HOAD in the heart.

Morphological response

Brood manipulation did not effect the structural size of 16 day old tree swallows (PC1 scores). This is consistent with previous studies of this species (Wiggins 1990, Wheelwright et al. 1991). It could be argued that the addition or subtraction of a single nestling may have been insufficient to elicit a response. This is unlikely for two reasons. First, addition of two nestlings (rather than one) exceeds the provisioning capacities of the parents in this population and frequently results in brood reduction (GPB unpublished. data). Second, at 16 days of age, individuals in Enlarged broods had smaller lipid stores and a lower body mass than those in Reduced broods, suggesting they were resource limited.

Previous studies have shown that under food restriction, other altricial species can maintain skeletal growth through catabolism of body tissues and lipid. Skeletal growth drops significantly, however, when energy reserves are depleted (Schew and Ricklefs 1998). Although I detected few differences in organ and muscle mass as a consequence of treatment, individuals in Enlarged broods had significantly lower lipid stores than those in Reduced

broods. Rather than utilize stored energy to maintain growth, in the present study it is more likely that nestlings first met energetic requirements for growth and maturation and then stored the remaining energy as lipid.

At 16 days of age nestlings were structurally larger in 1998 than in 1996. Systematic measurement error is unlikely as the same individuals (the same field assistant or myself) performed all measurements in both years. Interannual differences in morphology were more likely due to variation in weather conditions or food availability, as has been suggested previously for adults (Chapters 2 and 3).

Phenotypic variation and developmental plasticity

During periods of reduced nutrition, nestlings of some species delay tissue maturation and feather growth, or increase the duration of the nestling period (Schew and Ricklefs 1998). If this occurred in the present study, the phenotypic values of characters at 16 days of age may have little similarity to the values of those same characters a few days later when individuals actually left the nest. I argue that nestlings probably did not exhibit an adaptive suspension of maturation nor an extension of the nestling period.

There is a well established negative relationship between the hydration state of a muscle and its mature function (e.g., Ricklefs and Webb 1985). Water content, normalized to lean dry mass (an index of protein content), typically decreases with a nestling's chronological age (Konarzewski 1988). If nestlings in Enlarged broods were able to arrest their developmental program in response to unfavourable conditions, their muscles would likely have had an increased water content (less mature muscles) than nestlings of similar age from Reduced broods. This was not observed (Table 4.3).

Although exact dates of nest departure were not determined, no difference in the duration of the nestling period has been reported previously under similar brood manipulations (Wheelwright et al. 1991). In addition, the duration of the nestling period is inversely related to the length of the ninth primary feather at 16 days of age (De Steven

1980); the length of the primaries did not differ between treatments. Taken together, these data suggest that nestlings from each of the two treatments would have fledged at similar ages, with those from Enlarged broods being in poorer condition for a given structural size.

Heat increment of feeding

Following ingestion of a meal there is an unavoidable increase in metabolic rate, the heat increment of feeding (HIF; also called specific dynamic action). Conclusions drawn from my measurements of resting $\dot{V}O_2$ rely on the assumption that when nestlings in the two treatments were measured, they were of a similar absorptive state. Two lines of evidence indicate that this was the case: (1) As the magnitude and duration of the HIF increase linearly with increasing meal mass (Chappell et al 1997), I compared the mass of food in the small intestine of day 16 nestlings between treatments. Individuals in Reduced broods did not have more food in their intestines ($P>0.29$): Enlarged broods = 0.15 g (SD=0.05, N=7), Reduced broods = 0.12 g (SD=0.06, N=7). There was also no increase in resting $\dot{V}O_2$ with increasing mass of intestine contents ($P>0.69$). Finally, I performed an ANCOVA with brood manipulation as a main effect and both intestine contents and PC1 as covariates. Nestlings in the Reduced broods still had a significantly higher $\dot{V}O_2$ than individuals in the Enlarged broods ($F_{1,10}=9.146$, $P=0.013$); neither PC1, nor intestine contents were significant covariates ($P>0.10$). (2) In other passerines, as nestlings get older the duration of the HIF decreases (Chappell et al 1997). If differences in $\dot{V}O_2$ between treatments at 16 days of age were due primarily to a HIF, such differences should have been detectable on day 8. Contrary to the pattern seen on day 16, day 8 nestlings in the Reduced treatment had on average a lower mass-adjusted $\dot{V}O_2$ than individuals in the Enlarged broods (although not significantly, Fig. 4.1). Taken together these arguments indicate that if my measurements were affected by a HIF, both treatments were affected equally.

Phenotypic flexibility of organ size

The only organ that differed between brood sizes was the gizzard, being greater in individuals in the Reduced broods. As individuals in the Reduced broods were presumably receiving more food, variation in size of the muscular gizzard may be a result of an increased work load, analogous to a training effect (Piersma et al. 1993). For example, the gizzard of Japanese quail (*Coturnix japonica*) demonstrates repeated up- and down-regulation of size coincident with the fibre content of the diet (Stark 1999). Captive red knots (*Calidris canutus*) also display phenotypic flexibility, and decrease the size of their gizzards by ca. 75% upon switching from small bivalves to soft food pellets (Dietz et al. 1999). Morphological responses to changes in diet are rapid, and measurable within 24-48 hours of diet switching (Stark 1999). Interestingly, following diet switching experiments, Stark (1999) reported that the gizzards of experimental quail never returned to the same size as unchallenged controls. Whether differences I observed between nestling swallows are fixed is unknown.

Implications for post-fledging survival

There is a well established positive relationship between body mass at fledging and the probability of subsequent recruitment (e.g., Perrins 1965, Tinbergen and Boerlijst 1990, Magrath 1991, Both et al. 1999). In my study, brood manipulation resulted in significant variation in body mass just prior to fledging which likely had fitness consequences. The mechanism underlying the relationship between mass at fledging and recruitment is unclear. Body mass may represent a general indicator of health; for example, lipid stores may allow for survival during periods of adverse weather or reduced food intake. Although heavier individuals have greater lipid stores, they are often structurally larger. Garnett (1981) hypothesized that this may enhance survival by allowing large individuals to dominate smaller ones in competitive interactions. Recent work on great tits has shown that

individuals that are heavy when compared to others in the population have an increased probability of subsequent recruitment (Both et al. 1999).

Apart from structural size and large lipid stores, how physiological factors may influence the probability of survival and recruitment is unclear. An elevated size (or mass) - adjusted $\dot{V}O_2$, although in itself presumably detrimental, may be linked to an elevated $\dot{V}O_{2max}$ (aerobic capacity model, Bennett and Ruben 1979). Support for a correlation between resting $\dot{V}O_2$ and $\dot{V}O_{2max}$ is, however, equivocal (Hayes and Garland 1995). For example, in house sparrows (*Passer domesticus*) resting $\dot{V}O_2$ is correlated with $\dot{V}O_{2max}$ in juveniles but not adults (Chappell et al. 1999). An elevated resting $\dot{V}O_2$ has also been linked with dominance status (Røskaft et al. 1986, Bryant and Newton, 1994), although not in all species (Hammond et al. 2000, Vézina and Thomas 2000). Even with an estimate of aerobic capacity or dominance, the consequence of an elevated resting $\dot{V}O_2$ in tree swallow fledglings would be speculative without estimates of differential survivorship (e.g., Hayes and O'Connor 1999).

The only enzyme showing a clear response to brood manipulation was HOAD. Individuals in the Reduced broods had significantly higher cardiac HOAD activities, suggesting an increased capacity for oxidation of fatty acids. Mechanistically this may be simply coupled to their elevated fat stores. Marsh (1981) found a correlation between HOAD activity in the pectoral muscle and carcass fat levels in birds preparing for migration. However, during pre-migratory fattening, semipalmated sandpipers (*Calidris pusilla*) increase their capacity for fatty acid oxidation in skeletal muscle, but not the heart (Driedzic et al. 1993). I could not detect a correlation between carcass fat levels and cardiac HOAD activity ($P>0.50$). Although the amount of fat in the diet can affect enzyme activities in both skeletal (Fisher et al. 1983) and cardiac muscle (Power and Newsholme 1997) nestlings presumably received diets of similar fat content. At present the mechanisms underlying elevated heart HOAD activities are unclear.

Conclusion

Manipulation of brood size early in ontogeny had minimal affects on the physiology and biochemistry of tree swallows shortly before fledging. A lack of response suggests that these characters may be relatively insensitive to environmental variation. Consequently, the early rearing environment may play a relatively small role in determining variation in the adult physiological or biochemical phenotype. Some characters did respond to environmental variation, including lipid levels, cardiac HOAD activity, and resting $\dot{V}O_2$. The mechanism by which variation in these characters influences survivorship is unknown.

CHAPTER 5

GENERAL CONCLUSIONS

This is the first study to identify both physiological causes and ecological consequences of inter-individual variation in the aerobic metabolism of free-living birds. In this final chapter, I shall summarize the main findings of each of the 3 research chapters, present some general conclusions, and suggest areas of future research.

Research summary

Chapter 2. I used the doubly labelled water technique to estimate the energy expenditure of male and female tree swallows provisioning various natural brood sizes. I addressed two primary questions: (i) how does sustained metabolic rate affect Darwinian fitness, and (ii) what are the physiological and biochemical correlates of SusMR? Although there was no correlation between natural brood size and parental SusMR, nestlings in large natural broods grew at the same rate as those in smaller broods. One interpretation of this result is that adults with large natural broods were energetically more efficient than those rearing smaller natural broods. After statistically controlling for brood size, male and female parental SusMR increased with increasing nestling mass, and in females, with nestling growth over the previous 4 days (in one year only). Thus SusMR was positively related to a surrogate of Darwinian fitness.

In one of two years, individuals with relatively high SusMR had relatively large intestines. This suggests that a high digestive capacity was necessary to attain a high SusMR. This is the first demonstration of such a relationship in the field. It also suggests that capacity to maintain a high SusMR likely entails a cost in terms of increased resting metabolism.

Chapter 3. I measured the resting oxygen consumption rate and body composition of breeding tree swallows in two breeding seasons. I asked two primary questions: (i) what is the relationship between inter-individual variation in organ size and resting oxygen consumption rate ($\dot{V}O_2$), and (ii) do $\dot{V}O_2$ and body composition display inter-annual variation?

Individuals with relatively high resting oxygen consumption rates for their body mass had relatively heavy kidneys. This was the first intra-specific study of birds to demonstrate such a relationship (Burness et al. 1998). Contrary to previous (Konarzewski and Diamond 1995) and subsequent studies (Bech and Østnes 1999, Chappell et al. 1999) of other species, the size of the small intestine did not correlate with resting $\dot{V}O_2$. Although phenotypic flexibility in organ size is increasingly well recognized (reviewed in Piersma and Lindström 1997), this study was the first to demonstrate large scale inter-annual variation. Despite inter-annual variation in the sizes of organs of the abdominal cavity, resting $\dot{V}O_2$ did not vary between years.

Chapter 4: Numerous studies have investigated the role of the rearing environment in morphological development (structural size or body mass, Lindström 1999). In chapter 4, I performed the first study to ask how the environment during early development affects individual variation in physiology and biochemistry. To mimic "good" and "bad" environments, I either reduced or enlarged broods by one nestling. Within a few days of fledging, nestlings in "good" environments were significantly heavier, had greater mass of lipid, increased cardiac HOAD levels, heavier gizzards and higher size-specific resting $\dot{V}O_2$ than individuals raised under "poor" conditions. Perhaps more important than characters that differed as a consequence of treatment, are the number that did not. A lack of difference between treatments in the size of most organs suggests that development of many physiological and biochemical traits is relatively insensitive to this form of environmental variation.

Conclusions

Tree swallows rearing different sized broods did not differ in their energy expenditures. This suggests that within a single population of aerial insectivores there likely exists variation in foraging efficiency. Such variation has also been demonstrated in Eurasian kestrels; males with large natural broods had higher hunting yields per unit time, but similar energy expenditures than did males rearing smaller broods. In kestrels, however, differences in hunting yield were due to differences in territory quality (Masman et al. 1989).

In contrast to kestrels, tree swallows are aerial insectivores and do not hold feeding territories. As individual swallows rearing different sized broods did not differ in the physiological or biochemical traits that I measured, I propose that inter-individual differences in efficiency resulted from variation in foraging strategies. Although a positive relationship between SusMR and frequency of nest site visitations (an index of feeding frequency) has been shown previously in tree swallows (Williams 1988), feeding frequency is an incomplete measure of parental provisioning. In addition to feeding frequency, aerial insectivores may vary bolus size or prey composition (e.g., Wright et al. 1998). These behavioural strategies are not detectable through studies of energy expenditure or frequency of nest site visitations. Studies of foraging behaviour in this same population suggest that individuals with different natural brood sizes vary in their foraging decisions and capacity to respond to energetic challenges (R.C. Ydenberg, pers. comm.). These behavioural studies complement my physiological ones, and support the contention that individuals rearing different sized broods differ in quality, independent of their energy expenditure.

Despite a lack of relationship between SusMR and the number of nestlings an individual was rearing, among individuals of the same brood size, SusMR was related to nestling mass. This suggests that increased parental effort resulted in an increase in nestling growth. Because relatively heavy fledglings have greater chances of over-winter survival and recruitment (Both et al. 1999) variation in growth rates or nestling body mass probably have

fitness consequences. Operationally, natural selection is defined as the correlation between the variation in a phenotypic trait and variation in Darwinian fitness (Garland and Carter 1994). Under this definition, SusMR may have been under selection in one of two years. Although measures such as nestling mass or growth rates are incomplete measures of fitness, they provide a useful first step in analyzing evolutionary patterns of phenotypic variation (Garland and Carter 1994).

Although I consider parental energy expenditure to be a performance trait, it can also be viewed as an environmental effect that influences the nestling phenotype. The resemblance among offspring in a nest is a consequence of the interaction between genetics and the common nutritional environment provided by parental care. Consequently, selection on the environmental component of offspring size (e.g., Alatalo et al 1990) may result in selection on parental SusMR as a correlated response (Moreno et al. 1997). Recent evidence indicates that measurements of parental SusMR are repeatable between breeding seasons, suggesting that an individual's SusMR may retain a genetic component of variation (Potti et al 1999). If this is the case, SusMR may evolve under natural selection. Additional studies are required in order to estimate the seasonal stability and heritability of parental SusMR.

Individual tree swallows with relatively high SusMRs had relatively large intestines. Although this relationship has been shown in the lab (Konarzewski and Diamond 1994), this is the first study to demonstrate such a relationship in the field. It is well recognized that individuals will undergo intestinal hypertrophy under conditions of chronically high energy expenditure (Dykstra and Karasov 1992, Hammond and Diamond 1992, 1994, Hammond et al. 1994, Konarzewski and Diamond 1994). In my study, whether inter-individual variation in intestine mass was due to differential hypertrophy remains unknown.

One of the central theorems of life-history theory is that there exists a trade-off between current and future reproduction (Williams 1966, Charnov and Krebs 1974). This leads to the question: if among individuals with the same brood size a high energy expenditure is reproductively beneficial, what are the associated costs? One argument is that there is a

trade-off between the benefits of attaining a high SusMR and the energetic costs of maintaining large internal organs (Hammond and Diamond 1997). If such a trade-off exists I would predict that individuals with relatively large intestines for their body mass would have relatively high RMRs. Data presented in Chapter 3 failed to support this hypothesis; the only significant predictor of an individual's RMR (estimated by measuring resting $\dot{V}O_2$) was the mass of its kidney. A lack of correlation between RMR and intestine mass was surprising given that such correlations exist in laboratory mice (Konarzewski and Diamond 1994, 1995) and wild birds (Bech and Østnes 1999, Chappell et al. 1999). If I were to assume that a relationship between RMR and intestine mass did exist (and I simply failed to detect it), could the resultant increase in RMR reduce survival? In their study of dippers, *Cinclus cinclus*, Bryant and Newton (1994) calculated that the difference in RMR between dominant an subordinate individuals was small, constituting ~ 3% of the daily energy budget. Whether such differences would still be considered small under conditions of reduced food availability is unknown. Although there is an implicit trade-off between the costs and benefits of maintaining large internal organs (Hammond and Diamond 1997), future research should explicitly test this hypothesis.

Variation in parental care affects nestling survival, condition at fledging and the probability of subsequent recruitment (Tinbergen and Boerlijst 1990, Magrath 1991, Both et al. 1999). Few studies, however, have investigated the potential state variables that may link nestling condition with survivorship (McNamara and Houston 1992, Merilä and Svensson 1997). To date, the only physiological characters that have been studied with respect to variation in parental care are the size of subcutaneous fat reserves and the immunocompetence of nestlings (e.g., Merilä 1996, Saino et al. 1999). In the present study, although a number of physiological characters responded to brood manipulation (including, not surprisingly, lipid mass), it is perhaps more striking how few in fact showed variation. A lack of difference in body composition between treatments suggests that development of many physiological and biochemical traits is relatively insensitive to environmental variation.

One trait that did respond to variation was resting oxygen consumption rate. In some species RMR is related to dominance (Bryant and Newton 1994, Røskaft et al. 1986, but see Hammond et al 2000, Vézina and Thomas 2000), suggesting that a high RMR may be an indicator of 'quality'.

In blue tits, *Parus caeruleus*, an individual's lipid level established prior to 15 days of age is a good predictor of body condition up to four months later during migration (Merilä and Svensson 1997). How long inter-individual differences established during the nestling phase are maintained is unknown but it is tempting to speculate that the rearing environment may have contributed to variation of adult traits such as SusMR.

Future directions:

I conclude by identifying areas for future research:

Inter-individual variation in capacity to elevate SusMR. I have shown that individuals rearing different sized broods have similar energy expenditures. Whether under times of increased energetic demand (e.g., adverse weather conditions) all individuals have the same capacity to elevate their SusMR is unknown. Flexibility in parental effort would be best explored by manipulating work load directly without changing brood demand. This could be accomplished through the clipping of primary feathers of adults or through the addition of weights.

Inter-annual costs of high SusMR. No study has demonstrated an effect of variation in SusMR on fecundity the following year. This could be investigated through studying clutch size variation in the year following manipulation (above).

Repeatability and heritability of SusMR. To date, only a single study has shown significant statistical repeatability of SusMR (Potti et al. 1999). Additional studies should be establish how stable SusMR is for other avian species, and assess its components of variation.

Repeatability of body composition. Although an individual's physiology and biochemistry are phenotypically flexible, the significant repeatability of RMR measurements (Bech et al. 1999) suggests that individuals may show consistent differences in body composition. The increasing use of non-invasive technologies (e.g., ultrasonography, Dietz et al. 1999) may make a repeatability study of body composition during different life stages possible.

Environmental components of adult physiology. How important is the rearing environment in determining adult physiology? Although I have shown that some traits of nestlings are influenced by the rearing environment (e.g., resting $\dot{V}O_2$, lipid levels), it is unknown whether this variation is maintained in adults. To address this, the rearing environment should be manipulated and a sample of individuals should be studied as breeding adults.

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APPENDIX 1

Rates of CO_2 production and estimated sustained metabolic rates of male and female tree swallows rearing natural sized broods.

Nest	Year	Sex	Brood size	Mass[a] (g)	Mass change (g d^{-1})	rCO_2[b] (mL d^{-1})	SusMR[c] (kJ d^{-1})
2A-41	1996	M	5	19.38	-0.23	2179.7	57.1
D2-31	1996	M	5	18.63	-0.27	4614.6	120.9
D2-01	1997	M	5	20.63	+0.22	3907.7	102.4
2A-16	1997	M	5	18.63	-0.26	3830.5	100.4
2A-57	1997	M	5	19.50	0.00	4185.9	109.7
2A-63	1997	M	5	18.25	-0.48	3696.4	96.9
D2-20	1997	M	6	21.13	-0.24	4201.2	110.1
D2-32	1997	M	6	19.50	...[d]	4676.6	122.5
2A-14	1997	M	6	18.13	-0.25	3931.1	103.0
2A-56	1997	M	6	19.88	+0.25	4567.9	119.7
2A-76	1997	M	6	17.38	+0.25	4556.4	119.4
2A-77	1996	M	7	21.64	-0.62	3635.9	95.3
D2-12	1997	M	7	20.00	-0.61	3883.5	101.8
D2-44	1997	M	7	18.88	+0.74	3306.2	86.6
2A-17	1997	M	7	18.75	+0.46	4260.2	111.6
2A-21	1997	M	7	19.75	+0.46	4579.7	120.0
2A-78	1997	M	7	18.88	+0.25	5203.0	136.3
2A-59	1996	F	5	19.88	-1.24	4487.9	118.5
2A-41	1996	F	5	21.50	0.00	3804.0	99.7
D2-25	1996	F	5	18.75	...	2515.3	65.9
D3-15	1996	F	5	18.00	0.00	3622.3	94.9

2A-16	1997	F	5	17.50	0.00	4209.0	110.3
2A-57	1997	F	5	18.25	-0.51	3908.9	102.4
2A-63	1997	F	5	16.50	-0.49	4177.1	109.4
D2-01	1997	F	5	19.00	+0.47	4578.8	120.0
D2-11	1997	F	5	16.75	-0.48	3691.3	96.7
D2-21	1997	F	5	19.00	-0.46	3716.8	97.4
D2-29	1997	F	5	17.25	-0.54	4147.9	108.7
2A-37	1996	F	6	17.25	+0.42	3406.7	89.3
2A-85	1996	F	6	19.38	-0.75	4772.4	125.0
D3-09	1996	F	6	19.25	...	2141.5	56.1
2A-07	1997	F	6	18.00	0.00	4310.9	113.0
2A-25	1997	F	6	19.63	-0.23	4092.4	107.2
2A-33	1997	F	6	18.00	-1.06	3218.6	85.1
2A-73	1997	F	6	18.63	+0.78	3754.3	98.4
2A-40	1996	F	7	17.38	-0.24	3352.5	87.8
2A-77	1996	F	7	19.10	...	4551.0	119.2
D2-24	1996	F	7	16.63	-0.70	2205.6	58.3
D2-16	1996	F	7	17.50	0.00	2449.3	64.2
2A-17	1997	F	7	17.13	-1.25	3583.7	94.8
2A-21	1997	F	7	17.13	+0.23	3713.2	97.3
2A-78	1997	F	7	18.25	-1.00	4683.6	123.4
2A-80	1997	F	7	20.13	+0.25	4616.1	120.9
D3-01	1997	F	7	17.75	+0.46	3397.7	89.0
D3-16	1997	F	7	19.25	0.00	4641.4	121.6
D2-06	1997	F	7	17.25	+0.42	3759.4	98.5
D2-12	1997	F	7	18.50	-1.21	3169.5	83.9

[a]Average of initial and final mass, [b]Rate of carbon dioxide production, [c]Sustained metabolic rate. [d]Either the initial or final mass was missed.