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MONASH UNIVERSITY
THESIS ACCEPTED IN SATISFACTION OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

ON..... 5 August 2003 .....

.....[redacted].....

Sec. Research Graduate School Committee

ERRATA

p 14 para 2, line 6: replace "formulae:" for "formula:"
p 19 para 2, line 2: replace "was" for "were"
p 15 para 1, line 6: delete "in"
p 34 para 4, line 1: delete "numbers"
p 41 para 3, line 10: replace "...Everaarts, 1996; Davey *et al*..." for "...Everaarts, 1996 and Davey *et al*..."
p 45 para 1, line 1: replace "fairy-wren's" for "fairy-wrens"
p 45 para 1, line 2: replace "was" for "were"
p 47 para 2, line 12: replace "ie." for "i.e."
p 52 para 1, line 3: replace "was" for "were"
p 62 para 3, line 4: replace "0.29" for "0.29g"
p 63 para 3, line 2 and 5: replace " liver^{-1}" for "muscle^{-1}"
p 65 para 1, line 8: replace "...comparison test determines which..." for "...comparison tests determine which..."
p 67 para 1, line 7: replace "...comparison test determines which..." for "...comparison tests determine which..."
p 68 para 1, line 7: replace "...comparison test determines which..." for "...comparison tests determine which..."
p 80 para 1, line 16: replace "maybe" with "may be"
p 81 para 3, line 1: replace "ie." for "i.e."
p 81 para 3, line 5: replace "Withers, 1982" for "Withers, 1992"
p 81 para 3, line 7: replace "homogenate" for "homogenates"
p 85 para 1, line 1and 2: replace "ie." for "i.e."
p 107 para 1, line 1: replace "BMR" for "*BMR*"
p 107 para 2, line 21: replace "...has been previously been proposed..." for "...has previously been proposed..."
p 110 para 2, line 18: delete "an"

ADDENDUM

p VI, line 6: delete "...acclimatisation..." and replace with "Metabolic adjustments in fairy-wrens."
p VI, line 7: delete "...acclimatisation..." and replace with "Metabolic adjustments in spring."
p 3 para 2, line 3: delete "...expenditure involved in renewing..." and replace with "...energy content of feathers..."
p 3 para 2, add at the end of sentence 1, line 4: "However the total energy cost of synthesising feathers during moult is substantially higher."
p 3 para 2: delete the last sentence and replace with: "Typically, a passerine under 20g in mass expends between 471 and 1225 kJ·g^{-1} feathers produced, which equates to up to 110% of *BMR* in species such as the Bluethroat (Lindström *et al.*, 1993). The peak resting metabolic rates of moulting in African and European stonechats (*Saxicola torquata axillaris* and *Saxicola torquata rubicula*) were, on average, 31% and 32% higher than non-moult rates, respectively, in captive birds (Klaassen, 1995). The high energy costs of moult may, however, be compensated for by a decrease in activity and / or selective foraging for specific nutrients (Murphy, 1996)."
p 3 para 4, line 2: delete "...in the blood..." to read "...more oxygen to the flight muscles."
p 4 para 2, line 2: delete "...this creates an..." and replace with "...this increases the insulatory..."
p 4 para 3, line 3: delete "Metabolic rate needs to be adjusted in relation to thermoregulatory costs, and often..." and replace with "As a higher energy expenditure is usually associated with an increase in metabolic rate, often..."
p 5 para 1, line 6 and 8: add "...basal..." to read " ...basal metabolic rate..."
p 7 para 1, add at the end of sentence 5, line 10: "Female fairy-wrens control extra-group matings, and to a large extent prefer males from adjoining territories, particularly those that have acquired their bright nuptial plumage early (Mulder and Magrath, 1994; Mulder, 1997; Double and Cockburn, 2000). In fact studies by Mulder and Magrath (1994) found that 76% of offspring are sired by males from outside their social group."
p 8 para 2: Comment: A detailed temperature regime, including the actual overnight minimum temperature on the day of capture would have been useful but was not available from the Bureau of Meteorology and it would have been to impractical to measure temperature on the night before capture on each occasion. Thus long-term averages were used to gain insight into general seasonal trends, a practice adopted by many studies in this field.
p 8: add at the end of para 2, line 3: "Throughout the study, seasonal comparisons are made assuming winter encompasses the months of June, July and August; spring covers September, October and November; summer covers December, January and February; and autumn covers March, April and May."
p 9: add at the end of para 2, line 14: "Sexing of fairy-wrens by plumage was not validated in this study by dissection, as plumage traits are reliable on their own (Higgins *et al.*, 2001)."
p 11 para 1, line 8: add "transport and" to read "...blood oxygen transport and delivery."
p 11 para 1, line 9: delete "...include a decrease in blood haemoglobin oxygen affinity and / or an increase in the oxygen carrying capacity (*OCC*) of the blood..." and replace with "...include an increased oxygen carrying capacity (*OCC*), while a decreased blood haemoglobin oxygen affinity would improve oxygen delivery to tissues (Swanson, 1990b)."
p 11 para 1, line 12: add "compared to mammals" to read "...relatively rapid turnover rate of red blood cells compared to mammals."
p 11 para 2, line 12: delete "...in response to the cold stress of..." and replace with "...in winter..."
p 12 para 1, line 4: add "Piersma and Everaarts, 1996" to read "...Morton,1994; Piersma and Everaarts, 1996)."
p 13 para 3, line 1: delete "...artery..." and replace with "...an alar (brachial) vein with..."
p 14 para 1, line 1: delete "...whole blood quality control that had been analysed..." and replace with "...checked using a commercially prepared quality control (*BioRad*) that had been verified on a..."
p 15 para 4, line 6: delete "...all eight seasons..." and replace with "...all four seasons over the two years..."
p 17 para 1, line 6: delete "...over eight seasons in a two-year..." and replace with "...over each of the four seasons in the two-year period."
p 17 para 1, line 9: delete "...tests were performed to..." and replace with "...tests to..."
p 18 para 1, line 6: delete " ...the eight seasons..." and replace with "...the four seasons..."
p 18 para 2: Comment: As temperature was not recorded on the day of, or the night before capture, it was assumed to be similar within seasons and between years.
p 19 para 1, line 1: add "compared to females" to read "...in males compared to females in summer..."
p 19 para 2, line 3: delete "...or the sex bias..." to read "...body mass did not ..."

p 19 para 2, line 6: delete "...slope of the least square linear regression equations..." and replace with "...showed that the Pearson correlation coefficients for each of the blood parameters..."
p 19 all para 2 headed "Correlation between body mass and blood parameters" moved to p 18 before para 2 headed "Seasonal changes in blood parameters"
p 19 para 3: Comment: A more thorough appraisal of the extent of moult in all fairy-wrens trapped would have been more meaningful, but the possible effects that moulting might have had on blood parameters was only examined retrospectively and was not a planned comparison.
p 19: add at the end of para 3, line 7: "Moult score differences between the sexes would have been interesting to determine, but this was not possible due to the small sample size of each sex in each season."
p 24 para 1, line 2: delete "...functional..." to read "...of a relationship..."
p 27-28: Comment: Significance testing of Pearson correlation coefficients for each blood parameter is shown in Table 2.5.
p 30 para 1, line 1 and 2: delete "...a method..." and "...because it helps to combat..." to read "...fattening is employed by many small, overwintering bird species in the North Temperate Zone in response to cold conditions..."
p 30 para 2, line 1: delete "The..." and replace with "Body lipids are..."
p 30 para 2, line 2: delete "...the cells, not an increased..." and replace with "...content of individual adipocytes rather than increasing the number..."
p 32 para 3, line 2: delete "...immediate metabolic..." and replace with "...their short-term needs..."
p 33: add at the end of para 2, line 9: "Unfortunately this was unavoidable due to time constraints imposed by laboratory workload, involving calculation of blood parameters."
p 33 para 3, line 1: delete "...diurnal..." to read "...if fat-loading is..."
p 34 para 1, line 6: delete "Efficiencies in..." and replace with "Adjustments in the..."
p 34 para 2, line 1: delete "...directly determined..." and replace with "...tissues is affected by cardiac output and the blood *OCC*."
p 34 para 2, line 2: delete "...indicative of..." and replace with "Parameters influencing *OCC*..."
p 34 para 2, line 4: delete "...alternatively..." to read "...individual *Rbc* and *l* or by an..."
p 34 para 3, line 1: delete "...the flow rate of the blood would be reduced..." and replace with "...the work rate would increase due to the increased number of..."
p 35 para 1, line 1: add "and to hydration status" to read "...hormones and to hydration status, proteins..."
p 35: add at the end of para 2, line 5: "There are, however, other factors that effect *Hct* and *Hb* levels and these are considered further in the Discussion."
p 37 para 3, line 5: delete "...most likely more efficiently..." to read "...and more effectively than ..."
p 38 para 1, line 1: delete "...efficiency in..." and replace with "...increased effectiveness of the..."
p 38 para 3, line 12: add "between the two studies" to read "...difference between the two studies is..."
p 40 para 2, line 4: delete "...(including the grouping of data into seasons)..." and replace with "...analysis, grouping of data into calendar months (Rooke..."
p 40 para 3, add at the end of sentence 3, line 8: "Further, it may be that the cold challenge provided by the winter environment in south-east Australia is not sufficiently extreme to induce blood parameter changes."
p 40 para 3, line 10: delete "...instead..." to read "...are adjusted, such as..."
p 45 para 3, heading: delete "...size..." to read "...body mass on blood..."
p 46 para 2, line 1: add "compared to other higher vertebrates" to read "...most birds compared with other higher vertebrates, is the..."
p 46 para 2, line 5: delete "...series..." and replace with "...a complex group of..."
p 47 para 2, add at the end of sentence 4, line 13: "Blood testosterone levels in fairy-wrens start increasing several months prior to breeding, with significant increases starting in June and continuing through to December (Peters *et al.*, 2001). However, since there were no significant changes to blood *OCC* in winter, the indications are that testosterone was not having any substantial affect on blood parameters."
p 47 para 3: delete sentence 1 and replace with "Adjustment to the vascular system that can lead to enhancement of oxygen supply to metabolising tissues is not only achieved by increasing the rate of oxygen transport to the tissues, as discussed above, but also by relatively easier release of oxygen at the tissues."
p 47 para 3, line 3: delete "...determined..." and replace with "...unloading is influenced by blood..."
p 51 para 2, add at the end of sentence 4, line 11: "More specifically, *CS* is a regulatory enzyme in the Krebs cycle; *HOAD* oxidates fatty acids in the fatty acid cycle; *HK* catalyses the first step in the glycolytic pathway and *PHOS* converts glycogen to a chemical form that can enter the glycolytic pathway."
p 51 para 3: delete sentence 1 and replace with "Insulatory adjustment through increased feather mass in winter have also been shown to improve cold tolerance in some passerine species, such as the House finch and House sparrow, in which a 39% increase in feather mass occurred in winter (Dawson *et al.*, 1983; Barnett, 1970)."
p 52 para 3, add at the end of sentence 1, line 3: "Feathers were quickly removed (taking less than 10 - 15secs) before the bird's chest was opened in order to avoid their contamination with body fluids."
p 52: add at the end of para 3, line 10: "Removal of the liver and pectoralis muscles from fairy-wrens should make little difference to the calculation of stored body fat, as very little lipid is stored in these tissues (Blem, 1990)."
p 53 para 3, line 3: delete "...balance..." to read "...as water content and..."
p 53 para 3, line 13: add "method" to read "...the most accurate method."
p 55 para 4, line 8: add "but not including pectoralis muscles and liver" to read "...body mass (including plumage, but not the pectoralis muscles and liver) which..."
p 56 para 1, line 2: add "but not including pectoralis muscles and liver" to read "...body mass (including plumage, but not the pectoralis muscles and liver), comprising..."
p 61 last para, line 4: add "enzyme activities from each of" to read "...means for enzyme activities from each of the four seasons..."
p 62 para 1: delete sentence 2 and replace with "The mean seasonal dry mass of contour plumage was highest after post-breeding moult in autumn at 0.43g; mass declined due to loss and abrasion through winter (0.36g) and spring (0.31g). Total plumage mass was lowest in summer (0.24g), being 45% lower than in autumn."
p 63: add at the end of para 1, line 4: "A comparison of relative body lipids (% of wet mass) and relative water content (% of wet mass) shows a significant relationship (regression analysis: % water = 69.994 - 1.034 × % lipids; r = 0.65, $r^2 = 0.42$; $F = 27.58$, $P < 0.0001$), suggesting that higher body lipid levels lead to a relatively lower body water content."
p 63 para 2, add at the end of sentence 3, line 8: "Coefficients of variation were much higher in spring than in the other three seasons, perhaps indicating that glycogen levels were influenced by breeding. However, this could not be confirmed, as birds trapped in spring were not differentiated as breeding or non-breeding individuals."
p 63 para 3, line 8: add "measured" to read "... of all measured glycogen was..."

p 65 para 1, heading: add "contour" to read "...dry contour plumage mass and relative contour plumage mass..."
p 65 para 1, line 4: add "(1950-1996 averages) to read "... for Braeside Park (1950-1996 averages) are given..."
p 65 para 1: Comment: Actual temperatures on the day of collection were not recorded and are not available for this area from the Bureau of Meteorology. These data were collected to determine general seasonal trends.
p 70: add at the end of para 1, line 3: "Fairy-wren data are for carcasses without pectoralis muscle or liver."
p 75 para 1, add at the end of sentence 2, line 5: "The large surface area to volume ratio of small, homoeothermic animals leads to a proportionally higher loss of heat to the environment than in larger animals which have a relatively smaller surface area."
p 76: add at the end of para 1, line 2: "A consideration with the comparisons made in Table 3.5 is that there may be complications introduced by the fact that some birds, including the fairy-wrens, were in moult."
p 76 para 2, line 4: delete "...during..." and replace with "...in autumn after the moulting..."
p 76 para 2, line 16: delete "...alone does..." and replace with "...moulting may not place..."
p 76: add at the end of para 2, line 20: "Further, any costs associated with moulting may be offset by adjustments in activity."
p 77 para 2: delete sentence 1 and replace with "Heat-conserving behaviour, together with insulative adjustments, indicated by a 53% increase in contour feather mass in winter compared to summer, conceivably play a major role in the survival of fairy-wrens in cold winter conditions."
p 78 para 4: add at the end of sentence 3, line 6: "The slightly higher variability in fairy-wren and junco body lipids in winter is likely to be an artefact of the relatively small sample sizes in both studies. Alternatively, variation in ambient temperature on the nights before capture may have an influence on body lipid levels, although, this is unlikely in my study, as overnight temperature variation in other seasons is similar to that in winter."
p 78 para 1, line 3: add "(Lehninger, 1987)" to read "...less efficient active muscle (Lehninger, 1987)."
p 78 para 2: delete the last sentence and replace with "The reliance on oxygen for lipid catabolism effectively means that as muscle contraction increases, the proportional contribution of lipids as an energy source must decrease."
p 79 para 1: Comment: Constraints were imposed by the length of time required to kill and process the birds for measurement of enzymes and glycogen concentrations, which could take more than 10 hours and had to be performed on the same day.
p 79: add at the end of para 2, line 8: "Although there were no seasonal changes in body lipid levels in fairy-wrens, there was evidence that higher body lipid levels lead to a relatively lower body water content."
p 80 para 1, line 17: delete "...brooding and / or feeding of eggs or young." and replace with "...brooding of eggs or feeding of young."
p 80: add at the end of para 1, line 18: "If fasting is responsible for these differences in glycogen content, it would be expected that incubating females would have a higher glycogen content than males at this time of the year, but unfortunately brood patches were not examined in females captured in this study."
p 81 para 1, line 2: delete "...metabolic acclimatisation of..." and replace with "...in relation to metabolic changes involving high spring..."
p 82 para 2: Comment: *CS* activity may correlate directly with aerobic fitness; however, it may not change due to intense or sustained shivering thermogenesis if its level is already high as a result of the aerobic fitness level required for flying at all times of year.
p 82 para 2, line 5: add "at least" to read "...seasonal values at least 3-fold higher than..."
p 83 para 2, line 7: add ", particularly those located in regions which have severe low winter temperatures," to read "...in small passerines, particularly birds located in regions which have severe low winter temperatures, is for winter..."
p 84 para 3, heading: delete "...acclimatisation..." and replace with "Metabolic adjustments in fairy-wrens"
p 85 para 2, heading: delete "...acclimatisation..." and replace with "Metabolic adjustments in spring"
p 85 para 2, add at the end of sentence 2, line 7: "The 12.5mg of glycogen present in the liver and pectoralis muscles has an estimated energy content of about 0.21kJ, which is only sufficient for about 10 minutes of estimated daily energy expenditure."
p 85 para 2, line 7: add "However," to read "However, the use of muscle glycogen..."
p 86 para 1: Comment: The increase in flight activity associated with spring breeding is most likely fuelled in part by glycogen due to the anaerobic conditions in flight tissues during bursts of phasic activity. However, not all flight will be of this nature; in reality, most flight is most likely to be more restrained and therefore occurring under aerobic conditions. Hence increased *CS* activity levels indicate peak aerobic activity in spring.
p 88 para 2: Comment: Existence metabolism regression equations from Kendeigh *et al.* (1977) were used as only resting metabolic rate was measured in fairy-wrens.
p 88 para 3, line 4: delete "...this study..." and replace with "...mentioned in the latter live in ..."
p 89: add at the end of para 1, line 7: "Further, while the numerical abundance of insects has been shown to decrease in eastern Australian temperate forests in winter, the effective availability of insect prey for superb fairy-wrens might actually increase through reduced interspecific competition from migratory species such as silvereyes, whistlers and some honeyeaters (Haylock and Lill, 1988)."
p 91 para 1, line 7: delete "In temperate regions, metabolism in..." and replace with "Metabolic rate in euthermic birds resting..."
p 91 para 1: delete the last sentence and replace with "However, such cold-induced seasonal adjustments to metabolism are likely to have a high associated energetic cost (Dawson *et al.*, 1983a)."
p 91 para 3, line 2: delete "...it..." and replace with "...varies; both total and mass-specific *BMR* is recorded..."
p 91 para 3, line 3: delete "...but remaining..." and replace with "...*atricapillus*) while mass-specific *BMR* remains unchanged..."
p 92 para 1, line 3: delete "...the rate of..." to read "...dependent on thermal conductance..."
p 95 para 1, line 5: delete "...temperature-acclimatisation..." and replace with "...allow for thermal, steady-state equilibration and ..."
p 96 para 3, line 5: delete "...acclimatised..." and replace with "...slowly adjusted over..."
p 96 para 3, line 10: delete "...acclimatising..." and replace with "...for steady-state equilibration at each..."
p 97: add at the end of para 2, line 5: "Calculating an average using all measurements over the 60 minute period may possibly overestimate *BMR* if the animals are not quiescent through the entire experiment; the consequences of this are considered in the Discussion."
p 99 para 3, line 7: delete "...significant..." and replace with "...had an approximately 45% reduction..."
p 101 para 1, line 8: delete "...means..." and replace with "= indicates no..."
p 102 delete the second to last variable and replace with "Total $\dot{V}O_2$ (mL O_2 $bird^{-1}$ hr^{-1}) below T_{LC} at Mean Seasonal Minimum T_a"
p 105 para 1, line 6: delete "...with those predicted using the allometric equation for passerines, of Bennett and Harvey (1987)." and replace with "...within the 95% confidence interval of the allometric equation of Bennett and Harvey (1987) for passerines."
p 107: add at the end of para 1, line 2: "Further, intraindividual changes in *BMR* in kestrels (*Falco tinnunculus*) linked to body mass variation are thought to be a reflection of different maintenance regimens (Daan, Masman, Strijkstra and Verhulst, 1989)."
p 107 para 2: Comment: An increase in winter *BMR* is not mandatory for increased thermogenic capacity, although it has been recorded in several bird species. Alternatively, an improvement in the ability to maintain peak metabolic rate may compensate for any advantages offered by increasing *BMR* in winter. Thus it may be possible for some bird species in winter to actually lower *BMR*, for whatever reason, if for

example the ability to maintain peak metabolic rate is improved. It is possible, but unknown, if this occurred in silvereyes studied by Maddocks and Geiser (2000).

p 107 para 2, line 14: delete "...also..." to read "...Australia were reported to..."

p 108 para 1, line 10: add "the authors" and "improved tolerance to cold in winter" to read "...and summer, the authors suggesting that the bird's improved tolerance of cold in winter was independent..."

p 108 para 3, add at the end of sentence 1, line 2: "Hence T_{LC} is strongly affected by body mass, because smaller bodies have a relatively larger surface area which means that relatively more body-heat is lost through convection."

p 109 para 1, line 5: add "which may be related to their slightly larger mass" to read "...these two seasons, which may be related to their slightly larger mass."

p 109 para 1, line 10: add "and are marginally larger in size" to read "...much cooler areas and are marginally larger in size than both the..."

p 109 para 2, line 11: add "and Schleucher and Wither's (2001) prediction based on a pooled regression for non-passerine and passerine birds in the resting phase (0.21 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$)." to read "...by Aschoff (1981) for a 9.5g passerine resting at night (0.20 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$) and Schleucher and Wither's (2001) prediction based on a pooled regression for non-passerine and passerine birds in the resting phase (0.21 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$)."

p 109 para 2: Comment: Thermal conductance levels in this study refer to wet thermal conductance and assume that evaporative water loss, which was not measured in these experiments, was the same across all experimental groups.

p 110 para 2, line 1: delete "Given the highly significant increase in plumage mass of fairy-wrens in winter..." and replace with "Given the heavier plumage mass of fairy-wrens in winter than in summer (see Chapter 3)..."

p 110 para 2 and p 111 para 1: Comment: The calculations of total $\dot{V}O_2$ of 61, 60 and 58 mL O_2·bird are estimated oxygen consumption per hour in each of the seasons winter, spring and summer. However, comparisons should also be calculated on a 'per roost night' basis. Given average night lengths of 12, 10.5 and 9 hours, total overnight oxygen consumption rates per roost night would be approximately 732, 630 and 522 mL O_2·bird for winter, spring and summer, respectively. While this obviously indicates an increase in energy requirement in winter compared to summer, there is still no doubt it is reduced due to a decrease in the wet thermal conductance of winter-acclimatised birds.

p 111 para 2, line 14: delete "...relative to that in other seasons." and replace with "...below the thermoneutral zone closer to levels recorded in the other seasons and consequently minimise energy expenditure."

p 113 para 2: Comment: This assumes that the activity levels of the fairy-wrens were negligible in all determinations, as they were sleeping and there were no 'peaks' or 'spikes' of oxygen consumption recorded.

p 116 para 1, line 9: add "resident in the very cold regions" to read "...-sized passerines resident in the very cold regions of the North..."

p 118 para 2, line 21: delete the second to last sentence and replace with "Given that winter nights are, on average, about 3 hours shorter than those in summer, total oxygen consumption per roost night was estimated to be 29% lower in summer than winter. Therefore, although *RMR* was similar among the seasons, fairy-wrens had to maintain it for several more hours on cold winter nights. Therefore perhaps energy resources carried by fairy-wrens year-round are sufficient to sustain this without adjustments to either blood-oxygen delivery or energy stores."

p 119 para 2, heading: delete "The apparent lack of..." and replace with "Are there physiological adaptations to moulting in fairy-wrens?"

p 119: add at the end of para 2, line 7: "However, it is conceded that appraisal of moult in this study was relatively unsophisticated and therefore one cannot conclude that there were no physiological adaptations to moult."

p 143-157: add the following references:

Daan, S., Masman, D., Strijkstra, A. and Verhulst, S. (1989). Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition, and circadian phase in the kestrel, *Falco tinnunculus*. *Journal of Biological Rhythms* **4(2)**: 267-83.

Double, M. and Cockburn, A. (2000). Pre-dawn infidelity: females control extra-pair mating in Superb fairy-wrens. *Procedure Royal Society of London Ser B* **267**: 465-70.

Haylock, J. and Lill, A. (1988). Winter ecological energetics of two passerine bird species in temperate wet forest. *Australian Wildlife Reserve* **15**: 319-29.

Klaassen, M. (1995). Moult and basal metabolic costs in males of two subspecies of stonechats: the European *Saxicola torquata rubicula* and the East African *S.t. axillaris*. *Oecologia* **104**: 424-32.

Mulder, R.A. (1997). Extra-group courtship displays and other reproductive tactics of Superb fairy-wrens. *Australian Journal of Zoology* **45**: 131-43.

Mulder, R.A. and Magrath, M.J.L. (1994). Timing of prenuptial moult as a sexually selected indicator of male quality in Superb fairy-wrens (*Malurus cyaneus*). *Behavioural Ecology* **5**: 393-400.

Peters, A., Astheimer, L.B. and Cockburn, A. (2001). The annual testosterone profile in cooperatively breeding Superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behavioural Ecology and Sociobiology* **50**: 519-527.

Schleucher, E. and Withers, P.C. (2001). Re-evaluation of the allometry of wet thermal conductance for birds. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology* **129(4)**: 821-7.

PHYSIOLOGICAL RESPONSES OF SUPERB FAIRY-WRENS TO ENERGY CHALLENGES DURING THEIR ANNUAL CYCLE

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B.Sc. (Hons)

A thesis presented for the degree of

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ACKNOWLEDGMENTS

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I am also grateful for help received with fieldwork by Dr Margaret Stanley and Mr Peter Fell, who also assisted with technical support in the laboratory. Thank-you to Karen Leskie for help received in the laboratory during preliminary stages of enzyme activity determination.

Thanks also to Parks Victoria and the Department of Natural Resources and Environment for permission to conduct the fieldwork at Braeside Park.

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge and belief, no material previously published or written by another person, except when due reference is made in the text of the thesis.

Jeffrey Dean Box.

ABSTRACT

Seasonal trends in body mass, moult, blood parameters, insulation, body lipid levels, glycogen levels, catabolic enzyme activities and metabolic rate were investigated in wild Superb fairy-wrens in south east Australia. Changes in these physiological parameters were used to determine patterns of response to energy challenges imposed by cold winter conditions, breeding and, to a lesser extent, moult. Adaptations in most of these parameters are well documented for North Temperate passerines, but have received little attention in birds inhabiting temperate Australia.

The mean body mass of male fairy-wrens (9.8 - 10.1g) was significantly greater than that of females (9.1 - 9.6g), but did not vary seasonally. Likewise, total body lipids, which represent the largest stores of chemical energy available to the birds, and the activity of pectoralis muscle ß-hydroxyacyl-CoA dehydrogenase, a key catalyst involved in fat breakdown, also remained stable amongst all seasons (3.1 - 4.4% of wet body mass and 18.6 - 22.0 µmoles $min^{-1} \cdot g$ $muscle^{-1}$, respectively). Winter fattening is commonly recorded in small birds resident in the more northerly parts of the North Temperate Zone and is known to help them meet energy challenges presented by the region's severe winter weather conditions. The failure of fairy-wrens to accumulate fat stores in winter is probably a reflection of the comparative mildness of the austral climate in winter and the known fitness costs associated with fat storage.

Dry mass of contour plumage was measured as an index of insulation in fairy-wrens and was 77 and 53% higher in autumn and winter, respectively, than summer plumage mass. Moult varied seasonally, with the highest proportion of moulting birds being trapped in autumn (87%) and the lowest in winter (28%). Within the thermoneutral zone, the basal metabolic rate of fairy-wrens showed little variation over winter, spring and summer, with recorded values being 27.3 ± 1.8, 28.6 ± 2.6 and 25.3 ± 1.6 mL $O_2 \cdot bird^{-1} \cdot hr^{-1}$, respectively. However, below the bird's lower critical temperature (26-28°C), total metabolic rate at a

comparable ambient temperature was significantly lower in winter than in summer, due to differences in thermal conductance (1.55 versus 2.44 mL O_2·bird^{-1}·hr^{-1}·°C^{-1}). The reduction in thermal conductance in fairy-wrens in winter through improved insulation is thought to lessen the need for heat production through an increase in metabolic rate and thus it effectively reduces the energy requirements of the birds.

In spring, when most breeding occurred, fairy-wrens had mean stores of muscle and hepatic glycogen of 12.5mg per bird; these levels were more than twice those found in other seasons. Muscle glycogen is most often utilised during brief bursts of phasic activity, such as take-off, sudden manoeuvring and rapid accelerations. Similarly, the activity of both phosphorylase and hexokinase, key enzymes that catalyse the release of energy from stored glucose, were also highest in spring, with levels of 139 ± 3 and 2.0 ± 0.3 μmoles min^{-1}·g muscle^{-1}, respectively, being recorded. Activity levels of the enzyme citrate synthase were 20-40% higher in spring (137 ± 7 μmoles min^{-1}·g muscle^{-1}) than in other seasons. This indicated a seasonal enhancement of aerobic capacity and endurance of the pectoralis muscle that is likely to have been influenced by the energy demands of increased flight activity. Further, oxygen delivery to metabolising tissues was also significantly seasonally enhanced in spring, as indicated by a 17% increase in estimated blood oxygen carrying capacity. The combination of enhanced oxygen delivery, larger glycogen stores and an increased metabolic capacity of the pectoralis muscle in spring are believed to assist in meeting the additional energy requirements associated with breeding, particularly the likely increase in flight activity. The seasonal peak in blood oxygen carrying capacity and stored glycogen concentrations did not coincide with the time when moulting was most pronounced.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL METHODS

GENERAL INTRODUCTION

Small passerines resident in the temperate zone face several energy challenges during their annual cycle, particularly in relation to moulting, breeding and surviving cold winter conditions. The onset of winter in the North Temperate Zone presents a problem to many resident small birds, due to the combination of low temperatures, shorter day length and decreased food resources that may be covered by snow or ice (O'Connor, 1995a). Many birds avoid winter by migrating to warmer regions, but this involves a large energy expenditure on flight (Dawson *et al.*, 1983a). Flight involves an energy expenditure of between 2 and 25× the bird's basal metabolic rate (*BMR*), depending on the type of flight and size of the bird (Norberg, 1990). An estimate of flight costs, based on a 10-g bird flying close to its maximum speed, would be 7.7× *BMR* (Norberg, 1996). The benefits of migration must be substantial to offset its obvious costs.

Birds that remain at high northern latitudes avoid the costs associated with migration, but maintaining homoeothermy in winter cold presents a major energetic challenge too. Acclimatisation to cold in birds resident in the more northerly parts of the North Temperate Zone depends primarily on metabolic adjustments, which enhance the bird's ability to maintain a greatly elevated metabolic rate and heat production through shivering thermogenesis (Dawson and Carey, 1976; Dawson *et al.*, 1983a). Unlike mammals, birds apparently lack the ability for facultative, cold-induced, non-shivering thermogenesis (Connolly *et al.*, 1989). Insulatory adjustments, involving an increase in plumage mass and improved orientation of feathers, also assist in winter enhancement of cold tolerance, particularly in passerines exposed to moderate winters (Swanson, 1991).

In general, the energetic cost associated with reproduction in birds is high at several stages of their breeding cycle. In particular, gonadal development, egg production, incubation below thermoneutrality and rearing of nestlings have

estimated costs equivalent to 2-9%, 13-41%, 19-50% and 75% per chick respectively, of the daily *BMR* of adult passerines (Carey, 1996; Williams, 1996; Weathers, 1996). Further, increased parental effort may result in 'energetic stress', which may increase the parent's susceptibility to starvation, predation and disease (Merila and Wiggins, 1997).

Whilst the moulting process requires a wide array of adjustments in the physiology, energetics and nutrition of birds (Murphy, 1996), the estimated energy expenditure involved in renewing feathers is, however, surprisingly low, and may be the equivalent of less than 6% of *BMR* (Murphy and King, 1992). Typically, a passerine under 20g in mass expends between 471 and 1225 $kJ \cdot g^{-1}$ feathers produced (Lindström *et al.*, 1993), but this may be compensated for by a decrease in activity and / or by selective foraging for specific nutrients (Murphy, 1996).

Meeting these energy challenges associated with moulting, breeding and cold-induced thermogenesis involves enhancement of various facets of aerobic metabolism in small birds and these are considered below.

Extensive flying, such as that which occurs during migration, usually requires the delivery of more oxygen in the blood to the flight muscles. This can be achieved through an increase in blood oxygen carrying capacity (*OCC*). This is a relatively economical change that allows more oxygen to be delivered per unit of blood (Swanson, 1990b) and can be achieved relatively rapidly because of the high red blood cell turnover rate in birds (Rodnan *et al.*, 1957). Long-distance migration in sparrows and godwits is preceded by an increase in blood *OCC* (Morton, 1994; Piersma and Everaarts, 1996). Similarly, sustained shivering in overwintering passerines is likely to lead to an increased demand for oxygen in the pectoralis muscles and this is reflected in the seasonally elevated blood *OCC* of juncos, finches and goldfinches resident in harsh winter climates in the North Temperate Zone (Swanson, 1990b; Clemens, 1990; Carey and Morton, 1976).

Birds must maintain sufficient reserves of body lipids and glycogen for productive activities and stressful events, despite the limited storage capacity

imposed by flight demand and wing loading (Blem, 1990). Generally, most birds obtain their energy and nutrients for moult directly from their food and do not depend on body reserves (Murphy, 1996). In contrast, long-distance migration and overwintering in cold regions are accompanied by substantial increases in body lipid levels in many small North Temperate Zone passerines, such as finches and sparrows (O'Connor, 1995a; deGraw *et al.*, 1979). The exact role of carbohydrates in these conditions is unclear, with contradictory observations indicating either decreases, increases, or no change in, glycogen levels during migratory flight and cold-induced thermogenesis in passerines (Marsh, 1983; Marsh *et al.*, 1984; Carey *et al.*, 1978). However, it is clear that glycogen plays an important role during take-off, burst flight and fuel delivery to glucose-dependent tissues (Roth *et al.*, 1987; Schwilch *et al.*, 1996). Moreover, an increase in the use of stored body lipids or glycogen must involve an increased capacity to mobilise and catabolise these energy sources. The activities of key indicator enzymes responsible for this are elevated in finches and goldfinches in response to cold and in catbirds during migration (Carey *et al.*, 1989; Yacoe and Dawson, 1983; Marsh, 1981).

Insulation in birds can be regulated by varying the elevation of feathers using cutaneous muscles; this creates an insulatory layer of air within the plumage (del Hoyo *et al.*, 1992). Seasonal increases in contour plumage mass after moult also significantly improve a bird's insulation. Numerous small passerines overwintering in the harsh climate of the northern North Temperate Zone exhibit winter increases in plumage mass (Swanson, 1991; Dawson *et al.*, 1983), but the insulatory effects are apparently limited due to the bird's large surface area to volume ratio, which strongly affects convection (Dawson *et al.*, 1983a). However, this effect can be minimised by initiating behavioural thermoregulation, involving huddling and microclimate selection (Dawson and O'Connor, 1996).

A primary feature of metabolic acclimatisation to cold in birds in the North Temperate Zone is an enhanced ability to maintain a greatly elevated metabolic rate to support shivering thermogenesis. Metabolic rate needs to be

adjusted in relation to thermoregulatory costs, and often species with a high rate have significant winter body lipid reserves (Swanson, 1990a; O'Connor, 1995a). The rate of metabolism below thermoneutrality is dependent on, amongst other factors, thermal conductance, which is reduced in well-insulated birds (Dawson *et al.*, 1983a). Due to the relatively small size of juncos and chickadees and the extreme conditions they are exposed to when overwintering, their metabolic rate is higher in winter than in summer (Swanson, 1991; Cooper and Swanson, 1994). In contrast, Australian silvereyes (*Zosterops lateralis*) have a higher metabolic rate in summer than in winter, and this is probably linked to the high energy demands of breeding (Maddocks and Geiser, 2000) and possibly the relatively mild winter conditions.

Although there is a considerable body of knowledge concerning the annual energetic cycle of small passerines resident in North America and Europe, comparable information on birds in Australia is scarce. Of particular interest when comparing seasonal adaptations of birds in the two temperate zones is that there are fewer migratory species, the breeding season is more protracted (Ford, 1989), the winter climate is more moderate and summer is more harsh in temperate Australia. Given the existence of geographic variation in cold tolerance correlated with the severity of the winter climate (Dawson *et al.*, 1983), perhaps the relative role of metabolic contributions to acclimatisation would be predicted to vary with climate and region.

The Superb fairy-wren (*Malurus cyaneus*) is a small, non-migratory passerine that occupies a wide range of habitats up to 1400m above sea level in temperate eastern Australia. My aim was to determine whether there were annual patterns of variation in various physiological parameters of resident fairy-wrens that corresponded with the timing of the energy challenges imposed by breeding, winter conditions and moulting. Seasonal patterns in body mass and blood *OCC* were determined by measuring haematocrit, whole blood haemoglobin and the red blood cell count in samples collected from wild-trapped fairy-wrens in all four seasons of the year over two successive years. Seasonal comparisons of several relevant biochemical parameters of fairy-wrens were also

made over all four seasons of one year. These included calculations of total body lipid levels, the glycogen concentration in the liver and pectoralis muscle and the activities of phosphorylase, hexokinase, citrate synthase and ß-hydroxyacyl-CoA dehydrogenase, enzymes from the pectoralis muscle involved in the catabolism of triacylglycerols and glycogen / glucose. A seasonal comparison of contour plumage mass, used as an indicator of body insulation, was also made. The metabolic rate of resting fairy-wrens exposed to a range of ambient temperatures (T_a) was also determined and compared in spring, summer and winter. This allowed the estimation of *BMR* and lower critical temperature (T_{LC}) and also the effect that T_a has on metabolic rate below thermoneutrality in fairy-wrens. Where possible, physiological parameters determined for fairy-wrens were compared with values for similarly-sized passerines in the temperate zone of Australia, Europe and North America.

GENERAL METHODS

Study species

The Superb fairy-wren, *Malurus cyaneus* (Ellis, 1782) is resident in east and south-east Australia. It occurs along the coast and up to about 500km inland from central Queensland, south through New South Wales and Victoria to the Eyre Peninsula in South Australia and is also found in Tasmania. Superb fairy-wrens occupy a wide range of vegetation associations and landforms, except alpine and thick closed-forest. They are territorial year-round, living in groups of 2-9 which usually contain one breeding female and male and several helpers, which are usually males raised in the previous season. Fairy-wrens are socially monogamous but sexually promiscuous, with males seeking extra-pair copulations. They have a clearly defined breeding season, particularly where rainfall is relatively high and predictable. In Victoria, over 90% of fairy-wrens breed in the period from September to January, with over half nesting in October-November. Females construct the bulky, domed nest, taking about seven days. They lay 3-4 eggs at daily intervals. Incubation and brooding of the young take about 12-14 days each and are again performed solely by the female. All adult members of the group carry out feeding of the young, with immature helpers contributing only a little. Often up to three broods are attempted in one season (see Higgins *et al.*, 2001; Rowley and Russell, 1997; Rowley, 1965).

Study Site

Research was conducted in the Conservation Reserve of Braeside Park, which is situated approximately 25km south east of Melbourne (38° 00' S, 145° 11' E). The park covers an area of 295-ha and is less than 50m above sea level. It contains four distinct habitat types; Woody Heathland, Red Gum Woodland, Wetlands and Open Paddocks. The Woody Heathland in the north east corner of the park, covering about 54-ha in area, was the capture site of most fairy-wrens in this study. This site had good vehicle access and was relatively undisturbed

due to restricted public access. Vegetation in the Woody Heathland consisted mainly of Eucalyptus (predominantly Coastal Manna Gums *Eucalyptus pryoriana*, River Red Gums *E. camaldulensis* and Narrow-leaved Peppermint *E. radiata*), Kunzea (Prickly Kunzea *Kunzea continentale* and Heath Kunzea *K. myrsinoides*), Banksia (Coast Banksia *Banksia integrifolia* and Silver Banksia *B. marginata*) and numerous Wattle (*Acacia*) species. Ground cover consisted mainly of Austral Bracken (*Pteridium esculentum*) and various species of Club-rush, Sedge and Sword-sedge (Family: *Cyperaceae*).

Bureau of Meteorology climate averages from Moorabbin Airport (37° 98' S, 145° 08' E), approximately 3 km west of the park, were used as an indication of climate for Braeside Park during this study (Table 1.1).

Table 1.1. Climate averages used for Braeside Park.

Long term values for weather data collected by the Bureau of Meteorology at Moorabbin Airport, 3km west of Braeside Park, from 1950-1996.

	SPRING	SUMMER	AUTUMN	WINTER
Mean daily maximum temperature (°C)	19.2	25.1	20.4	14.1
Mean daily minimum temperature (°C)	9.4	13.5	10.9	6.4
Mean number of days where minimum ≤ 2°C	0.9	0	0.4	8.6
Mean seasonal rainfall (mm)	208	151	187	197

Additional birds used in metabolic experiments were captured at Koomba Park, Wantirna, which is situated approximately 23km east of Melbourne. This park is also less than 50m in altitude and contains similar habitat and has similar weather conditions to Braeside Park.

Capture of fairy-wrens

All fairy-wrens in this study were captured in mist nets measuring 10-12m long and 2 metres high. Typically 4-5 nets were erected in small clearings in the Woody Heathland reserve within about 500m of a base, where birds were processed. Nets were routinely checked every 15-20 minutes. Trapped fairy-wrens were individually held in cloth bags until processed. Netting sessions usually started around sunrise and lasted 4-8 hours, during which time up to 15 fairy-wrens were captured. On capture, fairy-wrens were processed within 30 minutes; this included banding, weighing, sexing and checking moult status. Sexing adult fairy-wrens during the breeding season was straightforward, with most males displaying a brilliant cyan-blue capped head and cheeks, and having a blue-black throat and breast. These colours were lacking in females, which are a mouse-brown, with whitish throat and breast. Immature and 'eclipse' males could usually be distinguished from females by their darker, olive-brown bodies and their black facial features.

Abbreviations, symbols and units

All abbreviations, chemical symbols and units of measurements and their meanings are set out in Appendix: Table I.

CHAPTER 2

SEASONAL CHANGES IN BODY MASS, BLOOD PARAMETERS AND MOULT OF FAIRY-WRENS.

(This chapter was slightly modified and published in the *Australian Journal of Zoology* in September 2002; the original paper is included in Appendix: Table II).

INTRODUCTION

The increased cold tolerance shown by some small passerines overwintering in areas of the North Temperate Zone represents a remarkable feat of endurance (Dawson *et al.*, 1983a). The sustained shivering responsible for increasing thermogenic capacity in these winter-acclimatised birds is apparently dependent in part on an increased oxygen supply to the skeletal muscles involved (Dawson and Marsh, 1989). It therefore follows that enhancement of the shivering rate could to some degree be achieved through the improvement of oxygen delivery. Potential mechanisms capable of leading to enhancement of blood oxygen transport at low metabolic cost include a decrease in blood haemoglobin oxygen affinity and / or an increase in the oxygen carrying capacity (*OCC*) of the blood (Swanson, 1990b). The ability to increase *OCC* is of particular importance due to the relatively rapid turnover rate of red blood cells (Rodnan *et al.*, 1957). Furthermore, to fuel overnight shivering, body fat storage in small passerines may be increased in winter compared to other seasons (Blem, 1990). This seasonal fat change, if significant, is often reflected by a change in body mass (Swanson, 1991).

Most of the few intensive studies of winter acclimatisation in small passerines from the North Temperate Zone suggest that adjustment of the vascular oxygen transport system is at least partially responsible for the phenomenon. More specifically, an increase in blood haematocrit or whole blood haemoglobin in winter has been recorded for Rosy finches (*Leucosticte arcton*) from California (Clemens, 1990), American goldfinches (*Spinus tristis*) from Michigan (Carey and Morton, 1976) and Dark-eyed juncos (*Junco hyemalis*) from Oregon (Swanson, 1990b). Superb fairy-wrens (*Malurus cyaneus)*, Brown thornbills (*Acanthiza pusilla*), Red-browed finches (*Neochmia temporalis*) and Eastern Yellow robins (*Eopsaltria australis*) from the low altitude regions of the South Temperate Zone in Australia also exhibit a decrease in red blood cell size and an increase in red blood cell numbers in response to the

cold stress of winter (Breuer *et al.*, 1995). There is also evidence that the potentially energetically costly activities of moulting and migration are accompanied by increases in blood *OCC* in some bird species, for example some sparrows and godwits, respectively (Murphy, 1996; Morton, 1994).

This section of the study of wild Superb fairy-wrens examines the key blood parameters, haematocrit, whole blood haemoglobin and red blood cell count, which influence the rate of blood oxygen delivery. A comparison is made among all four seasons of the year (summer, autumn, winter and spring) over two successive years to determine if adjustment of the vascular oxygen transport system occurs and to what extent. The observed changes are compared with those reported for other small passerines in both the similar and more extreme climatic conditions in the South and North Temperate Zones, respectively, and also with birds that undertake long migration movements. The effect that moulting may have on blood parameters is also considered. The body mass of the fairy-wrens is also examined to ascertain indirectly if winter fattening is occurring in response to cold stress.

METHODS

CAPTURE AND BLOOD SAMPLING

Superb fairy wrens were captured at a variety of sites in Braeside Park using mist nets, as outlined in Chapter 1: Generals Methods. Trapping occurred over a two-year period from June 1995 to May 1997. A total of approximately 265 hours was spent mist netting, mainly from sunrise to midday, although some netting took place in the afternoon. In all, 228 birds were caught, approximately 30 birds (range: 24 to 35) in each of the eight climatic seasons studied.

On capture, all fairy-wrens were banded, sexed, weighed and checked for moult. The sex of the bird was determined from its plumage (see Chapter 1: General Methods) and body mass was recorded to the nearest 0.5g using a Pesola spring balance. Moult was recorded by examining five body areas, namely the head, neck, body, primaries and rectrices. Moult was deemed 'light' if new feathers were present in one or two areas of the bird or 'heavy' if they occurred in three or more areas. Each bird was bled, weighed and checked for moult only once to achieve statistical independence.

Blood was collected by piercing a brachial artery with a 27.5-gauge hypodermic needle. The blood was withdrawn by capillarity into heparinised micro-capillary tubes and kept on ice. When possible, three tubes were collected from each bird, giving up to 75 μL of blood. The heparin in the tubes prevented the blood from clotting. Laboratory analysis of blood was performed within four-hours of collection.

MEASURED BLOOD PARAMETERS

Each haematological parameter was measured manually using standard techniques developed for human blood (Dacie and Lewis, 1991). The accuracy

of the methods was regularly checked using a whole blood quality control that had been analysed on a Technicon H2 autoanalyser.

Haematocrit

Haematocrit (*Hct*) is an estimate of the volume of blood occupied by red blood cells and expressed as a percentage of whole blood (Dacie and Lewis 1991). This was determined by centrifuging (*IEC: Micro-MB* centrifuge) blood filled capillary tubes at 3,000*rpm* for five minutes. The length of the column of packed red blood cells (*PC*) was measured and expressed as a percentage of the total length of the blood column (*BC*) according to the formulae:

$$Hct\ (\%) = (PC / BC) \times 100$$

Whole blood haemoglobin concentration

Whole blood haemoglobin concentration (*Hb*) was measured by cyanomethaemoglobin spectrophotometry using Drabkin's reagent as a diluent (Dacie and Lewis, 1991). Drabkin's reagent contained 200mg of potassium ferricyanide, 50mg potassium cyanide, 140mg potassium dihydrogen phosphate and 1mL Triton X-100 detergent made up to 1000mL with distilled water. A 10µL sample of evenly suspended whole blood was added to 1mL of Drabkin's solution, vortexed (*Thermolyne-Maxi Mix II* vortex) and left to stand for several minutes. The diluted sample was centrifuged (*MSE: Micro Centaur* eppendorf centrifuge) at 13,000*rpm* for five minutes; this separated the supernatant from the *DNA* material found in the red blood cells of birds. The absorbence of the supernatant was measured at 540nm against a Drabkin's blank on a *Pharmacia-Ultrospec III*® spectrophotometer. *Hb* is expressed in grams of haemoglobin per 100mL of blood and is calculated according to the equation:

$$Hb\ (\text{g} / 100\text{mL}) = (OD_{540} \times 64{,}500 \times 101) / (44 \times 1{,}000 \times 10 \times 1)$$

Where OD_{540} = Absorbance of supernatant at 540nm

64,500 = molecular weight of haemoglobin

101 = sample dilution factor (10μL sample and 1mL of Drabkin's solution)

44.0 = millimolar extinction coefficient of haemoglobin ($mM^{-1}cm^{-1}$)

1,000 = conversion factor - mg to g

10 = conversion factor - 1 litre to 100mL

1 = cuvette light path in (cm)

Red blood cell count

Red blood cell (*Rbc*) numbers were determined using an improved Neubauer haemocytometer. An aliquot of blood was diluted 201 times by adding 20μL of well mixed whole blood to 4mL of 0.9% NaCl, and mixed by gentle tilting and rotating for at least 2 minutes. The counting chamber was filled with the diluted blood and the *Rbc* were allowed to settle for several minutes. Using an *Olympus EHT 201910* compound light microscope with an ×10 objective and an ×10 eyepiece, the number of cells was counted in 80 small squares, which have a total volume of 0.02μL. As this was the least accurate of the methods, the cells in the diluted blood were counted three separate times for each bird and the average result used. The *Rbc* count is calculated according to the equation:

$$Rbc \text{ count } (\mu L^{-1}) = (N \times 201) / 0.02$$

Where N = number of *Rbc* counted in 80 small squares of the haemocytometer

201 = sample dilution factor

0.02 = volume of 80 small squares (μL)

To ensure a reliable cell count, five batches of diluted blood were made from a single fairy-wren in each of the eight seasons and counted in triplicate. This was analysed using a series of one-factor *ANOVA*s. The method used for counting *Rbc* proved to be reliable in that the cell count of each of the five batches of diluted blood taken from a particular bird were statistically indistinguishable in all eight seasons (Appendix: Table III).

CALCULATED BLOOD PARAMETERS

The mean red cell volume, the mean red cell haemoglobin concentration and the oxygen carrying capacity are calculated from *Hct*, *Hb* and the *Rbc* count with appropriate corrections to common units.

Mean red cell volume

The mean volume of a red blood cell (*MCV*) in femtolitres (fL) was calculated using the *PC* (as determined above for *Hct*) and *Rbc* counts by the equation:

$$MCV\ (\text{fL}) = PC\ /\ Rbc\ \text{count}\ (\mu\text{L}^{-1}) \times 10^{6}$$

Mean cell haemoglobin concentration

The mean haemoglobin concentration in a red blood cell (*MCHC*) in picograms (pg) was calculated using *Hb* and *Rbc* count by the equation:

$$MCHC\ (\text{pg}) = (Hb\ (\text{g}\ /\ 100\text{mL}) \times 10)\ /\ (Rbc\ \text{count}\ (\text{L}^{-1}) \times 10^{6}).$$

Oxygen-carrying capacity of whole blood

Each gram of haemoglobin in whole blood of a bird is capable of binding 1.34mL of oxygen (Withers, 1992). The oxygen-carrying capacity (*OCC*) of whole blood is therefore calculated from the *Hb* by the equation:

$$OCC\ (\text{mL}\ O_2\ /\ 100\text{mL blood}) = Hb\ (\text{g}\ /\ 100\text{mL}) \times 1.34$$

DATA ANALYSIS

Statistical analyses were carried out with Statistica 4.5, a Windows-based program by StatSoft, and Microsoft Excel 97. A power analysis was performed to determine the required sample size per season using data collected in the first season of this study (winter 1995) and data from the literature, including Breuer

(1992). This indicated that a sample size of at least 18 was required to be 80% confident of detecting a 20% change in any of the blood variables among seasons. Data for body mass and the measured blood parameters were independent and normally distributed making it unnecessary to transform data before analysis of variance (*ANOVA*) was performed. A three-factor *ANOVA* was carried out to examine body mass variation between the sexes over eight seasons in a two-year period. Data for males and females was then pooled and a two-factor *ANOVA* followed by eight *Post hoc* Tukey's multiple-comparison tests were performed to examine seasonal changes in blood parameters over two years. A series of single factor *ANOVA*s were carried out to determine if males and females differed in *Hc*t, *Hb* and *Rbc* numbers in each season.

The degree of correlation between body mass and each haematological parameter was examined visually using scatter diagrams. For each data set, the Pearson correlation coefficient (r), the least squares linear regression equation and the degree of variance of the linear relationship (r^2) were calculated. A series of single factor *ANOVA*s were also carried out on the slopes of the linear regression equations for each blood parameter. Pearson's X^2 test for Goodness of Fit was used to compare the proportions of moulting (both light and heavy) and non-moulting birds across the four seasons, pooling frequencies collected over the two years.

RESULTS

SEASONAL CHANGES IN BODY MASS

The mean body mass of adult male and female fairy-wrens in each season and the associated statistical analyses are summarised in Tables 2.1 and 2.2. Sexual dimorphism in body size has been recorded for most malurids, including fairy-wrens (Rowley and Russell, 1997). The mean seasonal body mass ranged from 9.8 to 10.1g for adult males and 9.1 to 9.6g for adult females. There were no significant differences in mass among the eight seasons or between years, but there was a significant overall difference between the sexes ($P < 0.0001$). In each season, the mean body mass of males was 3.1 to 7.7% heavier than that of females, with an average difference of 5.5%.

SEASONAL CHANGES IN BLOOD PARAMETERS

Seasonal trends in each blood parameter are shown in Figures 2.1 and 2.2 with all the data summarised in Appendix: Table IV. Statistical analyses are presented in Table 2.3. *Hb* and *Hct* values were highest in the spring of years 1 and 2. When data for the two years were combined the peak in *Hb* and *Hct* in spring was significantly higher (by 9-17% and 6-11%, respectively) than the winter, summer and autumn. In summer, *Hb* was also significantly higher than in winter. There were no significant differences between the two years in the mean values for particular seasons for either *Hb* or *Hct*. Within each of the years, the mean *Rbc* count appears to be highest in spring, although it was only significantly greater than autumn when the two years were combined. There were no significant differences in the mean values for particular seasons over the two years for *Rbc* count. There was no significant difference between males and females for *Hct*, *Hb* or *Rbc* count in spring or autumn (Table 2.4), when sample sizes permitted this comparison. There was however a higher *Hct* and *Rbc* count

in males in summer and winter, respectively. *MCV* ranged in size from 115 to 125fL over the two-year sampling period and exhibited no significant seasonal variation. Seasonal means for *MCHC* ranged from 31.6 to 37.0pg. Spring, summer and autumn values did not differ significantly, but they were all significantly higher than that for winter. *OCC* was derived directly from *Hb* and therefore showed the same seasonal variation pattern.

CORRELATION BETWEEN BODY MASS AND BLOOD PARAMETERS

Inadvertently, an unequal sex ratio of birds was sampled in all seasons. Since males were significantly heavier than females, data was tested to ensure body mass or the sex bias did not confound blood parameters. The scatter diagrams in Figures 2.3 and 2.4, together with the small r^2 values (all < 0.015), indicate that there is no significant relationship between body mass and any of the five blood parameters. In addition, statistical analysis showed that the slope of the least square linear regression equations for each of the blood parameters were not significantly different to zero (Table 2.5). Therefore any seasonal changes occurring in any of the blood parameters can not be explained by variation in body mass.

PATTERN OF MOULT

Seasonal moult in trapped fairy wrens is shown in Figure 2.5. As expected, the degree of moult varied significantly amongst seasons ($X^2_{(6)} = 72.9$, $P < 0.01$). In winter, 28% of birds had moult (both light and heavy) and this proportion increased through spring (58%) and summer (75%), until in autumn when 87% of birds were moulting. Spring and summer had approximately the same proportions of lightly and heavily moulting birds, whilst in autumn the majority of birds (65%) were in 'heavy' moult.

Table 2.1. Seasonal variation in body mass of adult fairy wrens.
Values are mean ± standard error with sample size given in parentheses.

SEASON		BODY MASS (g)	
		Male	**Female**
Winter 1		9.9 ± 0.1	9.3 ± 0.3
		[22]	[9]
Winter 2		9.9 ± 0.1	9.3 ± 0.1
		[16]	[16]
	Pooled	**9.9 ± 0.1**	**9.3 ± 0.1**
		[38]	**[25]**
Spring 1		10.0 ± 0.2	9.3 ± 0.3
		[16]	[8]
Spring 2		10.1 ± 0.1	9.6 ± 0.3
		[21]	[14]
	Pooled	**10.1 ± 0.1**	**9.5 ± 0.2**
		[37]	**[22]**
Summer 1		9.8 ± 0.1	9.1 ± 0.2
		[14]	[13]
Summer 2		9.9 ± 0.2	9.5 ± 0.1
		[10]	[17]
	Pooled	**9.9 ± 0.1**	**9.3 ± 0.1**
		[24]	**[30]**
Autumn 1		9.9 ± 0.3	9.6 ± 0.1
		[8]	[16]
Autumn 2		9.9 ± 0.2	9.6 ± 0.1
		[12]	[14]
	Pooled	**9.9 ± 0.2**	**9.6 ± 0.1**
		[20]	**[30]**

Table 2.2 f-ratios and probability levels from a three-factor *ANOVA* examining body mass variation between the sexes and among the seasons over a two year period.

MAIN EFFECTS:	TEST STATISTICS		
	f-ratio	**probability**	**degrees of freedom**
Year	2.564	0.111	1
Season	0.676	0.567	3
Sex	**35.190**	**< 0.001**	1
INTERACTIONS:			
Year x Season	1.034	0.379	3
Year x Sex	0.451	0.503	1
Season x Sex	0.859	0.463	3
Year x Season x Sex	0.019	0.996	3

Table 2.3. f-ratios and probability levels from a two-factor *ANOVA* examining variation in blood parameters amongst seasons over a two year period and *Post hoc* Tukey's multiple-comparison tests to determine which seasons differed.

Degrees of freedom were 1 (year), 3 (season) and 3 (year x season). Unlike means are separated by **X**.

FACTORS:	f-ratio (probability)		Interaction	Tukey's multiple-comparison test
	Year	Season	Year x Season	($p < 0.05$)
Hct	3.720 (0.056)	**20.353 (< 0.001)**	0.733 (0.534)	Spring **X** Winter, Summer, Autumn
Hb	0.705 (0.402)	**20.389 (< 0.001)**	1.424 (0.238)	Spring **X** Summer, Autumn, Winter Summer **X** Winter
Rbc	1.979 (0.162)	**5.389 (0.001)**	2.331 (0.760)	Spring **X** Autumn
MCV	0.008 (0.927)	1.565 (0.201)	**3.207 (0.025)**	Nil
MCHC	0.960 (0.329)	**11.150 (< 0.001)**	1.353 (0.260)	Spring, Summer, Autumn **X** Winter
OCC	0.679 (0.411)	**20.503 (0.000)**	1.401 (0.245)	Spring **X** Summer, Autumn, Winter Summer **X** Winter

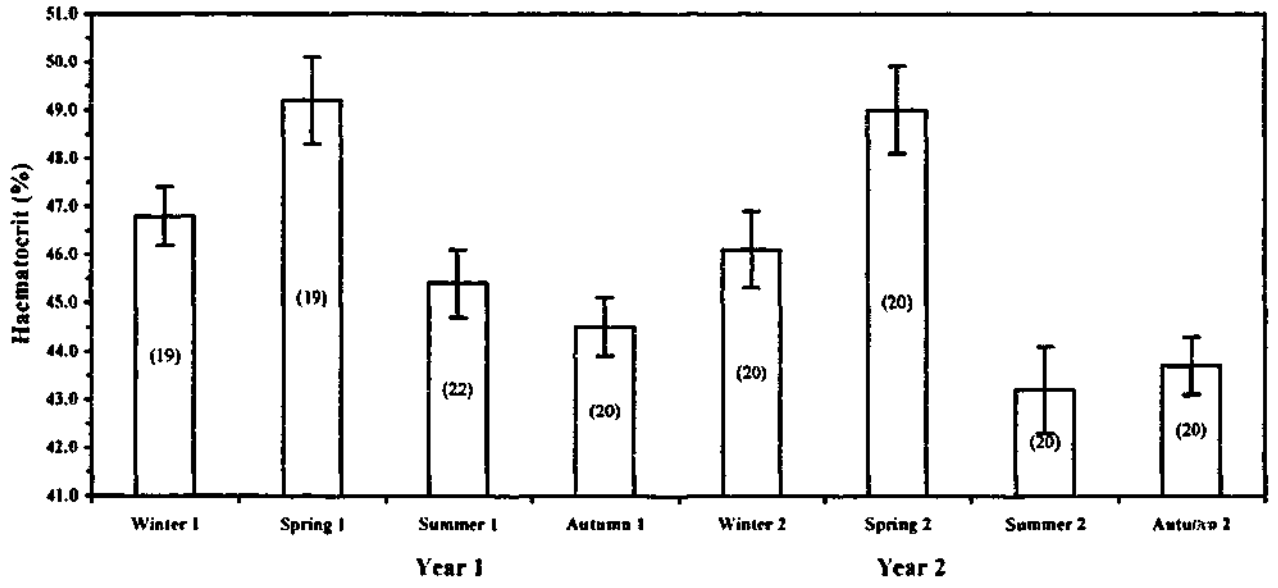
Table 2.4. f-ratios and probability levels from a series of one-factor *ANOVA* s examining variation in the *Hct, Hb* and *Rbc* count of male and female fairy-wrens.

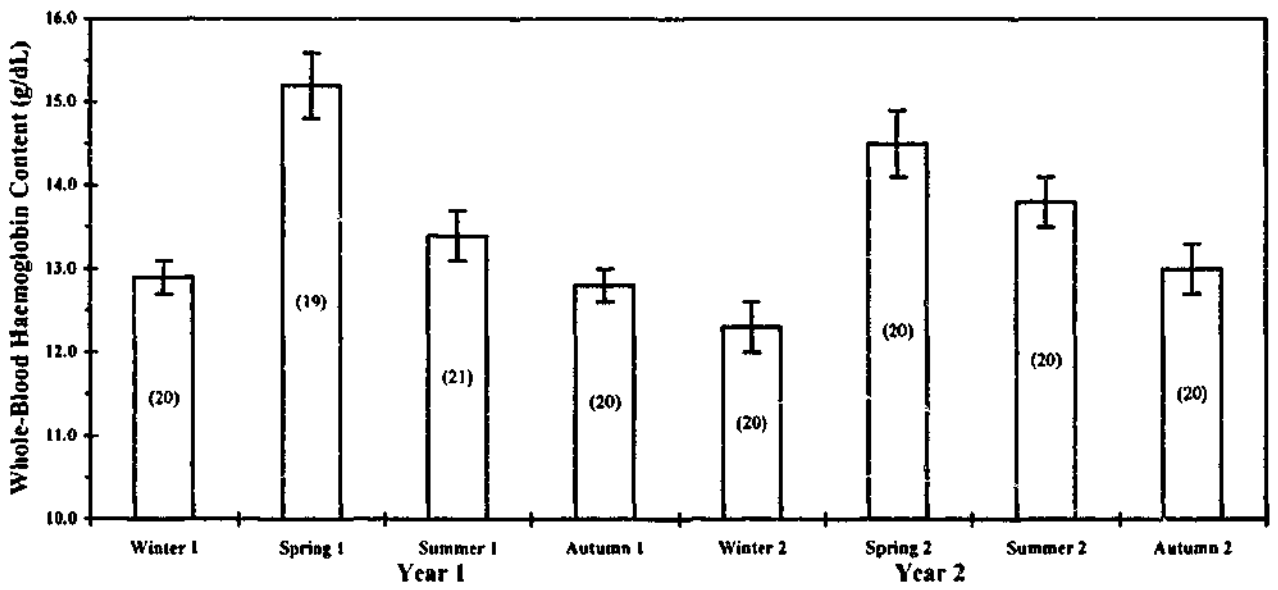
FACTORS	SEX (male / female)		
	f-ratio	probability	degrees of freedom
SPRING			
Hct	1.087	0.304	38
Hb	2.711	0.108	38
Rbc	1.924	0.174	38
SUMMER			
Hct	**4.085**	**0.020**	41
Hb	4.091	0.280	40
Rbc	4.098	0.325	39
AUTUMN			
Hct	4.098	0.526	39
Hb	4.098	0.805	39
Rbc	4.098	0.965	39
WINTER			
Hct	4.121	0.357	36
Hb	4.130	0.293	35
Rbc	**4.113**	**0.006**	37

Table 2.5. f-ratios and probability levels from a series of one-factor *ANOVA*s examining the existence of a functional relationship between body mass and each of the blood parameters measured.
The slopes of the linear regression equations, taken from Figures 2.3 and 2.4, are compared against a slope of zero.

FACTORS	BODY MASS			
	f-ratio	probability	degrees of freedom	sample size
Hct	0.835	0.362	1	160
Hb	1.997	0.160	1	160
Rbc	1.848	0.176	1	159
MCV	0.111	0.739	1	148
MCHC	<0.001	0.994	1	150

Figure 2.1. Bar graphs showing seasonal variation in mean (± standard error) values of haematocrit, whole blood haemoglobin content and red blood cell count.
Sample sizes are given in parentheses in each column.





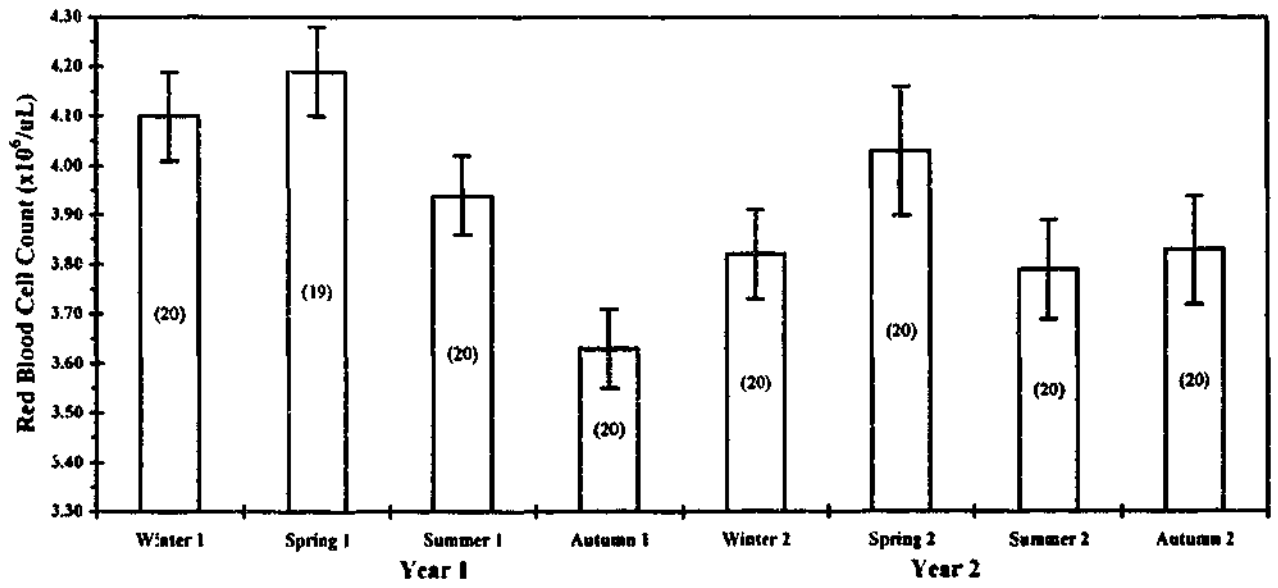


Figure 2.2. Bar graphs showing seasonal variation in mean (± standard error) values of mean cell volume and mean cell haemoglobin.
Sample sizes are given in parentheses in each column.

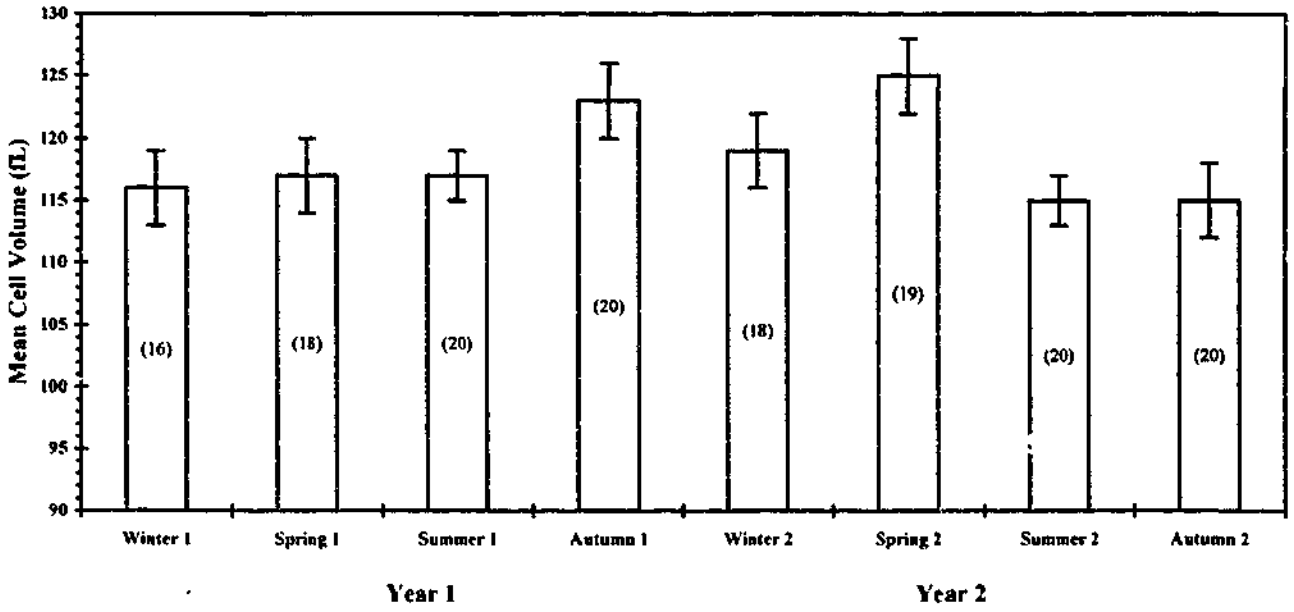
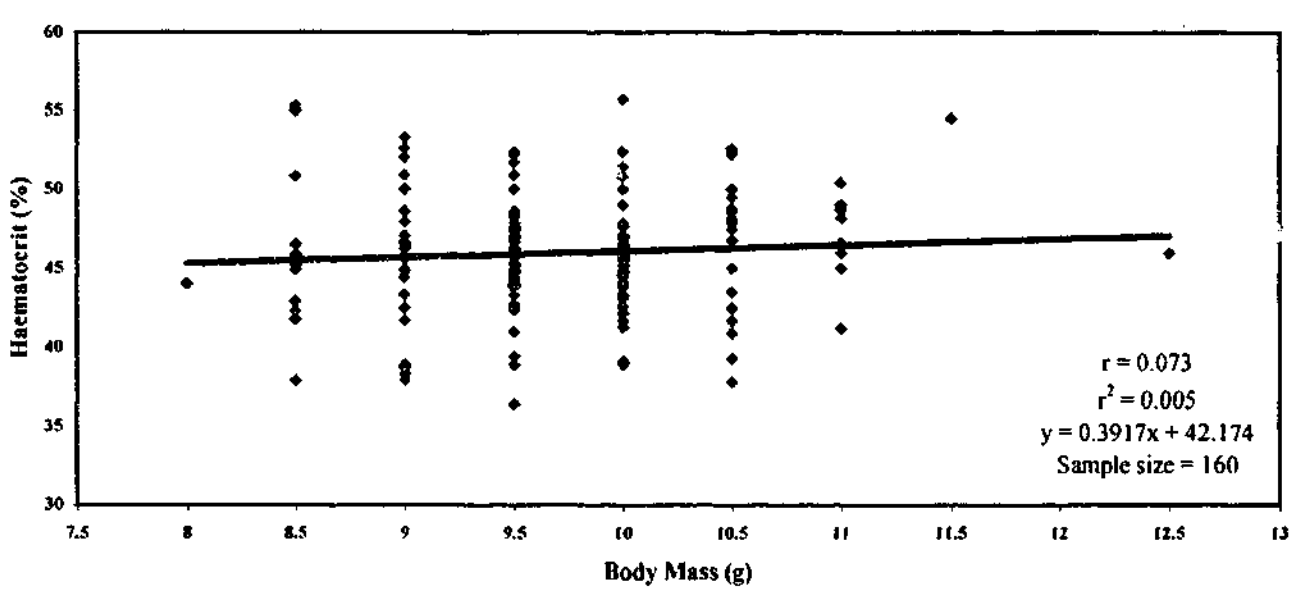
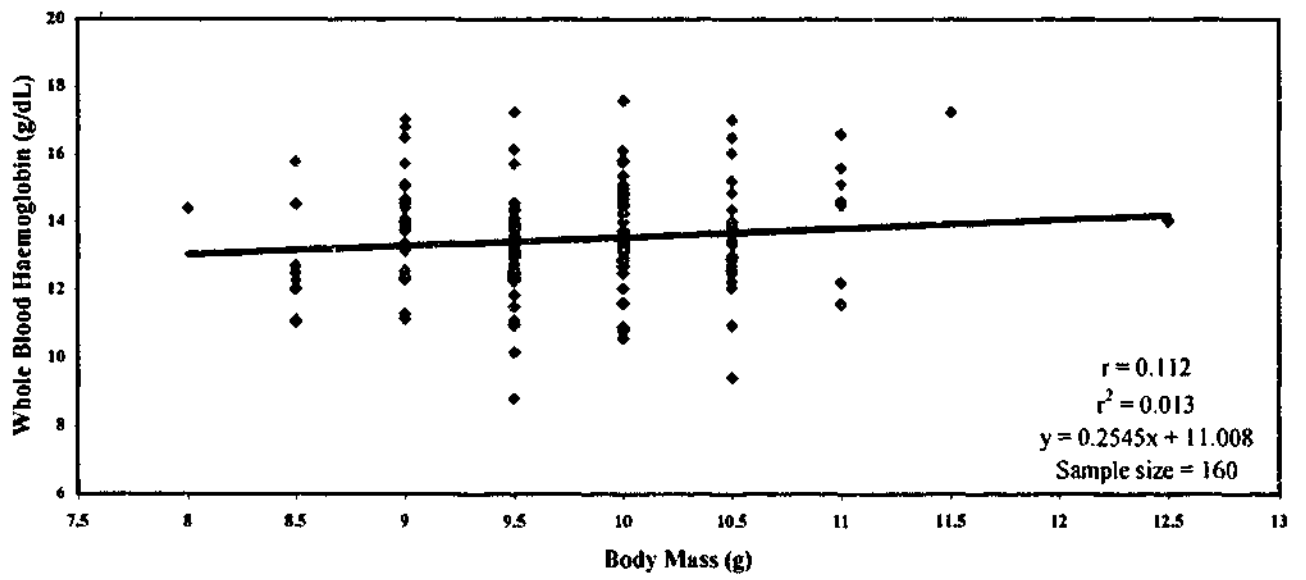


Figure 2.3. Scatter diagrams showing the relationship between body mass and haematocrit, whole blood haemoglobin and red blood cell count. Pearson correlation coefficient (r), r^2, the least squares linear regression equation and sample size in bottom right corner of each graph.





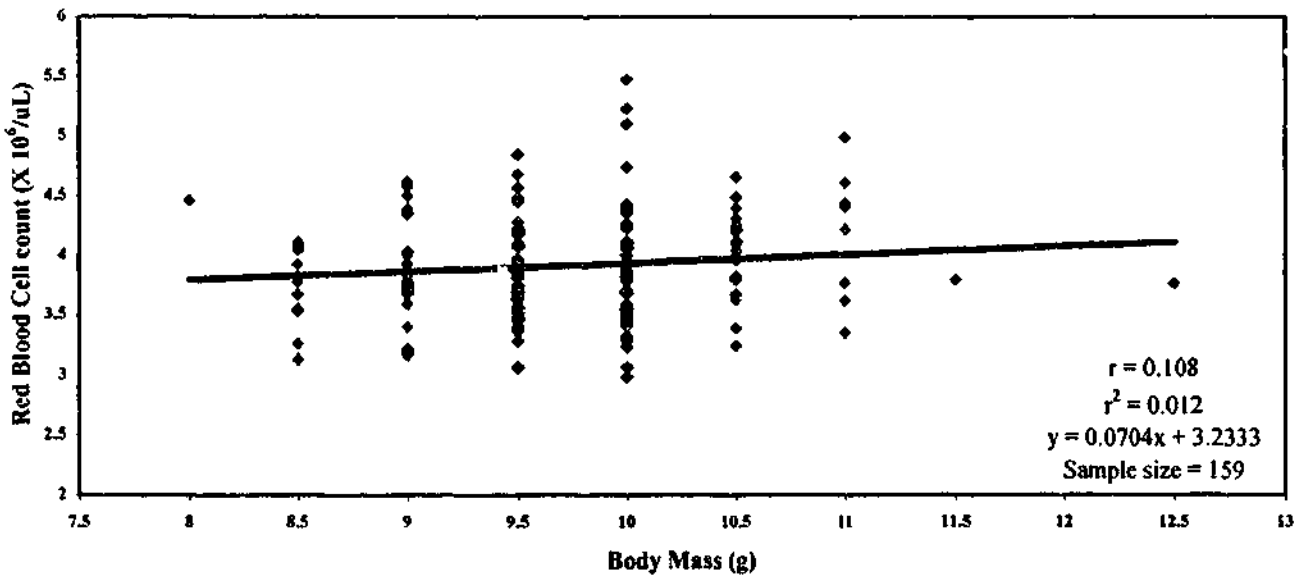
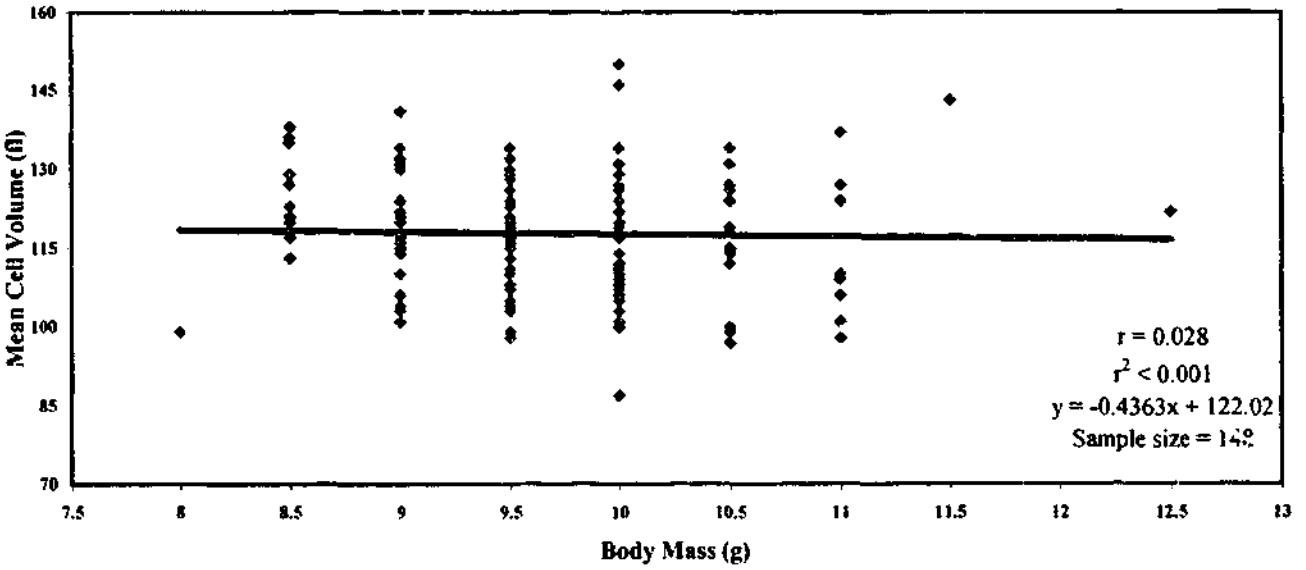


Figure 2.4. Scatter diagrams showing the relationship between body mass and mean cell volume and mean cell haemoglobin.
Pearson correlation coefficient (r), r^2, the least squares linear regression equation and sample size in bottom right corner of each graph.



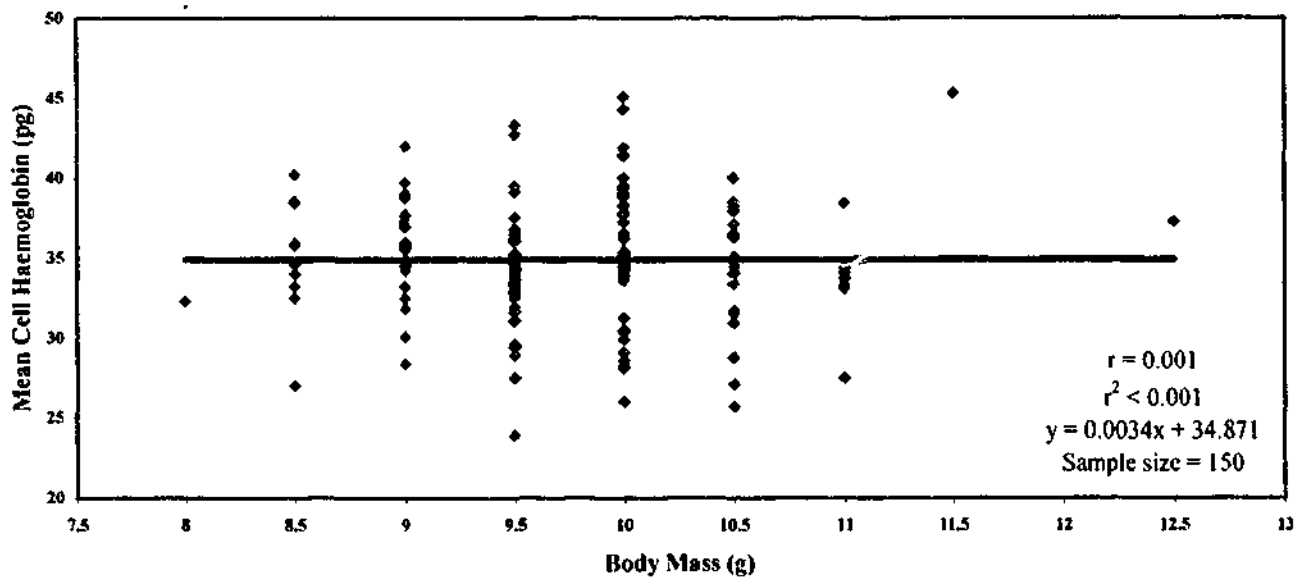
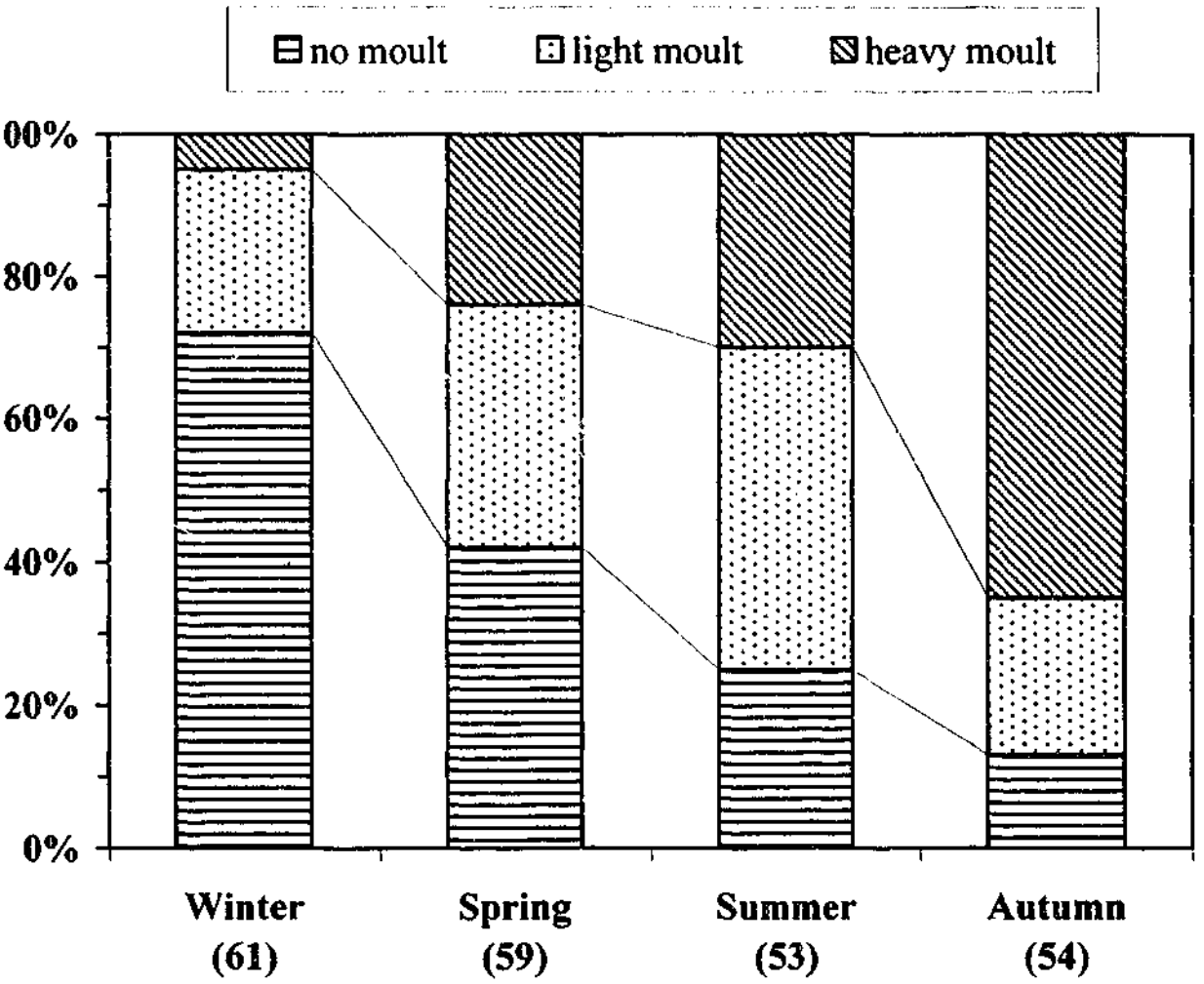


Figure 2.5 Seasonality of moult in trapped fairy-wrens showing relative proportion of 'no moult', 'light moult' and 'heavy moult' in each season.
Criteria for light and heavy moult are defined in Methods.
Sample sizes are given in parentheses under each season.



DISCUSSION

WINTER FATTENING IN RESPONSE TO COLD

Winter fattening in small passerines

Winter fattening is a method employed by many small, overwintering bird species in the North Temperate Zone because it helps to combat cold conditions. The change usually involves a significant increase in body mass due in part to increased body fat (lipids) deposits (Blem, 1990). Body mass may increase by more than 30% from summer to winter in some small passerines in the temperate zone, such as the American goldfinch, where up to 37% of the mass gain is attributed to lipid deposits (Carey *et al.*, 1978). The Dark-eyed junco was also recorded as gaining 9% in body mass in winter, 42% of which was due to elevated lipid stores (Swanson, 1991).

The lipids are mainly stored in the abdomen and beneath the skin and this involves an increase in the fat content of the cells, not an increased number of cells (Blem, 1990). The subcutaneous fat is usually deposited first and used last, possibly indicating that it also has a role as insulation (Blem, 1976). However, the main use for lipid depots is as the major metabolic energy reserve. Fat storage in birds requires behavioural, structural, physiological and biochemical changes, each of which has an energetic cost (Blem, 1990).

There are relatively few published studies systematically examining seasonal weight changes of birds in the South Temperate Zone, and even fewer that have shown that significant seasonal weight changes occur. Breuer (1992) found no winter to summer body weight change in Superb fairy-wrens or in other small passerines, including Brown thornbills, White-browed scrubwrens (*Sericornis frontalis*), Eastern yellow robins or Bell miners (*Manorina melanophrys*), in Southern Victoria. Only one species in her study, the Red-browed finch, showed a small, but significant, 3% weight increase in winter compared to summer, but it was concluded that such a small increase was most likely not due to fat deposition.

Silvereyes from Margaret River (Western Australia) showed no significant weight difference between winter months and other times of the year (Rooke *et al.*, 1986). Similarly, the body mass of winter-acclimatised silvereyes from Armidale (New South Wales) did not differ significantly from that of silvereyes in summer (Maddocks and Geiser, 2000)

In the present study, the body mass of male and female fairy-wrens differed significantly, but neither sex exhibited seasonal changes in mass. The average weight of male birds in each season was 9.9 to 10.1g and that of females 9.3 to 9.6g; these weights are comparable with those reported by Rowley and Russell (1997) for fairy-wrens from south-east Australia.

How much fat to store?

Winter lipid storage in small birds from the North Temperate Zone is not usually extensive enough to fuel survival for prolonged periods of time (Blem, 1976). For example, it has been estimated that the Dark-eyed junco in Oregon (U.S.A.) accumulates only enough fat each day to survive one night and part of the following day, approximately 13 hrs (Swanson, 1991), although winter-acclimatised juncos from the colder regions of Indiana and Ohio (U.S.A.) have estimated fasting capacities of 36 and 63 hours, respectively (Stuebe and Ketterson, 1982; Ketterson and Nolan, 1978). This indicates that fasting endurance and lipid storage in winter-acclimatised juncos may be adjusted, depending on the severity of the winter condition they encounter. In fact, there are numerous studies correlating the magnitude of lipid reserves in resident small birds in the North Temperate Zone with a suite of environmental variables, with strong indications that temperature is an ultimate, as well as a proximate, factor effecting winter fattening (see Blem and Shelor, 1986; Rogers, 1995).

Many of the small passerines studied in the North Temperate Zone experience relatively severe winter conditions in comparison to their southern counterparts; for example the mean daily maximum temperatures they encounter are often below 4°C (see for example: Swanson, 1990b). At Braeside Park, the mean overnight minimum and daily maximum temperatures in winter were 6.4°C and

14.1°C, respectively, with a mean of only 10.5 days over the 3 months of winter on which the overnight temperature fell below 2°C, see Chapter 1: General Methods. Despite the occasional low overnight temperature, foraging for food during the day is seldom prevented by inclement weather conditions, such as snow cover. In addition, there is a constant supply of flowing water through a creek to a catchment which holds water all year around. The mild ambient temperature regime of the study area indicates that if small passerines were fat-loading during the day, they would need to accumulate only enough fat for overnight survival, ie. up to about a 14 hours supply.

Seasonal body mass constancy in fairy-wrens

The extremely high lipid levels attained by some pre-migratory birds tend to indicate that winter fat storage in similarly sized, resident passerines is probably below maximum capacity (Blem, 1990). This is most likely due to the selective disadvantage of gaining and carrying extra fat, which is not offset by the benefits of the extra energy reserve (Blem, 1976). Comparative studies on small birds overwintering in a temperate climate indicate that a trade-off between predation risk and risk of starvation exists, resulting in an optimal body mass (Rogers, 1987). A trade-off that increases fat reserves can lead to higher survival in severe winter weather than would occur at minimum fat levels, but it would also increase exposure to predators while foraging and hence the risk of being preyed upon (Rogers and Smith, 1993). Moreover, other factors influence body fat levels, including the energetic costs associated with flight (Moreno, 1989; Freed, 1981) and the loss of agility when carrying extra fat levels (Blem, 1990).

It is possible that extensive fat deposition in fairy-wrens beyond their immediate metabolic needs might jeopardise the fitness of the bird through increased wing-loading. Any excess weight would presumably make flying more energetically expensive and would also restrict the agility and speed of the fairy-wren, and thus the bird's ability to escape predation. Further, the extra foraging required to accumulate more fat would increase exposure to predators seen at the study site, including predatory birds, such as Brown goshawks (*Accipiter fasciatus*),

Laughing kookaburras (*Dacelo novaeguineae*) and Grey butcherbirds (*Cracticus torquatus*).

Chan's (1995) body composition study on silvereyes found that the late-afternoon body mass of resident birds in the sample was not significantly different from that of migrants. However, there was a diurnal increase in lipid content with both resident and migrant birds gaining approximately 0.4g of fat (8% of live body mass) through the day. Complications that may have affected the determination of seasonal body mass changes in the present study include this daily fat-loading cycle. The majority of fairy-wrens were trapped and weighed between early and late morning, whereas fat level and hence body mass, should be at their highest in mid-to late afternoon in preparation for the overnight fast (Chan, 1995; Rooke *et al.*, 1986). Experimentally, using a balance accurate to 0.5g (approximately 5% of total body mass) may have been too coarse to detect small weight changes. Further, interpretation of body mass measurements in small birds as an indication of fat content may also be limited by the possibility of significant variation in other body components, such as water (Rooke *et al.*, 1986) and protein (Carey *et al.*, 1978) and in the amount of food in the digestive tract (Evans, 1969). Mean body mass of silvereyes from Margaret River was closely correlated with mean body fat content for six consecutive months, but to body water content for the remaining six months (Rooke *et al.*, 1986).

It is difficult to ascertain if diurnal fat-loading is occurring in winter-acclimatised fairy-wrens based solely on body mass changes. Inaccuracies may occur due to labile non-lipid components of body mass including the contents of the digestive tract, water balance and protein reserves (Blem, 1990). In Chapter 3, a more definitive assessment of seasonal fat loading is made, based on the total body fat content of fairy-wrens measured directly by carcass analysis on birds captured in the morning.

SEASONAL CHANGES IN BLOOD PARAMETERS

Blood oxygen transport

Haemoglobin is a red-pigmented, oxygen-binding protein found in the red blood cells of all higher vertebrates (Dacie and Lewis, 1991). Each haemoglobin molecule has a tetrameric structure which specifically binds with high affinity to oxygen molecules, transporting them from the lungs and unloading them at the relatively high oxygen tension found in tissues, such as muscles (Dacie and Lewis, 1991). Efficiencies in the vascular system, which can lead to enhanced oxygen supply to tissues include:

(1) an increased rate of oxygen delivery, and

(2) an increased oxygen unloading capacity

Oxygen delivery to the tissues is directly determined by the blood *OCC*. Parameters indicative of *OCC* include *Hct*, *Hb* and *Rbc* numbers and size, all of which are interrelated. An increase in *Hb* can be achieved by an increase in the haemoglobin content of individual *Rbc* or alternatively by an increase in the number of *Rbc* per unit volume of blood. An increase in *Rbc* numbers and / or size will lead to a higher *Hct*, assuming that plasma volume remains the same.

While an increase in *Hct* would lead to the delivery of more oxygen, the flow rate of the blood would be reduced as the increased number of red blood cells would lead to an increase in blood viscosity (Sturkie and Griminger, 1976; Promislow, 1991). Over time, this increase may jeopardise both cardiac and pulmonary functions by inducing right ventricular hypertrophy and pulmonary arterial hypertension (Morton, 1994). Consequently, a compromise is thought to be achieved in which there are sufficient red blood cells for efficient oxygen delivery, but not so many or of such size as to reduce the blood flow rate, thus leading to an optimal *Hct* (Wells and Baldwin, 1990).

Since *Hct* is a measure of relative volume and *Hb* and *Rbc* numbers are expressed in terms of blood volume, all these parameters will change in response to changes in the plasma compartment of the blood. A blood osmolality change due to

ever-varying levels of certain circulating hormones and proteins may also alter these parameters (Burtis and Ashwood, 1994).

Blood parameters in fairy-wrens

Jones and Johansen (1972) reported an average *Hct* of about 39.5%, an *Rbc* count of between 2.5 to 3.5 $\times 10^6$ / μL and a *Hb* from 9 to 21g / dL, for a wide range of passerines and non-passerines of varying body mass. Given the higher metabolic intensity of small passerines due to their small body size and high level of flight activity, there is a general tendency to have relatively high *Hct* and *Hb* in these birds (Palomeque *et al.*, 1980).

A comparison of blood parameters of fairy-wrens from this study with other wild-trapped, low altitude passerines of similar body mass shows similar values (Table 2.6). The mean seasonal values for *Hct* and *Hb* in the studied fairy-wrens ranged between 44.1 and 49.1% and 12.6 and 14.8g / dL, respectively. The *Rbc* count ranged from 3.73 to 4.11 $\times 10^6$ / μL, slightly higher, but still close to the ranges previously documented for other passerines. The higher variability in the red blood cell count across the studies may be explained by the methodology. Counting chambers, used for manual cell counts, vary among investigations and have improved over time to give more accurate and reliable counts. Importantly, in the present study the same technique and materials were used through this entire study, minimising variability when comparing the *Rbc* count in each season.

Table 2.6. A comparison of measured blood parameters of fairy-wrens in this study with those of other small passerines. All species were wild trapped, low altitude adult birds from both the North and South Temperate Zones, weighing between 7.5 and 13.5g. (^{S,W}: indicates the range of values recorded for summer and winter, ^{S}: indicates the value recorded for summer only; sample size is given in parentheses.)

Species	*Hct* (%)	*Hb* (g / dL)	*Rbc* Count $\times 10^6$ / µL
Superb Fairy-wren[a]	**44.1–49.1 (160)**	**12.6–14.8 (160)**	**3.73–4.11 (159)**
Superb Fairy-wren [b] (*Malurus cyaneus*)	44.1–45.8 ^{S,W} (60)	14.2–14.8 (60)	2.11–3.62 (60)
Red-browed finch [b] (*Neochmia temporalis*)	49.5–50.4 ^{S,W} (60)	12.6–12.7 (60)	2.32–3.51 (60)
Blackcap [c] (*Sylvia atricapila*)	44 ^{S,W} (8)	16.5 (8)	4.73 (8)
Pine siskin [c] (*Carduelis spinus*)	51 ^{S,W} (4)	17.5 (4)	5.37 (4)
American goldfinch [d] (*Spinus tristis*)	53.5–59.2 ^{S,W} (14)	16.5–17.6 (16)	3.86–4.63 (13)
Rosy finch [e] (*Leucosticte arctoa*)	52.3–58.4 ^{S,W} (15)	15.6–17.6 (17)	n/a
House finch [e] (*Carpodacus mexicanus*)	50.1 ^{S} (12)	15.2 (13)	n/a
Black-capped chickadee [d] (*Parus atricapillus*)	48.1 ^{S} (5)	12.6 (5)	3.29 (5)
Nashville warbler [d] (*Vermivora ruficapilla*)	43.1 ^{S} (5)	12.7 (5)	3.35 (5)
Audubon's warbler [d] (*Dendroica coronata*)	51.1 ^{S} (7)	14.5 (8)	3.63 (8)
Field sparrow [d] (*Spizella pusilla*)	51.0 ^{S} (9)	16.1 (9)	3.30 (6)
House sparrow [f] (*Passer domesticus*)	40.9 ^{S} (31)	11.7 (31)	4.10 (31)

[a] current study, [b] Breuer *et al.* (1995), [c] Palomeque *et al.* (1980), [d] Carey and Morton (1976)
[e] Clemens (1990), [f] Puerta *et al.* (1995)

SEASONAL CHANGES IN BLOOD *OCC* OF FAIRY-WRENS

The variations that fairy-wrens in this study showed in some blood parameters were quite marked between some seasons. These changes entailed a significant increase in *Hct* and *Hb* in spring relative to the other three seasons. *Rbc* numbers were also higher each year in spring than in autumn. The *MCV* remained constant over the four seasons, whilst *MCHC* was significantly lower in winter than in the other three seasons. These results indicate enhancement of blood *OCC* in spring and are probably a result of increased phasic activity rather than seasonal acclimatisation.

The main erythropoietic stimuli in birds is believed to be hypoxia and increased metabolic demands, such as those imposed by thermoregulation or long-distance flying during migration (Morton, 1994). Since the fairy-wrens in this study were not exposed to the hypoxic conditions found at high altitude or in burrows, and are not migrants, two questions need to be addressed:

(1) Why does the cold of winter have no significant effect on blood *OCC*?

(2) Why was blood *OCC* enhanced in spring?

Blood OCC in winter

The increased cold tolerance shown by some small birds wintering in the North Temperate Zone depends primarily upon metabolic adjustments resulting in increased heat production (Saarela *et al.*, 1989). Typically, the seasonal variation in thermogenic capacity of small passerines results from winter-acclimatised birds being capable of sustaining heat production for longer and most likely more efficiently than summer-acclimatised birds (Dawson and Carey, 1976). The process believed to be responsible for this increased thermogenic capacity involves enhancement of shivering thermogenesis (see Calder and King, 1974; Hohtola and Stevens, 1986). It is reasoned that sustained shivering due to cold stress leads to increased demand for oxygen in the muscles involved, the pectoralis major and, to a lesser extent, the pectoralis minor (del Hoyo *et al.*, 1992). It follows then, that

adjustments leading to increased efficiency in the vascular oxygen transport system may be involved.

Winter enhancement of the vascular oxygen transport system has been recorded for a handful of non-migratory bird species in the North Temperate Zone. A summer versus winter comparison of *Hct* and *Hb* in dark-eyed juncos in Oregon, a region with moderate winter weather conditions, revealed increases of 11.1% and 8.6%, respectively, in these parameters (Swanson, 1990b). Rosy finches in California (U.S.A.) exhibited a similar pattern, with mean increases of 11.7% and 12.8% in winter *Hct* and *Hb*, respectively, in comparison with summer (Clemens, 1990). American goldfinches wintering in the more extreme conditions of Michigan (U.S.A.) were also recorded as having an 11% increase in mean *Hct* levels relative to summer values (Carey and Morton, 1976). In each of these studies, it is apparent that increased oxygen demands in the winter-acclimatised birds are met, in part, by an increased *OCC* of the blood.

There are fewer studies detailing seasonal blood parameter differences in small passerines in the South Temperate Zone. Breuer *et al.* (1995) found no summer to winter changes in the *Hct* or *Hb* of four small passerine species in south-east Australia, but did record significant changes in *Rbc* number and size. Red blood cell counts in Brown thornbills, Red-browed finches, Eastern yellow robins and Superb fairy-wrens were 45-72% higher in winter than in summer, while red cell volume decreased by 40-73%. It was concluded that the smaller winter *Rbc*, with their greater surface area to volume ratio and consequently shorter diffusion pathway, should enhance blood oxygen transport efficiency. These results and the conclusions drawn from them contrast sharply with those obtained in this study. A comparison of the blood parameters of fairy-wrens that were measured directly, summarised in Table 2.7, shows the most significant difference is the *Rbc* count in summer birds. While a winter / summer (and autumn) comparison of *Rbc* numbers in this study reveal no significant change, Breuer *et al.* reported a 72% increase in the winter count relative to summer. Consequently, *Rbc* size, which was not measured directly, was calculated to show a 42% reduction in cell size. Breuer *et al.* logically argued that *Rbc* numbers increased in winter to 3.62×10^6 / μL based on

the fact it was higher than the summer count (2.11×10^6 / μL). Given Breuer *et al.*'s winter *Rbc* count is within the range of this study (3.56 to 4.28×10^6 / μL) it is possible the summer *Rbc* numbers reflect a decrease of 42%, rather than an increase in winter.

Table 2.7. A comparison of measured blood parameters of fairy-wrens between this study and that of Breuer *et al.* (1995). Blood values are mean ± standard error, and sample size is given in parentheses. Mean maximum and minimum temperatures (°C) for each site are given for winter and summer.

Season		*Hct* (%)	*Hb* (g / dL)	*Rbc* count $\times 10^6$ / μL
Winter				
(14.1 / 6.4)	Current study (Braeside)	46.5 ± 0.5 (39)	12.6 ± 0.2 (40)	3.96 ± 0.06 (40)
(10.7 / 6)	Breuer *et al.* (Healesville)	44.1 ± 0.8 (30)	14.8 ± 0.4 (30)	3.62 ± 0.14 (30)
Summer				
(25.1 / 13.5)	Current study (Braeside)	44.6 ± 0.6 (42)	13.6 ± 0.2 (41)	**3.87 ± 0.07 (40)**
(29.1 / 15)	Breuer *et al.* (Healesville)	45.8 ± 0.7 (30)	14.2 ± 0.3 (30)	**2.11 ± 0.05 (30)**

It is difficult to explain the discrepancy in the summer *Rbc* count of the two studies, particularly as the study sites are geographically close, being approximately 60km apart, with similar mean daily maximal and minimal temperatures in both winter and summer. It may be possible that *Rbc* numbers are more labile than other blood parameters within fairy-wren populations. This is difficult to determine due

to the lack of comparative intraspecific studies to draw on. Further blood work covering all seasons at Healesville may be helpful in determining the lability of blood parameters within bird species.

Apart from the limited number of published studies cited, a meaningful comparison of results from the present study with those of other investigations is difficult for several reasons, notably small sample sizes (Clemens, 1990), inadequate statistical analysis (including the grouping of data into seasons) (Rooke *et al.*, 1986, deGraw *et al.*, 1979), the use of captive birds (deGraw *et al.*, 1979) and experimental design (no data collected in spring or autumn) (Clemens, 1990, Swanson 1990b, Breuer *et al.*, 1995, Carey and Morton, 1976).

A summer to winter comparison of fairy-wren blood parameters in the present study shows that *Hct* and *Rbc* count were not statistically different, while *Hb* was actually lower in winter than in summer, due to a lower mean cell *Hb* content. This may suggest that the effect of cold stress has no apparent effect on the vascular oxygen transport system of fairy-wrens, a finding in direct contrast to the studies outlined above. A possible explanation is that while muscle activity may increase when fairy-wrens are shivering, oxygen supply to the muscle may not be limiting, so that an increase in vascular oxygen transport efficiency would not be of any benefit. It is also possible that other aspects of the metabolic process involved in sustained shivering are adjusted instead, such as the mobilisation and efficient breakdown of stored energy within the shivering muscles. This is explored in detail further in Chapter 3.

Enhancement of blood OCC in spring

The main muscle used to power flight in birds is the pectoralis, which, on average, comprises about 15.5% of total mass (del Hoyo *et al.*, 1992). During sustained flight, the rate of oxygen consumption by birds is among the highest reported for vertebrates, being up to 14 × higher than resting rates, although factors of 5-10 × are more usual (Brackenbury, 1984).

Carey and Morton (1976) suggested that muscular activity during flight might stimulate *Hb* production or *Hct* maintenance. This was based on the fact that wild-

captured birds regularly have higher *Hb* than laboratory birds. For example, Trust's (1968) (see Carey and Morton, 1976) study on Horned larks (*Eremophila alpestris*) showed this trend.

Blood work on White storks (*Ciconia ciconia*) showed that *Hct* and *Hb* increased with age in nestlings, but increased still further in fledglings until the bird was a fully-developed flier (Puerta *et al.*, 1989; Alonso *et al.*, 1991). Similarly, fledgling Common cranes (*Grus grus*) do not attain the same *Hct*, *Hb* and *Rbc* numbers as adults until after their first migratory trip (Puerta *et al.*, 1990). Accordingly, these authors suggested that the increase in the levels of these blood parameters, leading to an increased *OCC*, was probably related to the higher oxygen demands of flying, particularly during migration.

During the rapid vernal migration that White-crowned sparrows (*Zonotrichia leucophrys*) undertake annually, Morton (1994) found that the bird's *Hct* rose significantly, with newly-arrived individuals having the highest *Hct*. These values declined thereafter until the end of postnuptial moult, increasing again prior to their autumnal return migration. A similar pattern of blood parameter changes in White-crowned sparrows from a different region had previously been recorded by deGraw *et al.* (1979). Similarly, long distance migrants, such as the Bar-tailed godwit (*Limosa lapponica*) and the Short-tailed shearwater (*Puffinus tenuirostris*), show increasing *Hct* and *Hb* in the weeks leading up to departure on migration (Piersma and Everaarts, 1996 and Davey *et al.*, 2000).

Superb fairy-wrens are highly sociable, generally living in a close-knit, extended family group of 5-12 birds, occupying a territory year-round. Groups typically consist of an adult pair, their young of the season and sometimes additional males (Rowley and Russell, 1997). Outside the breeding period, activity is low, particularly in summer and autumn, territory borders are relaxed and time is spent foraging in the mornings and afternoons, whilst during the midday hours the group switch to mutual preening and resting (Rowley and Russell, 1997). In contrast, spring induces a marked increase in the activities of all group members.

Breeding of fairy-wrens in south-east Victoria is highly seasonal, occurring mostly in spring, starting in September and continuing through to December

(Tidemann and Marples, 1987). The breeding female is responsible for nest site selection, nest building and incubation, and the breeding male for defending the established breeding territory, which may be up to one hectare in area (Serventy, 1982). Breeding males may also be responsible for feeding females on the nest or escorting them to a foraging site. While all the 'helper' members of the group feed the hatchlings, the female re-nests, often producing up to 3 or 4 broods in a season.

Given the high energetic costs involved with breeding (see Merila and Wiggins, 1997; Carey, 1996; Williams, 1996; Weathers, 1996) and the associated increase in the amount of flying, it seems quite likely that the increase in *Hct* and *Hb* observed in spring in this study assists in meeting the energetic demands of increased flight activity. Whilst the increase in flight-related activities may not be as dramatic as that in fledglings preparing for flight or adult birds undertaking extensive migration, there may still be a need for enhancement of blood oxygen delivery through increased *OCC*.

OTHER FACTORS THAT MAY INFLUENCE BLOOD *OCC* IN FAIRY-WRENS

The oxygen-carrying capacity of bird blood may be influenced by a variety of underlying causes. Ideally, to study the effect that seasonal changes have on blood oxygen transport, the other potentially influential factors need to be considered. The following factors and their likely effect are discussed below: moult, hydration status, age, stress levels associated with capture and handling and sex-related factors, including body mass and reproductive hormones.

Effects of moult on OCC

Moulting has been shown to impose energy demands on birds of 6-26% of daily energy expenditure (Murphy, 1996; Whittow, 1976). The process of renewing body plumage is partly hormonally controlled and is usually integrated into the bird's annual cycle, so that the energy needs and physiological alterations required

when moulting do not interfere with such activities as breeding or migration (del Hoyo *et al.*, 1992).

Changes in blood parameters and constituents during moult have been recorded for small passerines, such as White-crowned sparrows (Chilgren and deGraw, 1977; deGraw *et al.*, 1979) and Harris' sparrows (*Zonotrichia querula*) (deGraw and Kern, 1985). Chilgren and deGraw (1977) found that during the postnuptial moult of captive White-crowned sparrows the birds *Hct* decreased by 13%, while total body water increased dramatically. They hypothesised that the decline in *Hct* during moult was due to a concurrent increase in blood volume, or more specifically in plasma volume. It is known that an increase in body water content is associated with the expansion of the circulation needed for nurturing and growing feathers and pulp (Murphy, 1996). Further work on captive Harris' sparrows (deGraw and Kern, 1985) involving direct measurement of whole blood and plasma volumes confirmed that reductions in *Hct* (by 12-14%), *Rbc* number, plasma protein levels and plasma osmolality during postnuptial moult were due to an expansion of plasma and blood volume by 42% and 26%, respectively.

Fairy-wrens in this study moulted mainly in autumn, when 65% of the birds were in heavy moult, with significant feather replacement occurring in three or more areas of their body. However, *OCC* of the blood did not increase at this time. This suggests that whilst moulting may impose high energy demands on fairy-wrens, meeting these costs does not require an increase in oxygen transport rate. Moreover, in spring when 76% of birds captured had little or no moult, blood *OCC* was at its maximum.

Similarly to the situation in both of the above-mentioned sparrow species, the postnuptial moult of fairy-wrens in autumn in this study coincided with low mean seasonal values for both *Hct* and the *Rbc* count, although these values were not significantly lower than other seasons.

Effects of dehydration on blood OCC

Changes in plasma volume have a direct influence on the blood parameter values measured, whereby a decrease in plasma volume will automatically lead to

increases in *Hct*, *Hb* and *Rbc* numbers, as all are measured relative to volume. Conversely, an increase in plasma volume will lead to decreased levels of these parameters.

During extended migratory flight, respiratory water loss may exceed metabolic water production by about 30% (Berger and Hart, 1974), depending on the ambient temperatures encountered. Morton (1994) estimated that during migration, mountain White-crowned sparrows would lose 8% of their body mass during one overnight migration flight for this reason.

Given that fairy-wrens are sedentary, occupy a permanent territory (Blakers *et al.*, 1985) and that the study site had a constant supply of drinking water and relatively moderate daily maximum temperatures (see Chapter 1: General Method), the hydration status of the birds is most unlikely to have changed in spring or any other season. However, as neither total body water nor total blood and plasma volumes were measured in this study, dehydration cannot be unequivocally ruled out as a factor contributing to high spring *Hct* levels.

Effects of age on blood OCC

Intraspecific age-related differences in blood parameters have been documented for both non-passerines and passerines. Work on the blood of wild nestling Noisy miners (*Manorina melanocephala*) indicates both a change in the shape of *Rbc* from spherical to a more ellipsoidal form and a continuous 2.4-fold increase in the oxygen-carrying capacity of the blood (Bolton, 1996). Young miners fledge quickly in a relatively immature state, with relatively poor flight capacity and a long dependency on parental care, and so it is not surprising that these fledglings do not have adult levels of blood oxygen transport (Bolton *et al.*, 1999). In contrast, nestling Short-tailed shearwaters exhibit discontinuous and markedly variable rates of change in blood oxygen transport parameters during their long development stage (Arnold *et al.*, 1999). Additionally, fledglings have attained adult levels of blood oxygen transport upon fledging, possibly explaining their relatively strong flight capabilities (Arnold *et al.*, 1999).

In this study, to avoid the probable confusion the fairy-wrens age would have on the blood parameters, data was only collected from apparently healthy adult birds. Immature birds were distinguished from the similarly-coloured adult females by the lack of greenish blue wash in their brown tail as described in Chapter 1: General Methods. Also, on leaving their nest, fledglings are very vulnerable and generally spend the next several weeks hiding in dense shrubby cover, where they are fed by their parents (Serventy, 1982), reducing the likelihood of capturing dependent young birds. Nestlings were also avoided in this study.

Effects of stress during capture on blood OCC

The intensity of stress to birds, particularly wild-trapped individuals, when performing experimental studies needs due consideration when interpreting the resulting data. During handling and blood sampling, humoral indices of stress, such as blood catecholamines, corticosterone and lactate, can increase several fold quickly, in an outwardly-appearing, quiet, unstressed bird (Le Maho *et al.*, 1992).

The effects of stress or stress-related hormones on blood parameters of birds in the short term (< 30 minutes) are unclear. In this study, routine handling and blood sampling of fairy-wrens after capture took approximately 15-20 minutes. That the procedure was applied in a similar fashion to each fairy-wren should lead to a broadly similar stress response from each bird. Given that the main objective of this study is a relative comparison among the different seasons, the effect of stress-induced changes to any of the blood parameters, if it does occur, should be minimised.

Effects of body mass size on blood OCC

On a broad scale, larger mammals have been shown to have fewer and larger red blood cells than smaller mammals due to the differing oxygen requirements dictated by their mass-specific metabolic rates (Promislow, 1991). A similar trend can be found in birds (Hartman and Lessler, 1963). In this study, the body mass of over 160 fairy-wrens of both sexes was measured and found to range from 8 - 12.5g. Despite the relatively wide spread, body mass was not correlated with any of the

blood parameters measured. This suggests that variation amongst the blood parameters was not attributable to the physical size of the bird. Similarly, Breuer (1992) also found no significant relationship between these blood parameters and the body mass of fairy-wrens, or in the three other species studied, the Brown thornbill, the Red-browed finch and the Eastern yellow robin, which collectively ranged in weight from 6.3 to 20.8g.

Effects of reproductive hormones on blood OCC

A unique aspect of most birds is the seasonal development of the gonads and the reproductive tract. Cues triggering breeding activity, such as an increase in the photoperiod, can lead to a 200-300 times increase in the testis size of males and the size of the ovaries and oviducts of females (del Hoyo *et al.*, 1992). Physiologically, these changes are controlled by a complex series of hormones that circulate around the body in the vascular system. Two of the hormones, Follicle Stimulating Hormone and Luteinizing Hormone, are both responsible for seasonal growth of the gonads. In turn, gonadal growth induces the production of the sex hormones testosterone and oestrogen in the testes and ovaries, respectively.

Extensive studies on adult quail and chickens have shown that testosterone generally has an erythropoietic effect, enhancing *Rbc* numbers and thus *Hct*. In contrast, oestrogen generally has a depressing effect, resulting in the lowering of *Hct* levels (Sturkie and Griminger, 1976). However, studies of the effects of the major sex hormones on the blood parameters influencing the *OCC* of small passerines are limited and the trends sometimes conflicting.

Studies on captive male House sparrows (*Passer domesticus*) by Puerta *et al.* (1995) found that testosterone treatment of adult birds had no significant effect on any red blood cell parameters, while the same treatment on young males caused an increase in both the *Hb* and *Rbc* numbers. A study by deGraw *et al.* (1979) on White-crowned sparrows found a sharp decline in *Hct* in the breeding months in both captive and wild individuals. Further, other laboratory studies on the same species indicated that oestrogen and testosterone both reduced the *Hct*, although the

authors concluded that changes in the levels of gonadal steroids were unlikely to be solely responsible for these changes (Kern *et al.*, 1972).

Male and female White-crowned sparrows of the subalpine meadows of California were found to have their highest levels of testosterone and oestrogen, respectively, during the breeding period (Morton *et al.*, 1990; Wingfield and Farner, 1978). Given that these sex hormones alter blood characteristics in wild birds, they might be expected to exert their maximum effect over the breeding season (Carey and Morton, 1976) and their smallest effect in the middle of the non-breeding season. Although not measured, the sex hormones testosterone and oestrogen in fairy-wrens are presumably at their lowest levels in autumn and at their highest in spring. In spring, the main breeding season for fairy-wrens, and autumn, there were no significant differences between the blood parameters of males and females. This lack of difference suggests that the effect on blood parameters of testosterone and oestrogen is the same ie. either they both have little or no effect or they are both responsible for increasing *Hct* and *Hb* in males and females in spring. Given that data on the effects of oestrogen and testosterone on the blood parameters influencing *OCC* in small passerines is relatively sparse and somewhat contradictory, it is difficult to form firm conclusions on their effects, particularly without directly measuring sex hormone levels in fairy-wrens.

FURTHER STUDY

Efficiencies in the vascular system that can lead to enhancement of oxygen supply to metabolising tissues include not only oxygen delivery but also oxygen unloading at the tissues. Oxygen unloading is determined by blood oxygen affinity, where a decreased haemoglobin oxygen affinity results in the unloading of more oxygen at the tissues. There are few investigations into seasonal trends in the blood oxygen affinity of birds, particularly passerines. This is most likely due to the complexity of the method involved (see Swanson, 1990b; Clemens, 1990;

Palomeque *et al.*, 1980) and possibly the apparent intraspecific variability within some of the more widely-studied species (Lutz, 1980).

Despite these difficulties, a study of Dark-eyed Juncos found that winter-acclimatised birds had an increased blood *OCC*, while blood oxygen affinity remained unchanged (Swanson, 1990b). In contrast, Rosy finches at high altitude in California exhibited an increase in blood oxygen affinity, with little change to blood *OCC* (Clemens, 1990). This supports Lutz's (1980) contention that changes in *OCC* occur as a short-term adjustment to seasonal changes, while oxygen affinity is altered as a long-term adaptation.

Consideration could be given to the measurement of blood oxygen affinity in fairy-wrens to increase the scope of understanding of adjustments to the vascular system which lead to an enhancement of oxygen transport and delivery.

CHAPTER 3

SEASONAL CHANGES IN INSULATION, ENERGY SUBSTRATE LEVELS AND CATABOLIC CAPACITY OF FAIRY-WRENS.

INTRODUCTION

Seasonal variation in the capacity for sustained shivering in response to cold has been recorded for numerous small passerines inhabiting regions with both relatively harsh and mild winters within the North Temperate Zone (Carey *et al.*, 1989; Swanson, 1991). The nature of the seasonal change in capacity for shivering thermogenesis, resulting in increased shivering endurance in winter acclimatised birds, is not well understood, but it is known to occur through metabolic adjustments (see for example Swanson, 1990; O'Connor, 1995a).

Shivering thermogenesis in fasting birds involves moderate muscle twitching, usually for long periods of time, and hence blood glucose and, in particular, fat, are believed to be the primary energy substrates used (O'Connor, 1995b; Marsh and Dawson, 1989). The amount of body fat available to fuel shivering in passerines in the North Temperate Zone can increase dramatically in winter; for example, stored total body lipids in the Dark-eyed junco, in winter are double those in summer (Swanson, 1991). The extent of the use of glycogen as a fuel when shivering at low ambient temperatures varies among species, although its contribution appears, at best, to be minor (Marsh and Dawson, 1982; Marsh *et al.*, 1990; Dawson *et al.*, 1992). However, it is known that in maximally active muscles the demand for ATP for contraction can be so great that blood flow cannot provide oxygen and free fatty acids or glucose fast enough (Weber, 1987). Under these conditions, which may occur during burst flight or take-off, the predominant fuel source used is stored muscle glycogen (Rothe *et al.*, 1987).

Numerous studies document increased winter lipid storage in seasonally-acclimatised passerines, with some also reporting increased glycogen concentrations in winter compared to summer (see, for example, Carey *et al.*, 1978; Swanson, 1991). However, elevated energy stores alone cannot account for improved thermogenic endurance (Dawson *et al.*, 1992). There must also be an increased capacity to mobilise and catabolise the energy source. The relative activity of key enzymes in catabolic pathways involved in the breakdown of

stored energy can be used to assess seasonal changes in the metabolic capacity of particular tissues and the type of fuel used (Marsh, 1981; Yacoe *et al.*, 1982).

This section of the study of wild fairy-wrens examines seasonal variation in several biochemical parameters in order to evaluate the extent to which metabolic acclimatisation may be occurring. Analysis will include measuring total body lipids, which consist mainly of triacylglycerols, and stored glucose, in the form of glycogen present in the liver and pectoralis muscles. Changes in the capacity of the pectoralis muscles to carry out oxidation of fatty-acids and carbohydrates will also be estimated using the activity of the key metabolic enzymes citrate synthase, ß-hydroxyacyl-CoA dehydrogenase, hexokinase and phosphorylase as indicators. These enzymes were chosen for their key positions in the catabolic pathways involved in fat and glycogen / glucose breakdown and aerobic energy production (Yacoe *et al.*, 1982). Seasonal changes in each of these measured parameters will be compared, where possible with those recorded for small passerines inhabiting the more climatic extreme North Temperate Zone.

Insulatory adjustments have also been shown to assist in the winter enhancement of cold tolerance in some passerine species, such as the House finch and House sparrow, in which a 39% increase in summer feather mass compared to that in winter was recorded (Dawson *et al.*, 1983; Barnett, 1970). In the present study, the dry mass of contour plumage of fairy-wrens was measured in each season as an index of insulation and compared to those of four similarly sized resident North Temperate Zone species.

METHODS

CAPTURE AND PROCESSING OF FAIRY-WRENS

Superb fairy-wrens were captured at several sites in Braeside Park using mist nets, as outlined in Chapter 1: General Methods. Trapping occurred over a one-year period from September 1996 to August 1997. A total of 40 birds was caught, 10 in each season. Only one or two birds were collected on each visit due to the amount of laboratory analysis required and in order to spread sampling out over each season.

On capture, all fairy-wrens were sexed from plumage traits and transported to the laboratory in separate cloth bags. Birds were killed by asphyxiation with pure nitrogen within one hour of capture and weighed on a *Mettler HK60* balance (±0.01g).

All contour and inner down feathers were removed from each bird, leaving only the tail feathers and the primaries, secondaries and coverts on the wings. The removed feathers were stored in airtight, polythene bags for subsequent drying and weighing. A longitudinal cut was made down the breast and abdomen and the liver and left pectoralis muscle were quickly removed, weighed and immediately frozen in liquid nitrogen for measurement of glycogen concentration. The right pectoralis muscle was also removed, weighed and placed on ice for determination of catabolic enzyme activities. The remainder of the carcass was placed in a tightly sealed polythene bag and stored at -70°C for subsequent determination of total body lipid content.

PLUMAGE MASS DETERMINATION

Polythene bags containing feathers and down from the 40 fairy-wrens were freeze-dried in a Dynavac Freeze Drying Unit at -50°C and 5 Torr for 48

hours and immediately weighed (± 0.0001g) on a *Mettler AE166* micro balance. Moisture-absorbing desiccant (silica gel) was used when temporarily storing the bags until each could be weighed. The feathers were then removed from the bags and the bags freeze-dried again to determine their weight and hence by subtraction the dry mass of the feathers.

Mean seasonal values are presented as total plumage mass (g) and relative plumage mass (%), which indicates the percentage of total bird mass contributed by the plumage. The wet mass used in calculating this latter value was the mass measured just after killing, which was approximately two hours after capture. Comparisons among means for the four seasons were made with one-factor *ANOVA*s for both total and relative plumage mass. The data were normally distributed, making transformation unnecessary. A *Post hoc* Tukey's multiple-comparison test was used to identify homogeneous subsets of means that were statistically indistinguishable.

MEASUREMENT OF TOTAL BODY LIPID LEVELS

While the magnitude of lipid reserves can be estimated using body mass, this is not accurate due to labile non-lipid components such as the contents of the digestive tract as well as water balance and protein reserves (Blem, 1990). Likewise, subjective estimation of fat 'classes' which roughly correlate to lipid mass is also not as accurate as direct methods. Other indirect measurements involve mathematical estimation, which may include weighing the animal in water, determination of potassium-40 levels and multiple measurements of skin thickness, but these are not easily applied to birds (Blem, 1990). Another nondestructive method involves measurement of total body electrical conductivity in a fat analyser. While this is largely accurate it is only effective for birds larger than 40g in mass (Walsberg, 1988). Although tedious and time-consuming, direct lipid measurement by solvent extraction of whole bodies of killed birds is the most accurate.

Solvent extraction of lipids

Organic tissue needs to be dried to constant mass before the extraction of lipids but there is no standardised drying temperature or technique. However, lipid extracted from homogenised House sparrows and Meadow voles (*Microtus pennsylvanicus*) using petroleum ether did not differ among oven drying temperatures ranging from 40-120°C and freeze drying (Kerr *et al.*, 1982).

Lipid extraction from an organic material is typically performed with a solvent, which is washed over a dry, chopped-up tissue sample on a continuous basis, employing a countercurrent process (Mayo *et al.*, 2000). The extraction time and the solvent involved in removing all lipids from a carcass using this system is reported to be quite variable (Dobush *et al.*, 1985; Sawicka-Kapusta, 1975). In this study, petroleum ether was used as the solvent because of its speed of action in a Soxhlet apparatus, which is at least eight times faster than other common solvents, including chloroform-methanol or chloroform-petroleum ether-methanol mixtures (Blem, 1990; Dobush *et al.*, 1985). Unlike chloroform mixtures, petroleum ether extracts little non-lipid material, and being non-polar, easily extracts the main neutral storage lipid, triacylglycerols while leaving phospholipids found in cell membranes and nervous tissue (Dobush *et al.*, 1985; Nelson, 1975). A preliminary study using two fairy-wrens showed that extraction of 98% of extractable body lipids occurred in the first two hours of processing using petroleum ether heated to 75 ± 5°C (see Appendix: Table V).

Tissue Preparation

All weights (± 0.0001g) were measured on a *Mettler AE166* microbalance and all oven drying was performed at 75°C in a controlled-temperature cabinet (*Memmert*). Whole fairy-wren carcasses stored at -70°C were weighed and thawed. Each bird was then freeze-dried whole, in a *Dynavac* Freeze Drying Unit for at least 48 hours at -50°C and 5 Torr and then weighed to determine dry mass and relative body moisture content. Individual birds were then cut into small pieces (<5mm in dimension) with scissors. The entire sample

(approximately 2.5-3.0g dry mass) was placed in a 22 × 80mm porous extraction thimble (*Advantec MFS*), which was plugged with dry cottonwool and dried further at 75°C until constant mass was attained, usually after at least 24 hours. This removed any moisture absorbed from the atmosphere while preparing the sample.

Lipid Extraction

Body lipid was extracted from fairy-wrens using petroleum ether (Selby-Biolab HPLC/GC grade) with a boiling point range of 35-60°C, in a Whatman Soxhlet Extractor apparatus. The apparatus comprises three glass components; a condenser, an extractor (70mL) and a quick-fit round bottom flask (250mL).

After cooling in a desiccator and weighing, the dry extraction thimble was placed into the extraction well. A cold water supply was attached to the condenser, and the round bottom flask of the Soxhlet apparatus was lowered over a water bath set at approximately 75 ± 5°C. Vaporised petroleum ether dripped into the extraction thimble, until the extractor was full causing the siphon arm to empty the extractor. This extraction cycle was continuous and was left to run for approximately 90-100 cycles through the apparatus, taking about two hours to complete. The thimble containing the fairy-wrens was air dried for about one hour then placed in a 75°C cabinet overnight before cooling in desiccant and weighing.

Calculations and data analysis

Total body fat stored as lipids in fairy-wrens was calculated as the difference in the dry weight of the thimble containing the fairy-wren before and after extraction. Although fairy-wrens used in this experiment had their liver and pectoralis muscle removed (to determine stored glycogen concentrations and catabolic enzyme activity), very little lipid is stored in liver or muscle tissue (Blem, 1990; O'Connor, 1995b; Rothe *et al.*, 1987). Mean (± standard error) seasonal values are presented as total lipid mass (g) and relative lipid content (%), which is the percentage of total wet or dry body mass (including plumage)

which comprises stored lipids. Mean (± standard error) seasonal relative water content (%), the percentage of total body mass (including plumage), comprising water, is also given.

Comparisons among means for the four seasons were made with one-factor *ANOVA*s for body lipid mass, relative lipid content and body water content. Data were normally distributed, making transformation unnecessary. Where appropriate, a *Post hoc* Tukey's multiple-comparison test was used to identify statistically different pairs of seasons.

MEASUREMENT OF METABOLITES

LIVER GLYCOGEN LEVELS

Tissue glycogen concentrations were determined using the method of Keppler and Decker (1974).

Tissue Preparation

Deproteinization: The liver tissue was crushed while frozen, then homogenised in 5 volumes of ice cold 0.6M perchloric acid at top speed using an *Utra-Turrax* T_{25} homogeniser, and then sonicated. A 0.2mL aliquot of the homogenate was transferred to a glass tube in an ice bath to be used for glycogen hydrolysis, whilst the remaining homogenate was used to determine the free glucose content of the tissue.

Assay system

Free Glucose: To determine the free glucose in the tissue, the treated tissue homogenate was spun at 13,000*g* for 5 minutes and the supernatant decanted into an eppendorf tube. The supernatant was neutralised with solid potassium hydrogen carbonate ($KHCO_3$), using 0.05% Methyl Orange as an indicator, and then placed on ice, ready for glucose determination.

Total Tissue Glucose: The amount of free glucose plus glycogen in the tissue was determined by hydrolysing all the glycogen to glucose in the tissue homogenate. The glycogen in the treated sample was hydrolysed by the addition of 100µL of 1M $KHCO_3$ solution, 2.0mL of 0.2M acetate buffer (pH 4.8) and 200µL amyloglucosidase solution, to 200µL of homogenate. The tube was stoppered and then incubated in a water bath at 40°C for 2 hours. Incubation was stopped by the addition of 1.0mL of 0.6M perchloric acid; the sample was then centrifuged for 5 minutes at 13,000*g* at 4°C and the acid supernatant fluid decanted into a glass tube. This was then neutralised with solid $KHCO_3$ and placed on ice, ready for glucose determination. Amyloglucosidase solution was prepared by adding 20mg of the enzyme protein (from *Aspergillus niger*) to 20mL of 0.2M acetate buffer (pH 4.8).

Glucose Determination: Glucose concentration was measured spectrophotometrically, whereby glucose in the presence of the enzymes hexokinase (*HK*) and glucose 6-phosphate dehydrogenase (G 6-PDH) and the chemicals adenosine 5'-triphosphate (ATP) and nicotinamide-adenine dinucleotide phosphate (NADP) is converted to gluconate-6-phosphate, adenosine 5'-diphosphate (ADP) and reduced nicotinamide-adenine dinucleotide phosphate (NADPH).

$$\textbf{Glucose + ATP} \xrightarrow{HK} \textbf{G-6-P + ADP}$$

$$\textbf{G-6-P + NADP}^{+} \xrightarrow{\text{G 6-PDH}} \textbf{Gluconate-6-phosphate + NADPH + H}^{+}$$

The amount of NADPH formed in the reaction is stoichiometric to the amount of glucose. The increase in NADPH was measured against a blank by means of its light absorbance at 340nm in a *Pharmacia Ultrospec III*® spectrophotometer, with a light path of 1cm and a final cuvette volume of 1.510mL at 25°C (refer to Appendix: Table VI for more detail). A glucose standard solution for assay control was also prepared and used.

Calculations

Liver glycogen contents were calculated as µmoles of glucose per gram of wet mass tissue (µmoles glucose·g liver^{-1}) after correction for the free glucose content of the tissue, according to the equation:

Liver Glycogen Content = Total Tissue Glucose – Free Glucose

(µmoles glucose·g liver^{-1})

Given: $$\textbf{Total Tissue Glucose} = OD_{340} \times \frac{1.510 \times 20 \times 17.5}{6.218 \times 1} \times \frac{(L.M. + 0.5)}{0.5}$$

$$\textbf{Free Glucose} = OD_{340} \times \frac{1.510 \times 20}{6.218 \times 1} \times \frac{(L.M. + 0.5)}{0.5}$$

Where: OD_{340} = optical density of reaction at 340nm

1.510 = final cuvette volume (mL)

20 = unit conversion factor (50µL sample to 1mL)

17.5 = sample dilution factor after hydrolysis

L.M. = total weight of liver used (g)

0.5 = dilution factor (volume of acid (mL) used to homogenise tissue)

6.218 = extinction coefficient of NADPH at 340nm ($1 \times mmol^{-1} \times cm^{-1}$)

1 = light path length (cm)

PECTORALIS MUSCLE GLYCOGEN LEVELS

The same procedure and calculations were used as for liver using frozen pectoralis muscle tissue.

CATABOLIC ENZYME ACTIVITIES

The right pectoralis muscle was analysed to determine the activities of four catabolic enzymes: hexokinase (*HK*, E.C. 2.7.1.1.), ß-hydroxyacyl-CoA dehydrogenase (*HOAD*, E.C. 1.1.1.35.), phosphorylase (*PHOS*, E.C. 2.4.1.1.) and citrate synthase (*CS*, E.C. 4.1.3.7.).

Tissue preparation

The muscle tissue was diced coarsely with scissors and then homogenised (*Ultra-Turrax* T_{25}) for approximately 30 seconds in 3mL of homogenising buffer maintained on ice. The crude homogenate was sonicated to break open cells, then spun in a *MSE-Micro Centaur* eppendorf centrifuge for 1 minute at 13,000*g* at 4°C, resulting in a supernatant free of cells and fat. The homogenising buffer contained 50mM-imidazole, 50mM-KCl, 7mM-$MgCl_2$ and 5mM-ethylene-diaminetetracetic acid (EDTA - di sodium salt) adjusted to pH 7.4 with HCl.

Assay system

Spectrophotometric assays were performed at 37°C using a *Pharmacia Ultrospec III*® spectrophotometer with a *Haake-FK* water bath. A wavelength of 340nm was used for *HOAD*, *HK* and *PHOS* and 412nm for *CS*, using a light path length of 1cm and a final cuvette volume of 1mL. Enzymes were assayed by a modification of the procedures used by Yacoe *et al.* (1982). Numerous trial assays involving varying substrate concentrations were performed on freshly prepared tissue samples to determine maximum enzyme activity rates. The final assay preparations used were:

HK: 0.4mM-$NADP^+$, 2.5mM-ATP, 10mM-creatine phosphate (CP), 1mM-glucose, 1IU-creatine phosphokinase (CPK), 1IU-glucose 6-phosphate dehydrogenase (G 6-PDH) and assay buffer (50mM-imidazole, 7.5mM-$MgCl_2$, 0.8mM-EDTA, 1.5mM-KCl and 1mM-Clelands reagent adjusted to pH 7.4 with HCl) in a final volume of 1mL.

HOAD: 0.2mM-NADH, 0.1mM-acetoacetyl Co-A and assay buffer (50mM imidazole adjusted to pH 7.4 with HCl) in a final volume of 1mL.

PHOS: 0.4mM-$NADP^+$, 4µM-glucose-1, 6-diphosphate (G-1, 6-diP), 1.6mM-AMP, glycogen (2mg), G 6-PDH (in excess), phosphoglucomutase (PGM) (in excess) and assay buffer (50mM sodium phosphate buffer, pH 7.0 and 10mM $MgCl_2$) in a final volume of 1mL.

CS: 0.2mM-5, 5'-Dithiobis-[2-Nitrobenzoic acid] (DTNB), 0.5mM-oxaloacetate, 0.3mM-acetyl Co-A and assay buffer (50mM Tris (hydroxymethyl) amino-methane adjusted to pH 8.1 with HCl) in a final volume of 1mL.

These enzyme assay preparations, including substrate volumes, are tabulated in more detail in Appendix: Table VII.

Control assays

Preliminary experiments on muscle tissue from fairy-wrens captured in spring determined that *HK*, *HOAD* and *CS* homogenates had no significant change in activity when stored for 48 hours at –70°C when compared to enzyme activity of fresh tissue homogenate. The activity of *PHOS* dropped by approximately 50% when frozen under the same conditions (see Appendix: Table VIII for details). As a consequence, *PHOS* activity was determined within one hour of tissue preparation, while the activities of the remaining enzymes were determined the following day from aliquots of supernatant frozen at –70°C. Interestingly, *PHOS* extracted from the leg muscle of mice and rapidly frozen and stored at –70°C did not differ significantly from activities of the same enzyme measured in fresh tissue (Yacoe *et al.*, 1982).

Calculations

All activities measured were proportional to enzyme concentration and were measured at saturating substrate concentrations. Control assays were run for each of the enzymes in the absence of substrate. Triplicate assays were performed in each case on each enzyme and the means are reported in µmoles substrate used per minute per gram of fresh mass tissue (µmoles $min^{-1} \cdot g$ muscle^{-}

[1]) at 37°C. Enzyme activity was calculated using the following equations (see Kaplan and Pesce, 1989):

$$\textit{HOAD / HK / PHOS} \quad (\mu\text{moles min}^{-1}\cdot\text{g muscle}^{-1}) = \frac{OD_{340} \times 1000}{6.128 \times \text{sample vol} \times 1} \times \frac{(\text{tissue mass} + 3)}{\text{tissue mass}}$$

$$\textit{CS} \quad (\mu\text{moles min}^{-1}\cdot\text{g muscle}^{-1}) = \frac{OD_{412} \times 1000}{13.61 \times \text{sample vol} \times l} \times \frac{(\text{tissue mass} + 3)}{\text{tissue mass}}$$

Where:

6.128 = extinction coefficient of NADPH and NADH at 340nm ($1 \times \text{mmol}^{-1} \times \text{cm}^{-1}$)

13.61 = extinction coefficient of DTNB at 412nm ($1 \times \text{mmol}^{-1} \times \text{cm}^{-1}$)

Sample vol = volume of tissue homogenate added to cuvette

1000 = conversion factor (sample volume to 1mL)

3 = dilution factor (volume of buffer (mL) used to homogenise the muscle)

Tissue mass = total weight of muscle tissue used (g)

l = light path length (cm)

DATA ANALYSIS

Mean (± standard error) seasonal values for glycogen concentrations (μmoles glucose·g liver^{-1}) in the liver and pectoralis muscle, and catabolic enzyme activities (μmoles min^{-1}·g muscle^{-1}) are presented. Comparisons among means for the four seasons were made with a one-factor *ANOVA* followed by *Post hoc* Tukey's multiple-comparison test to identify statistically different seasons.

RESULTS

SEASONAL CHANGES IN INSULATIVE CAPACITY

The mean plumage mass and relative plumage mass of adult fairy-wrens in each season and the associated statistical analyses are summarised in Table 3.1. The mean seasonal dry mass of contour plumage was lowest in summer at 0.24g; this increased by 50 and 79% respectively, to 0.36g in winter and 0.43g in autumn. The plumage mass in autumn was significantly higher than in the other three seasons; in summer, plumage mass was significantly lower than in any other season.

Relative plumage mass of fairy-wrens in autumn and winter comprised, on average, 4.66 and 4.04%, respectively, of the bird's total wet mass. This was significantly greater than in spring and summer, when mean relative plumage mass was 3.19 and 2.64%, respectively, of adult mass.

SEASONAL CHANGES IN BODY LIPID AND WATER CONTENT

Mean seasonal total body lipid mass, relative body lipid content and body water content of fairy-wrens is summarised in Table 3.2. The mean mass of stored body lipids in fairy-wrens ranged from 0.28g in summer to 0.40g in winter, with autumn and spring birds storing a mean of 0.29 and 0.31g, respectively. There was no significant difference ($P > 0.05$) in body lipid mass over the four seasons. These levels indicate that seasonal lipid mass constituted between 3.1 and 4.4% of the wet mass of fairy-wrens and 10.4 to 13.7% of dry mass. Again, body lipid levels relative to wet or dry body mass showed no significant seasonal variation ($P > 0.05$).

The relative body water content of fairy-wrens was stable in spring and summer, at 67.6% of total body mass (including feather mass). Fairy-wrens in

winter had the lowest mean seasonal body moisture content at 64.3%, significantly less than in spring and summer ($P < 0.001$). Autumn birds had a mean body water content of 66.2%, which was not significantly different from that in the other three seasons.

SEASONAL VARIATION IN TISSUE GLYCOGEN LEVELS

Mean seasonal glycogen concentrations of the liver and pectoralis muscle and the associated statistical analyses are summarised in Table 3.3. Liver glycogen levels were similar ($P > 0.05$) in summer, autumn and winter with mean seasonal values ranging from 15.1 to 17.6 µmoles glucose·g liver^{-1}. However, in spring, birds had liver glycogen concentrations significantly higher than those in the other three seasons ($P = 0.01$), with a mean concentration of 34.7 µmoles glucose·g liver^{-1}, a value which is approximately twice that found in the other seasons. Given a liver mass of about 0.35g, the birds had stored on average 2.2, 1.1, 1.1 and 1.0mg of glycogen in spring, summer, autumn and winter, respectively.

Muscle glycogen levels followed a similar pattern, with mean seasonal concentrations varying between 20.6 and 22.0 µmoles glucose·g liver^{-1} in summer, autumn and winter ($P > 0.05$), whilst levels were significantly higher in spring ($P < 0.001$), being more than two times greater at 47.9 µmoles glucose·g liver^{-1}. Given a mass of about 1.20g for the paired pectoralis muscles, fairy-wrens had stored on average 10.3, 4.6, 4.8 and 4.5mg of glycogen in spring, summer, autumn and winter, respectively. In each season, 81-82% of all glycogen was stored in the pectoralis muscle of the fairy-wrens.

SEASONAL CHANGES IN THE CATABOLIC CAPACITY OF THE PECTORALIS MUSCLES

Seasonal means and the associated statistical analyses for the mass-specific activity levels of *HOAD*, *HK*, *CS* and *PHOS* are summarised in Table 3.4. Three of the four catabolic enzymes measured showed some significant inter-seasonal variation. *HK*, which catalyses the phosphorylation of glucose, was significantly higher in spring than in the other three seasons ($P < 0.001$). Similarly, both *CS*, which catalyses the first step in the tricarboxylic acid cycle, and *PHOS*, which regulates glycogen breakdown, also had significantly higher ($P < 0.001$) activity levels in spring than in the other three seasons. However, the activity of *HOAD*, an enzyme of the ß-oxidative pathway involved in lipid breakdown, showed no significant seasonal variation ($P > 0.05$).

The effects of freezing on catabolic enzyme activities

In this study, freezing muscle tissue homogenate for 48 hours at –70°C had no significant effect on the activity of *HOAD*, *HK* and *CS*, but it did for *PHOS*, reducing activity by approximately 50% (see Appendix: Table VIII). O'Connor and Root (1993) also reported no significant difference in the activity of *CS* or *HOAD* in House sparrows when tissue homogenates were sonicated and then frozen (-70°C) within 60 minutes of extraction. Similarly, Marsh (1981) showed that storage at 0°C for eight hours caused no change in activity of *HOAD* and *CS* in Gray catbird (*Dumetella carolinensis*) pectoralis muscle. Earlier reports (see Srere, 1969) indicate that activity of mitochondrial enzymes, such as *CS*, may be increased by freezing the sample, due to the cell lysing that occurs. Thorough cell lysing of all freshly prepared homogenates by sonication ensures that subsequent freezing of muscle samples does not have the effect of significantly enhancing the cell-lysing process (O'Connor and Root, 1993).

Table 3.1. Mean dry plumage mass and relative plumage mass of adult fairy-wrens for each season and associated statistics. Values are mean ± standard error and sample size is 10 in each season. Mean Max and Min temperatures (°C) for Braeside Park are given for each season.
F-ratios and probability levels from two one-factor *ANOVA* s examines variation in plumage mass and relative plumage mass between seasons. Tukey's multiple-comparison test determines which seasons differed. Degrees of freedom were 3 (seasons). Unlike means are separated by X.

SEASON	Mean Temp. Max / Min (°C)	Total Plumage Dry Mass (g)	Relative Plumage Dry Mass (%)
Autumn	20.4 / 10.9	0.43 ± 0.02	4.66 ± 0.15
Winter	14.1 / 6.4	0.36 ± 0.01	4.04 ± 0.10
Spring	19.2 / 9.4	0.31 ± 0.02	3.19 ± 0.20
Summer	25.1 / 13.5	0.24 ± 0.02	2.64 ± 0.20
f-ratio (probability)		**27.025 (< 0.001)**	**26.524 (< 0.001)**
Tukey's multiple-comparison test ($P < 0.05$)		Aut X Win, Spr, Sum Win, Spr X Sum	Aut, Win X Spr, Sum

Table 3.2. Mean total lipid mass, lipid levels relative to wet and dry body mass and relative water content of adult fairy-wrens for each season.
Values are mean ± standard error and sample size is 10 in each season.
The full range of values in each season is given in parentheses.

SEASON	Total Lipid Mass (g)	% Lipid Levels Relative To: Wet Mass	% Lipid Levels Relative To: Dry Mass	Relative Water Content (%)
Spring	0.31 ± 0.04 (0.15 - 0.56)	3.2 ± 0.4 (1.8 - 5.6)	11.0 ± 1.1 (6.3 - 18.5)	67.6 ± 0.5
Summer	0.28 ± 0.03 (0.13 - 0.36)	3.1 ± 0.3 (1.4 - 4.1)	10.4 ± 0.9 (5.1 - 12.9)	67.6 ± 0.6
Autumn	0.29 ± 0.04 (0.14 - 0.51)	3.1 ± 0.4 (1.6 - 5.2)	10.5 ± 1.2 (5.6 - 16.3)	66.2 ± 0.5
Winter	0.40 ± 0.05 (0.20 - 0.67)	4.4 ± 0.5 (2.3 - 7.0)	13.7 ± 1.4 (7.5 - 20.8)	64.3 ± 0.5

Table 3.3. Mean glycogen content of liver and pectoralis muscle tissue for each season and associated statistics. Values are mean ± standard error with a sample size of 10 in each season. The full range of values in each season is given in parentheses. Glycogen concentrations are measured in μmoles glucose [g tissue]$^{-1}$ F-ratios and probability levels from two one-factor *ANOVA* s examines variation in plumage mass and relative plumage mass between seasons. Tukey's multiple-comparison test determines which seasons differed. Degrees of freedom were 3 (seasons). Unlike means are separated by X.

SEASON	Mean Glycogen Concentration	
	Liver	**Muscle**
Spring	34.7 ± 8.3 (13.3 - 81.9)	47.9 ± 5.0 (25.9 - 73.4)
Summer	17.0 ± 1.5 (8.8 - 23.5)	21.5 ± 1.2 (16.7 - 27.5)
Autumn	17.6 ± 1.8 (4.7 - 25.2)	22.0 ± 1.9 (13.0 - 32.3)
Winter	15.1 ± 2.1 (8.6 - 26.7)	20.6 ± 1.3 (15.0 - 29.2)
f-ratio (probability)	**4.256 (0.011)**	**22.048 (< 0.001)**
Tukey's multiple-comparison test ($P < 0.05$)	Spr X Sum, Win, Aut	Spr X Sum, Win, Aut

Table 3.4. Mean seasonal activities of catabolic enzymes extracted from the pectoralis muscle of fairy-wrens and associated statistics. Values are mean ± standard error with a sample size of 10 in each season. The full range of values in each season is given in parentheses. Enzyme activities are measured at 37°C in µmoles min. g muscle^{-1} .
F-ratios and probability levels from four one-factor *ANOVA* s examines variation in *HOAD* , *HK* , *CS* and *PHOS* between seasons. Tukey's multiple-comparison test determines which seasons differed. Degrees of freedom were 3 (seasons). Unlike means are separated by X.

SEASON	Enzyme Activities			
	HOAD	***HK***	***CS***	***PHOS***
Spring	21.9 ± 1.7 (10.1 - 27.7)	2.0 ± 0.3 (0.7 - 3.0)	131 ± 7 (100 - 162)	139 ± 3 (128 - 164)
Summer	22.0 ± 0.4 (19.4 - 24.0)	0.8 ± 0.1 (0.4 - 1.0)	109 ± 6 (79 - 135)	90 ± 5 (70 - 126)
Autumn	19.2 ± 1.1 (11.8 - 23.9)	0.5 ± 0.1 (0.2 - 1.4)	101 ± 4 (83 - 125)	111 ± 12 (68 - 182)
Winter	18.6 ± 0.8 (14.8 - 22.4)	0.6 ± 0.1 (0.2 - 1.0)	92 ± 5 (72 - 120)	110 ± 3 (104 - 125)
f-ratio (probability)	2.620 (0.065)	**20.597** **(< 0.001)**	**9.608** **(< 0.001)**	**8.469** **(< 0.001)**
Tukey's multiple-comparison test ($P < 0.05$)	N / A	Spr X Sum, Aut, Win	Spr X Sum, Aut, Win	Spr X Sum, Aut, Win

Table 3.5. A summer - winter comparison of mean relative plumage mass (%) of fairy-wrens and four other small passerine species.
Sample size is given in parentheses.

SPECIES	Relative Plumage Mass Summer (%)	Winter (%)	Change between Summer and Winter (%)
Superb fairy-wren [a] (*Malurus cyaneus*)	**2.6 (10)**	**4.0 (10)**	**+54**
American goldfinch [b] (*Spinus tristis*)	2.9 (13)	3.9 (25)	+34
American goldfinch [c] (*Spinus tristis*)	3.4 (16)	4.0 (36)	+18
House finch [d] (*Carpodacus mexicanus*)	3.3 (17)	4.6 (32)	+39
Dark-eyed junco [e] (*Junco hyemalis*)	3.4 (14)	4.1 (14)	+21
House sparrow [f] (*Passer domesticus*)	3.6 (9)	5.0 (10)	+39

[a] Current study.
[b] Dawson and Carey (1976).
[c] Carey *et al.* (1978).
[d] Dawson *et al.* (1983).
[e] Swanson (1991).
[f] Barnett (1970).

Table 3.6. A seasonal comparison of body lipid mass and relative body lipid content in fairy-wrens and four other small passerines species.
B.M.: Total Wet Body Mass (g), **L.M.**: Body Lipids Mass (g), **% B.L.**: Relative Body Lipid Content (%), **% B.W.**: Relative Body Water Content. Sample size in each study is given in parentheses.

	Spring				Summer				Autumn				Winter			
	B.M.	**L.M.**	**% B.L.**	**% B.W.**	**B.M.**	**L.M.**	**% B.L.**	**% B.W.**	**B.M.**	**L.M.**	**% B.L.**	**% B.W.**	**B.M.**	**L.M.**	**% B.L.**	**% B.W.**
Fairy-wren[1] *(Malurus cyaneus)*	**9.3** (10)	**0.31** (10)	**3.2**	**67.6**	**8.9** (10)	**0.28** (10)	**3.1**	**67.6**	**9.3** (10)	**0.29** (10)	**3.1**	**66.2**	**9.0** (10)	**0.40** (10)	**4.4**	**64.3**
Dark-eyed Junco *(Junco hyemalis)*					17.8 (59)	0.5 (5)	2.8	69.8					19.4 (56)	1.2 (6)	6.0	66.8
House Finch[3] *(Carpodacus mexicanus)*					22.5 (21)	1.1 (21)	4.9	61.0					23.5 (12)	2.4 (12)	10.2	64.5
American goldfinch[4] *(Carduelis tristis)*	13.5 (30)	0.8 (30)	5.9	61.9	11.9 (16)	0.5 (16)	4.0	63.6	11.9 (29)	0.5 (29)	3.8	64.8	14.6 (36)	1.5 (36)	10.5	59.9
House Sparrow[5] *(Passer domesticus)*	28.9 (35)	1.6 (35)	5.6	61.9	27.1 (32)	1.4 (32)	5.3	62.7	27.8 (24)	1.44 (24)	5.2	61.9	28.2 (39)	1.7 (39)	6.0	61.0
House Finch[6] *(Carpodacus mexicanus)*	21.8 (5)	1.4 (5)	6.3	62.6	21.4 (11)	1.4 (11)	6.5	65.7	21.1 (5)	1.1 (5)	5.4	62.6	21.7 (12)	1.6 (12)	7.5	61.0

[1] This study; [2] Swanson (1991); [3] O'Connor (1995a); [4] Carey *et al.* (1978); [5] Barnett (1970); [6] Dawson *et al*. (1983).

Table 3.7. A summer - winter comparison of glycogen concentrations in the liver and pectoralis muscle of fairy-wrens and two other small passerine species. Glycogen concentration are shown in mg/g tissue.
Values are mean ± standard error with the sample size given in parentheses.
N.S.: indicates no significant difference between summer and winter values.
Sig.: indicates a difference between summer and winter values.
N.I.: significant difference not indicated in study.

SPECIES	Liver		Pectoralis Muscle	
	Summer	Winter	Summer	Winter
Superb Fairy-wren [a] (***Malurus cyaneus***)	**3.37 ± 0.30 (10)**	**2.99 ± 0.42 (10)**	**4.26 ± 0.24 (10)**	**4.08 ± 0.26 (10)**
	N.S.		**N.S.**	
House Finch [b] (*Carpodacus mexicanus*)	0.41 ± 0.20 (10)	2.29 ± 0.88 (8)	8.72 ± 1.05 (10)	6.75 ± 0.79 (8)
	N.S.		N.S.	
American Goldfinch [c] (*Carduelis tristis*)	1.97 (4)	0.42 (7)	4.3 (4)	4.62 (7)
	N.S.		N.S.	
American Goldfinch [d] (*Carduelis tristis*)	0.21 ± 0.02 (8)	0.49 ± 0.17 (8)	0.84 ± 0.19 (8)	2.07 ± 0.34 (9)
	N.I.		Sig.	

[a] Current study. Data from this study has been converted from µmoles / g tissue to mg / g tissue to allow for direct comparisons.

[b] Marsh *et al.* (1984). +30°C experimental birds.

[c] Marsh and Dawson (1982). +30°C experimental birds.

[d] Carey *et al.* (1978).

Table 3.8. A comparison of catabolic enzyme activities from the pectoralis muscle of fairy-wrens with those of two other small passerine species.
Enzyme activities are shown in μmoles min^{-1}. g $muscle^{-1}$, at the temperature indicated.

	HOAD					CS					HK					PHOS				
	Spr	**Sum**	**Aut**	**Win**		**Spr**	**Sum**	**Aut**	**Win**		**Spr**	**Sum**	**Aut**	**Win**		**Spr**	**Sum**	**Aut**	**Win**	
Fairy-wren[1] *(Malurus cyaneus)*	21.9 (10)	22.0 (10)	19.2 (10)	18.6 (10)	**Not Sig.**	130.5 (10)	108.6 (10)	101.4 (10)	91.5 (10)	**Spr > Sum, Aut, Win**	2.0 (10)	0.8 (10)	0.5 (10)	0.6 (10)	**Spr > Sum, Aut, Win**	139.3 (10)	90.4 (10)	111.3 (10)	109.8 (10)	**Spr > Sum, Aut, Win**
House Finch[2] *(Carpodacus mexicanus)*	21.8 (29)			19.6 (12)	**Not Sig.**	148.3 (34)			159.6 (12)	**Not Sig.**										
House Finch[3] *(Carpodacus mexicanus)*	10.7 (9)	12.7 (6)		19.6 (9)	**Win > Spr, Sum**	113.4 (9)	100.6 (6)		119.7 (9)	**Not Sig.**	1.12 (9)	0.92 (6)		1.19 (11)	**Not Sig.**					
American goldfinch[4] *(Carduelis tristis)*	54.2 (10)	43.8 (10)		91.5 (10)	**Win > Spr, Sum**	319.3 (10)	251.6 (10)		288.9 (10)	**Spr > Sum**	0.71 (10)	0.70 (10)		0.42 (10)	**Not Sig.**		10.4 (9)		15.0 (8)	**Win > Sum**
American goldfinch[5] *(Carduelis tristis)*		45.0 (7)		68.4 (6)	**Win > Sum**		210 (7)		203 (6)	**Not Sig.**										

[1] This study, (assayed at 37°C); [2] O'Connor (1995), (assayed at 25°C); [3] Carey *et al.* (1989), (assayed at 25°C);
[4] Yacoe and Dawson (1983), (assayed at 25°C); [5] Marsh and Dawson (1982), (assayed at 25°C).

Figure 3.1 Seasonal changes in relative plumage mass (%) of 36 fairy-wrens over a one year period.
Each point represents a single individual.

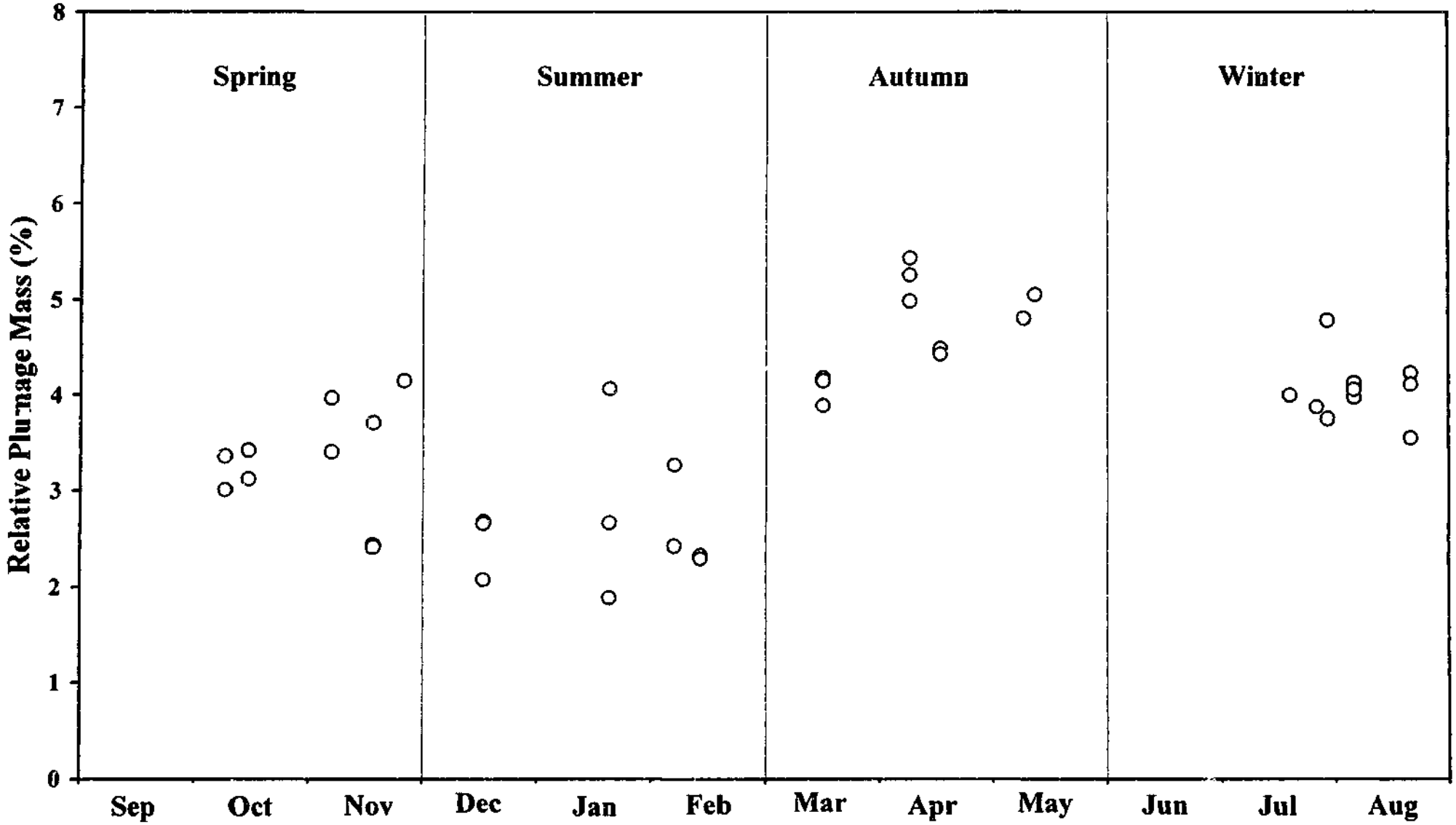
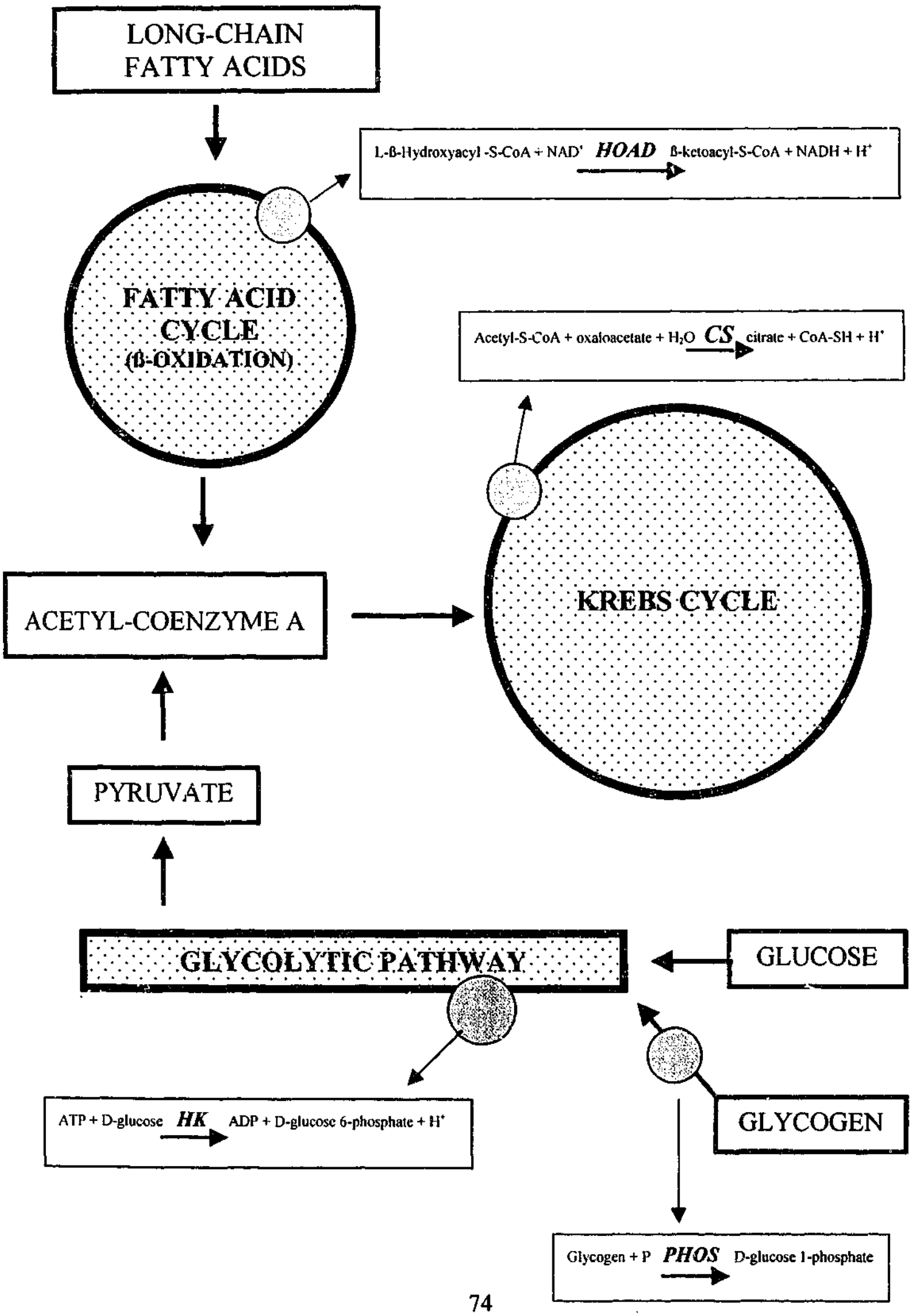


Figure 3.2 Reactions catalysed by the catabolic enzymes measured in this study, and their relative position in the metabolism of lipids (fatty acids), glycogen and glucose (adapted from Lehninger, 1987; Stein and Myers, 1994).



DISCUSSION

THE IMPORTANCE OF INSULATIVE CAPACITY IN OVER-WINTERING FAIRY-WRENS

The dry mass of contour feathers has been used by numerous authors as an index of insulation in determining the extent of seasonal insulative acclimatisation in birds of many sizes. The extent to which birds rely on insulative changes to combat cold conditions is, however, questionable, particularly in small passerines. Large birds, such as Black grouse (*Lyrurus tetrix*) which weigh about 1000g, have been shown to compensate for low ambient temperatures mainly by improving thermal insulation (Rintamaki *et al.*, 1983). In contrast, 21g House finches (*Carpodacus mexicanus*) overwintering in California and Colorado (U.S.A.) have pronounced differences in cold resistance, a reflection of the temperature difference between the two regions, but they show no significant increase in winter contour plumage mass in winter (Dawson *et al.*, 1983). This finding led the authors to conclude that plumage insulation probably has a minimal role in winter acclimatisation in small passerines. Other authors, including Swanson (1991) and Carey *et al.* (1978), have reached a similar conclusion, despite finding significant seasonal changes in plumage mass in the small passerine under study.

Fairy-wrens in the current study exhibited a significant 53% increase in relative plumage mass between summer and winter and an 81% increase between summer and autumn. These seasonal changes are shown clearly in Figure 3.1; plumage mass steadily increased from summer through to autumn and winter and decreased again in spring, a trend similar to that in the American goldfinch (Dawson and Carey, 1976). A summary of summer to winter changes in plumage mass of several small, resident passerines from the North Temperate Zone is shown in Table 3.5. This reveals that the 53% increase in plumage mass of fairy-wrens appears to be considerably greater than that found in similarly

sized passerines from the North Temperate Zone, which have summer - winter increases ranging from 18-39%.

A large significant difference in insulative capacity was apparent in fairy-wrens between winter and summer, but is this indicative of insulative acclimatisation? Total contour plumage mass of fairy-wrens was heaviest in autumn during the moulting period, declined over the inter-moult period through winter and spring, until it reached a minimum in summer. However, relative plumage mass, arguably a better indicator of insulation, was greater in autumn and winter, than in spring and summer. Intuitively, if plumage mass were vital for survival when it is coldest, it would be heaviest in winter and possibly spring, the two coldest seasons which have mean overnight temperatures below 10°C. So while there are seasonal changes in plumage mass and relative plumage mass, they do not entirely coincide with the coldest times of the year. The authors whose studies are summarised in Table 3.9 either did not collect data over all four seasons, or did not categorise the data into seasons, making it difficult to determine if a similar pattern occurs in other small passerines. A likely scenario is that feather mass in fairy-wrens is increased during the autumnal moult in preparation for the succeeding cold months. Although moulting alone does not place much energetic stress on birds (Murphy, 1996), the combination of reduced daylight hours for foraging, cold temperatures and the general scarcity of food in winter may be energetically challenging, without the added demands of feather production.

Aside from the seasonal timing of peak insulative capacity, the relatively large surface area to volume ratio of fairy-wrens would likely impose constraints that would make significant seasonal insulative acclimatisation difficult (Saarela *et al.*, 1989). However, in addition to the quantity of plumage that birds have, the insulative capacity of feathers can also be improved by ptiloerection, the fluffing of feathers, which increases the thickness of the insulative layer (Calder and King, 1974). Further, selection of favourable overnight roosting positions can greatly reduce exposure to adverse weather conditions (Kelty and Lustick, 1977). Fairy-wren group members roost together overnight, usually huddling on

a horizontal branch of a dense shrub or tree (Rowley and Russell, 1997; Higgins *et al.*, 2001). Dense cover is believed to provide a thermal benefit by shielding birds from wind; in some cases, it creates a microclimate several degrees warmer than the surrounding night-time air (Walsberg, 1985). In addition, communal roosting significantly reduces the bird's surface area that is exposed to the cold, essentially decreasing their surface area to volume ratio (Walsberg, 1990). At cool temperatures, swiftlets from New Guinea often cluster together, a behaviour that led to a 30% reduction in energy expenditure (McNab and Bonaccorso, 1995). Similarly, huddling in Bushtits (*Psaltriparus minimus*) and Goldcrests (*Regulus regulus*) reduced energy expenditure by 21 and 37%, respectively, during overnight roosting (Chaplin, 1982; Gavrilov, 1972).

Given the 53% increase in contour feather mass in winter, in addition to behavioural habits such as communal roosting at night, there is considerable evidence suggesting that fairy-wrens show seasonal insulative acclimatisation.

SEASONAL CHANGES IN ENERGY SUBSTRATE LEVELS IN FAIRY-WRENS

Stored energy substrates: Glycogen and Lipids

Glycogen is the main storage polysaccharide in animal cells; it occurs intracellularly in the form of large clusters or granules. It is typically most abundant in the liver and in skeletal muscle (Lehninger, 1987). During increased muscle activity, adrenaline is secreted which stimulates the formation of blood glucose from the glycogen stored in the liver and the breakdown of glycogen to lactate in the muscle tissue.

Glycogen is most useful under conditions where the demand for ATP for muscle contraction is so great that blood flow cannot provide oxygen and fuel fast enough. The muscle glycogen is broken down to lactate by anaerobic glycolysis, with a net yield of three ATP molecules per glucose unit degraded. The disadvantage of glycolysis, particularly that found in skeletal muscle, is that

there is a limited supply of glycogen available; moreover, the accumulation of lactic acid and the consequent decrease in pH lead to a less efficient active muscle. The excess glucose consumed is converted into lipids for long-term storage, due to the very limited capacity to store glycogen.

Tissues containing stored lipids are amorphous and widely distributed in the body; the lipids consist of triacylglycerols, the storage form of free fatty acids. These lipids have a very high energy content and can be stored in very large amounts in adipose tissue. When needed by skeletal muscles or the heart, free fatty acids are transported around the body in the bloodstream bound by serum albumin. The breakdown of free-fatty acids in the Fatty Acid Cycle to acetyl CoA, leading to the Krebs cycle, occurs aerobically (Boyer, 2002). The reliance on oxygen for lipid catabolism effectively means that lipids cannot be used as an energy source when maximal muscle contraction is occurring.

Seasonally stable body lipid levels

Mean stored body lipid mass of fairy-wrens was constant over all seasons. However, there was relatively high individual variability in stored lipid mass, some birds having approximately three times as much as others in each of the four seasons. Lipid mass constituted, on average, 3.1 to 4.4% and 10.4 to 13.7%, respectively, of total wet and dry body mass and did not vary seasonally.

Relative lipid levels for fairy-wrens are generally lower in each season than seasonal means obtained for some resident North Temperate Zone birds (see Table 3.6). Dark-eyed juncos from Oregon, a region of relatively mild winter climate had similar summer lipid levels to those of fairy-wrens, showing no winter increase. As with the fairy-wrens in the present study, there was much variability in lipid levels among juncos in winter. The American goldfinch, House sparrow and House finch in more climatic extreme regions exhibited significant fat loading in winter relative to summer levels. This change was also reflected in significant seasonal mass changes in the goldfinches and sparrows.

In all the studies listed in Table 3.6, with the exception of that of Dawson *et al.* (1983), body lipid mass was determined on birds that were wild-trapped

between early and late morning. However, Dawson *et al.* captured House finches in California throughout the entire day discovering that lipid content although variable, showed a weak correlation with time, increasing slightly through daytime hours. Birds that were trapped in mid-afternoon had accumulated about 50% more lipids than birds caught mid-morning. O'Connor (1995a) found a similar trend in House finches in Michigan. A similar diurnal pattern of lipid accumulation was also documented in Golden-crowned kinglets (*Regulus satrapa*) in Virginia (U.S.A.) which had, on average, approximately 50% more lipid mass in the mid-afternoon than in mid-morning (Blem and Pagels, 1984). Similar to the above-mentioned birds, resident and migrant Silvereyes in Tasmania in the South Temperate Zone exhibited, on average, a 30% increase in lipid stores over the same portion of the day (Chan, 1995). Although it is likely that stored lipid mass in fairy-wrens would be highest just prior to sunset, due to practical constraints all birds in the present study had to be trapped between early and late morning and so birds probably had not completely replenished fat stores depleted overnight. Consequently, seasonal variation in fat stores of fairy-wrens may have been underestimated. However, the studies by Swanson (1991), Carey *et al.* (1978) and Barnett (1970) involved wild-trapping juncos, goldfinches and sparrows before midday and these birds showed extensive lipid accumulation, even after fasting overnight. Therefore any significant lipid deposition by fairy-wrens should have presumably been apparent regardless of the time of day at which the birds were collected. This suggests the fairy-wrens fail to store body lipids in winter to the same degree as other temperate-wintering passerines.

The mean relative body water content of fairy-wrens in winter was significantly lower than in the remaining three seasons, which were statistically indistinguishable. However, all four seasonal means are within the range of values obtained for other passerine species (Table 3.6). Interestingly, mean winter values for all four North Temperate Zone species are the lowest seasonal values recorded. This is presumed to occur as lipid contains little water relative to other tissues, therefore the accumulation of body lipids leads to a relative decrease in total body water (Carey *et al.*, 1978).

High glycogen levels in spring

Fairy-wrens stored an average of 12.5, 5.7, 5.9 and 5.4mg of glycogen in spring, summer, autumn and winter, respectively, with 81-82% being stored in the pectoralis muscles. The concentration of glycogen in the liver and pectoralis muscle of fairy-wrens was constant over summer, autumn and winter. However, in spring, on average, both tissues stored at least twice as much glycogen as was found in the other seasons. Moreover, the lowest glycogen concentration measured in the pectoralis muscle of an individual bird in spring was 25.9 μmoles glucose · g tissue^{-1}, which was higher than the seasonal averages for summer, autumn and winter. The results also indicate that for both tissues, glycogen concentrations were quite variable among individuals in spring. Fasting causes a change in metabolism of birds, so that they use more fat and save carbohydrates. This usually results in higher concentrations of fat metabolites in the blood whilst glycogen stores usually decline (Rothe *et al.*, 1987). For example glycogen stored in pectoralis muscle tissue of domestic hens decreased by 20% after six hours of fasting (Riesenfeld, *et al.*, 1981). The variation in stored glycogen concentrations found in fairy-wrens maybe due to different levels of fasting in the birds associated with brooding and / or feeding of eggs or young.

A comparison of stored glycogen levels of American goldfinches and House finches and the fairy-wrens in this study is shown in Table 3.7. In summer and winter, fairy-wren pectoralis muscles held higher concentrations of glycogen than the liver, as was also the case for House finches in Colorado (Marsh *et al.*, 1984) and American goldfinches in Michigan (Marsh and Dawson, 1982; Carey *et al.*, 1978). Neither the current study, nor those of Marsh *et al.* (1984) and Marsh and Dawson (1982) found a summer to winter change in glycogen levels stored in either of the two tissues. Conversely, Carey *et al.* (1978) found that American goldfinches amassed more muscle glycogen in winter than in summer, although no seasonal change was observed in the liver. No data were found for glycogen tissue levels in other small passerines in

autumn or, in particular, spring, a season when high glycogen levels are presumably required. The possible implications in relation to metabolic acclimatisation of high spring glycogen concentrations and the lack of a summer to winter change in this stored fuel, are considered later in this Discussion.

FUEL MOBILISATION AND CATABOLIC CAPACITY OF FAIRY-WREN PECTORALIS MUSCLE

The measurement of metabolic flux can be used to assess the maximum metabolic capacity of a tissue, although a more simple, but indirect, method uses the measurement of key indicator enzymes (Marsh, 1981). The major difficulty with this latter approach lies in the choice of enzymes and the optimum *in vitro* conditions for enzyme assays that yield maximum potential activities. The four enzymes used as indicators in this study were chosen for their key position in each of the pathways involved in the catabolism of fatty acids and glycogen / glucose. The positions of each enzyme and the entry point of the two energy sources in the overall energy-producing process are shown in Figure 3.2.

Optimal *in vitro* conditions, ie. those which yield the highest activity for each of the enzymes, were determined by experiment. This involved the measurement of reactions using a range of concentrations of both substrates and enzymes at both approximate room temperature (25°C) and approximate fairy-wren body temperature (37°C) (Withers, 1982). Also, given the considerable length of time required to measure the activities of the four enzymes, the effects of freezing muscle homogenate before assaying were also checked.

CS, *HOAD*, *HK* AND *PHOS*: WHAT DO THEIR RESPECTIVE ACTIVITIES INDICATE?

Aerobic capacity

CS is a regulatory enzyme, which catalyses a possible rate-limiting step of

the Krebs cycle. It is involved in the first reaction of the cycle, condensing acetyl-CoA and oxaloacetate to citrate. The activity of *CS* has previously been used as an indicator of the tissue's capacity for aerobic work in small passerines (see Marsh, 1981). *CS* activity in the long-distance migratory Gray catbird from Michigan and Florida (U.S.A.) indicates significant increase in total aerobic capacity in preparation for migration (Marsh, 1981). Work on perfused hearts of endurance-trained rats also indicated that an increase in *CS* activity was correlated with the endurance of the whole animal (Holloszy and Booth, 1976), whilst Wickler (1980, 1981) reported that winter acclimatisation in mice involved substantial enhancement of tissue-level aerobic capacity through increased *CS* activity.

The present study revealed that there was seasonal variation in *CS* levels in the pectoralis muscle of fairy-wrens, with a significant increase in activity occurring in spring, indicating an increase in aerobic endurance of the birds at this time. Yacoe and Dawson (1983) also recorded an increase in *CS* activity in spring in American goldfinches, with seasonal values 2.5-3-fold higher than those of fairy-wrens in the current study (see Table 3.8). Unfortunately the significance of this change in activity was not discussed. My study, like the four others reviewed in Table 3.8, revealed no significant difference in *CS* activity between winter and summer. This may indicate that increased metabolic activity due to shivering has no influence on the aerobic fitness of the bird, perhaps because, as has been suggested by Carey *et al.* (1978), the maximum aerobic activity of small passerines is most likely influenced by flying, a more energy demanding activity. Thus, relatively moderate muscle twitching during shivering thermogenesis would presumably have little, if any, impact on *CS* activity.

ß-oxidative capacity

HOAD is one of four ß-oxidative enzymes responsible for fatty acid oxidation in the fatty acid cycle. Long-chain fatty acids are oxidised to yield acetyl-CoA, which is used to produce ATP via the Krebs cycle and electron transport chain. More specifically, *HOAD* catalyses the dehydrogenation of L-ß-

hydroxyacyl-CoA to ß-ketoacyl-CoA. The activity of *HOAD* is commonly used to indicate tissue capacity to oxidise fatty acids (O'Connor, 1995b; Marsh, 1981).

Results in this study showed that the pectoralis muscles of fairy-wrens exhibited no significant seasonal variation in their capacity to catabolise lipid. This was perhaps not surprising since body lipid levels, previously determined, were also seasonally constant. O'Connor (1995b) measured *HOAD* in House finches in Michigan in spring and winter, also finding a lack of significant seasonal difference in fat catabolism capacity. Despite these exceptional findings, the general trend in small passerines is for winter *HOAD* levels to be higher than in spring and / or summer (Table 3.8). As expected, House finches and American goldfinches in the studies of Carey *et al* (1989), Yacoe and Dawson (1983) and Marsh and Dawson (1982), listed in Table 3.8, also had significantly increased body lipid contents in winter, which indicated a need to store and use more lipid in winter.

Aerobic glucose oxidation capacity

HK catalyses the first reaction involved in catabolising glucose via the glycolysis pathway. During this reaction, D-glucose is phosphorylated by *HK* to irreversibly yield glucose 6-phosphate. Glycolysis eventually produces some ATP and, under aerobic conditions, acetyl CoA, which is used to produce more energy via the Krebs cycle and electron transport chain. The activities of most glycolytic enzymes are not affected by endurance training in mammals, except *HK*, which increases significantly (Carey *et al.*, 1989).

The increased activity of *HK* in fairy-wrens in spring indicates a 2.5-4-fold increase in the capacity of the pectoralis muscle to oxidise glucose, compared to that in other seasons. In contrast, both House finches and American goldfinches showed no seasonal variation in the activity of *HK* (Table 3.8), with spring levels being no different to those in summer and winter.

Glycogenolytic capacity

PHOS catalyses the single-step reaction that phosphorylates stored

glycogen to produce glucose 1-phosphate, which then enters directly into the glycolytic pathway. The capacity of fairy-wrens to break down glycogen to a useable energy source is significantly greater in spring, when the activity of *PHOS* is highest. This is augmented by the fact that liver glycogen levels are also highest in spring. Further, a significant increase in glucose 1-phosphate production from glycogen may require increasing the activity of other key enzymes in the glycolytic pathway, as occurred with *HK* in this study. That *PHOS* activity is similar in winter, summer and autumn and glycogen levels in both the pectoralis muscle and liver at these times are also similar, indicates that the use of, or need for, glycogen does not change over the three seasons.

The paucity of studies that use *PHOS* as an indicator of glycogen breakdown in muscle tissue makes a comparison with other species difficult. Yacoe and Dawson (1983) found a significantly higher concentration of *PHOS* in winter than in summer in American goldfinches but the full relevance of this is difficult to determine, as seasonal tissue glycogen levels were not measured. Yacoe *et al.* (1982) found the activity of *PHOS* from the pectoralis muscle of ten species of South American bats to be relatively high. The authors reasoned that glycogen, and its subsequent breakdown is important during take off and rapid acceleration when aerobic capacity may be momentarily exceeded.

METABOLIC ACCLIMATISATION IN FAIRY-WRENS

Fairy-wrens have been shown to store significantly greater quantities of glycogen in spring than in the other three seasons. In addition, key catabolic enzymes responsible for glycogen and glucose breakdown, *PHOS* and *HK*, have increased activities in spring compared with summer, autumn and winter. In contrast, total body lipid levels remained seasonally constant, together with the enzyme *HOAD*, a key catalyst responsible for lipid breakdown. *CS* activity in fairy-wrens also indicated an increase in the aerobic capacity of the pectoralis muscles in spring. In which season(s), if any, does metabolic acclimatisation

appear to be occurring in fairy-wrens? And, is this a reflection of the season, ie. due to the climatic conditions, or, of behavioural aspects of the birds, ie. breeding?

Metabolic acclimatisation in spring

Despite there being no significant summer to winter change in the stored glycogen levels in the liver and pectoralis muscle of fairy-wrens, there was a significant increase in both stores in spring when levels were approximately double those in other seasons. The increase in stored glycogen concentration, along with the increase in activity of both *PHOS* and *HK* in spring, suggests that fairy-wrens require and use more energy during this period, and that mainly carbohydrates supply it. The use of muscle glycogen, which constituted over 80% of the glycogen stored by fairy-wrens, could be most adaptive under anaerobic conditions, such as those that occur when muscle contraction is so fast that the blood cannot supply oxygen and exogenous fuels, such as glucose and free fatty acids, fast enough (Weber, 1987). In domestic pigeons the large white fibres of the pectoralis muscle contains relatively low numbers and small-sized mitochondria, no fat stores and relatively large amounts of glycogen (Rothe *et al.*, 1987). These white 'fast twitching' fibres serve for brief bursts of phasic activity such as take offs, rapid acceleration or sudden manoeuvres (Rothe *et al.*, 1987) and draw mainly upon readily available, muscular and hepatic carbohydrate stores (Schwilch *et al.*, 1996).

Fairy-wrens have short rounded wings which provide good lift and take-off, and thus are suited to quick movement among and between small, thick patches of cover, rather than to flying long distances (Rowley and Russell, 1997). In most cases, foraging for food involves hopping and jumping with both feet, whether on the ground or within a tangle of branches (Rowley and Russell, 1997). Behavioural studies indicate that male and female fairy-wrens are both more noticeably active during the breeding season. Territory intruders, including other fairy-wrens, cuckoos and predators, are vigorously chased away and the birds are also building nests and feeding young (Higgins *et al.*, 2001). All this

additional activity in spring presumably increases the amount of time spent flying. In particular, observations reveal fairy-wrens undertake a lot of brief bursts of phasic activity, described earlier to mainly use carbohydrates as a fuel. Further, in this study peak aerobic activity, as determined by *CS* activity, occurred in spring, further indicating an increased flight capacity at this time. It is possible that the increase in activities associated with breeding is fuelled in part at least, by intramuscular glycogen. This accords with the argument in Chapter 2, that increased activity in fairy-wrens during spring breeding explains the observed seasonal changes in the blood *OCC* concerned with oxygen transport.

It is unlikely that metabolic adjustments made by fairy-wrens in spring are directly related to the climate, given the general mildness of day and night temperatures and the relatively high rainfall at this time, compared to the other season.

Winter acclimatisation

For the many small birds exposed to the cold winter temperatures found at high latitudes in the North Temperate Zone, survival is dependent on their ability to sustain high rates of thermogenesis (Marsh and Dawson, 1989). Thermogenic endurance of these birds varies seasonally (see, for example, Dawson and Marsh, 1989) and is a definitive characteristic of metabolic acclimatisation to cold (Dawson *et al.*, 1983). Regulated heat production in birds is believed to involve shivering of skeletal muscle, in particular the pectoralis and, to a lesser extent, the leg muscles (Cannon and Nedergaard, 1988; Hohtola and Stevens, 1986). During this process, muscle tissue continually contracts and relaxes in an asynchronous pattern and needs to be sustained for extended periods of time (Calder and King, 1974). Lipid is assumed to be the major form of stored energy used to fuel shivering (O'Connor, 1995a), but there is also evidence that glycogen plays some role (Marsh and Dawson, 1982). Under cold conditions, energy stores, whether of lipids or glycogen, substantially increase in winter-acclimatised birds to support thermogenesis (Dawson, 1983a). To determine if

winter acclimatisation was occurring in fairy-wrens, stored muscle and liver glycogen levels and total body lipid content were determined and compared among seasons and also with values for other passerine species in the North Temperate Zone.

Muscle contraction during cold stress in mammals results in increased use of carbohydrates, even under conditions that favour oxidation of fatty acids (see Marsh and Dawson, 1989). Similarly, experiments by Carey *et al.* (1978) on American goldfinches found that winter-acclimatised birds used muscle glycogen under severe cold experimental conditions, and, importantly, that winter muscle glycogen levels were initially higher than those found in summer-acclimatised birds. In contrast, glycogen in both the liver and muscle tissues of fairy-wrens did not differ significantly between summer and winter, as also reported for American goldfinch (Marsh and Dawson, 1982) and House finches (Marsh *et al.*, 1984). In fact, manipulative experiments by Marsh *et al.* (1984) showed that muscle and liver glycogen concentrations did not vary between summer and winter-acclimatised House finches exposed to three different thermal regimes, indicating that the reliance on glycogen in winter is no more than that in summer. Therefore, given glycogen is stable in winter and summer in fairy-wrens, any increase in the rate of thermogenesis in winter is most likely not fuelled by glycogen.

Small passerines overwintering in the North Temperate Zone are known to accumulate larger amounts of lipids in winter than in summer (see for example Swanson, 1991). Excess fat is presumably stored to support the increase in shivering thermogenesis required during long, cold nights and also during short periods of inclement weather conditions, such as snow cover (O'Connor, 1995a). Seasonal analyses of relative body lipid and stored glycogen levels in fairy-wrens showed no significant difference between winter and summer values. Therefore, if metabolic acclimatisation to winter cold does occur, it would appear not to involve an increase in the amount of stored body lipids or glycogen above what is required in other seasons. Further, none of the four key enzymes investigated in this study showed significant changes in activity between summer and winter.

Since a significant increase in the use of either energy source should be accompanied by increased activity of the catabolic enzyme that is involved in their breakdown, it is unlikely that winter metabolic-acclimatisation, if it is occurring, involves changes in fuel usage. This raises the question of how much fat do fairy-wrens need to store to fuel overnight survival in winter.

Lipid requirements of fairy-wrens for overnight survival

Although there was no significant winter fat loading in fairy-wrens, it is possible that the bird's overnight fuel requirements are relatively low, and that the amount of fuel, in the form of fat, required for overnight survival in winter is stored all year round by the birds. The amount of stored body lipids available to fuel overnight thermogenesis in fairy-wrens could not be determined in this study, as birds were trapped in the morning, after fasting overnight. But, if we assume that lipids have an energy content of 39.7 kJ / g (see Blem, 1990), theoretically fairy-wrens had about 15.9 kJ (39.7 kJ / g × 0.40g) of fat available for catabolism in winter after fasting overnight. Existence metabolism regression equations from Kendeigh *et al.* (1977), indicate a 9g passerine would require approximately 55 kJ / day (24 hours) assuming an overnight temperature of 0°C for 10-15 hours. Therefore, after fasting overnight in winter, fairy-wrens theoretically still have enough stored fuel to survive for about 7-8 hours without feeding. Although in practice birds usually succumb to starvation before all stored body lipids are used up (Blem, 1990), fairy-wrens at low altitude are not commonly exposed to temperatures as low as 0°C and so their energy requirements would be even lower than estimated here.

Winter fat loading in North Temperate Zone bird species has been shown to provide fuel for survival, in some cases for several days; for example, winter-acclimatised Dark-eyed juncos in Ohio have sufficient body lipids to last 72 hours (Ketterson and Nolan, 1978). Most of these species mentioned in this study live in regions in which it snows in winter. Snow cover, snow storms and decreased daylength would understandably make feeding, whether on seeds or invertebrates, difficult and unpredictable. In fact, seasonal changes in body lipid

levels appear to be most common for ground foraging birds such as sparrows and finches (Blem, 1990). Therefore it is possible that excess lipids in such species are accumulated as insurance for periods of inclement weather when foraging for extended lengths of time is impossible or difficult. However, fairy-wrens in this study are restricted to regions that never have snow, as such foraging near the ground for invertebrates is never impeded, possibly negating the need for energetically expensive winter lipid accumulation.

CHAPTER 4

SEASONAL CHANGES IN RELATION TO METABOLIC RATE AND AMBIENT TEMPERATURE FOR FAIRY-WRENS.

INTRODUCTION

In order to maintain their constant body temperature and high levels of activity, birds generally have a relatively high metabolic rate, which scales allometrically, such that smaller species have higher mass specific energy requirements (del Hoyo *et al.*, 1992). Metabolic rate is also influenced by the bird's habitat, with desert species usually having reduced rates compared to similarly-sized birds in more mesic regions (Tieleman and Williams, 2000; Williams and Main, 1976). In temperate regions, metabolism in birds resting at low T_a is often increased to produce heat via shivering thermogenesis (Swanson, 1991). However, such cold-induced seasonal adjustments to metabolic rate has to be made in relation to the energetic costs associated with thermoregulation (Dawson *et al.*, 1983a).

The most prominent metabolic adjustments to low temperatures, particularly in some birds resident in the North Temperate Zone, involve enhanced shivering endurance and increased 'summit' metabolism (O'Connor, 1995a). The increases in metabolic rate needed to sustain shivering in extreme climates typically involve higher fuel requirements and this is often reflected in a significant increase in body lipid reserves. For example, significant increases in metabolic rate and body fat content were reported in winter-acclimatised House finches in Michigan compared to birds trapped in summer (O'Connor, 1995a). Similarly, increases in maximum oxygen consumption ($\dot{V}O_{2\text{-max}}$) and cold tolerance, which characterise winter metabolic-acclimatisation in Dark-eyed juncos, were accompanied by a significantly heavier body mass stemming from a higher body fat content than that recorded in summer (Swanson, 1990a).

Other metabolic responses to low T_a include changes in basal metabolic rate (*BMR*). However, the direction of change in *BMR* in winter varies; it is recorded as increasing in the Black-capped chickadee (*Parus atricapillus*), but remaining unchanged in American goldfinches relative to summer levels (Cooper and Swanson, 1994; Dawson and Carey, 1976). Further, both these species were

reported to have a relatively low, Lower Critical Temperature (T_{LC}), in winter than in summer, due to seasonal differences in thermal conductance. Given resting metabolic rate (*RMR*) below thermoneutrality is dependent on the rate of thermal conductance, improving thermal conductance effectively leads to a lower *RMR* (Schmidt-Nielsen, 1990). Thermal conductance is reduced in birds that are well insulated by high subcutaneous body fat levels and, in particular, an increased feather mass (Dawson *et al.*, 1983a). Seasonal *RMR* changes linked to changes in plumage mass have been recorded in several North Temperate Zone species, but the physiological importance of such changes is often considered to be minor. This is thought to be due to the relatively small size and hence large body surface area to volume ratio of the birds involved and the extreme climates in which they live (see for example Swanson, 1991; Dawson and Carey, 1976).

Investigations into seasonal variation in metabolic rate in birds resident in Australia are few. From the studies examining metabolism in small passerines found in temperate, low altitude regions of Australia, there is a strong indication that seasonal variation in thermal energetics exists. However, unlike the situation in numerous small North Temperate Zone species, seasonal variation in body mass, linked to fat deposition, is uncommon in birds in Australia, in which seasonal changes in metabolism have been reported. Two Australian bird species from temperate and arid regions respectively, the Silvereye and White-browed scrubwren, exhibit a significant seasonal change in *BMR*, but with little associated change in body mass (Maddocks and Geiser, 2000; Ambrose and Bradshaw, 1988).

The aim of this section of the study was to determine whether wild-trapped fairy-wrens, which show seasonal variation in plumage mass, but do not exhibit significant fat-loading, exhibit seasonal-acclimatisation in their thermal energetics. This was approached by investigating seasonal changes in metabolic rate, as measured by oxygen consumption, over a range of T_a naturally encountered by fairy-wrens in the wild. This allowed calculation of the approximate temperature range defining the zone of thermoneutrality, within which *BMR* was estimated, and the relation of *RMR* to T_a below this zone, for

fairy-wrens in winter, spring and summer. These parameters were compared to those of similarly sized passerine species in both the North and South Temperate Zones.

METHODS

CAPTURE AND PROCESSING

Metabolic rates, measured as oxygen consumption, $\dot{V}O_2$ (mL $O_2 \cdot hr^{-1}$), were determined for 78 fairy-wrens, 54 of which were wild-trapped at Braeside Park using mist nets (as outlined in General Methods in Chapter 1). The other 24 birds were captured by Alan Lill at Koomba Park in Wantirna (Victoria), approximately 22km east of Melbourne, in the winter and summer of 1994/95. This park is also less than 50m in altitude and contains similar habitat and has similar weather conditions to Braeside Park. The birds from Braeside were captured in winter, spring and summer during the years 1998-2001 with 17, 18 and 19 birds trapped in each season, respectively. On each visit to the site, one or two fairy-wrens were captured within three hours of sundown and transferred to the laboratory, usually within one hour of capture, in separate cloth bags. All birds were kept in bags in the dark at room temperature for several hours to ensure they were post-absorptive. Data were pooled over all study areas and the total numbers of fairy-wrens and the sex ratio (male : female) used in the calculation of seasonal metabolic rates were 32 (16 : 16), 18 (7 : 11) and 28 (13 : 15), in winter, spring and summer, respectively.

MEASUREMENT OF OXYGEN CONSUMPTION RATE

$\dot{V}O_2$ in fairy-wrens was measured in an open-circuit respirometry system similar to the method described by Withers (2001) and (1977). All tubing used in the system was impervious to gas flux. Individual fairy-wrens were placed in a cylindrical, clear, perspex metabolic chamber with a volume of approximately 1500cm^3. The chamber had a wire mesh floor and terminal entry and exit points to permit airflow. The chamber was positioned in a thermostatically controlled-

temperature cabinet. The T_a in the cabinet was kept constant during the experiment, with each fairy-wren being exposed to a single temperature in the range of 0 - 40°C. Each bird was placed in a chamber in the dark with an air flow going through it for at least one hour prior to any data collection to allow for temperature-acclimatisation and physical settling of the bird. Measurement of oxygen consumption rate commenced after sunset (approximately 18:00 in winter and 21:00 in summer), so that the values reported are for post-absorptive birds in the inactive phase of their daily cycle at constant T_a.

The flow-rates of air used in the experiments ranged between 7.0 - 17.7 $L \cdot hr^{-1}$ and were regulated upstream by a *Bronkhorst Hi-tec* flow rate meter. Whilst fractional concentrations of oxygen were determined for air leaving the chamber using either an *Analytical Development Company 7000* oxygen analyser or an *Applied Electrochemistry S-3A / I* oxygen analyser. The flow rate meter, gas analyser and a digital thermometer attached to a thermocouple inserted 1cm into the chamber were all linked to a computer with customised software used to record the flow rate (± 0.01 $L \cdot hr^{-1}$), fractional oxygen concentration (± 0.01%) and temperature (± 0.1°C) of the air leaving the chamber. Each variable was measured automatically at regular time intervals, usually every 30 seconds over a one-hour period, giving approximately 120 readings. The flow rate was adjusted until the fractional oxygen concentration was maintained above 20.4%, usually around 20.5 to 20.8%. T_a within the chamber were kept constant (± 0.5°C) for the entire experiment. Soda-lime ($Ca[OH]_2$ and NaOH: *ADC*, 8-14 mesh), a CO_2 absorbent material and colour-indicator silica gel (precipitated silicic acid) removed CO_2 and H_2O vapour, respectively, from the air supplied to the chambers. Before measuring the oxygen concentration of air leaving the chamber, the air entering the gas analyser was dried using the desiccant anhydrous calcium sulfate (Drierite, 8 mesh; *Hammond* Co.) and the CO_2 again removed with soda-lime. The gas analysers were calibrated each experimental day before use with dry, CO_2-free room air and pure nitrogen (100%), which have oxygen concentrations of 20.95% and 0%, respectively. The flow rate meter was calibrated periodically, and the electronic thermocouple was calibrated

frequently against a standard spirit thermometer. A diagram of the complete metabolic system is shown in Appendix: Table IX.

Each bird was used in only one experiment and at a single temperature, except where stated, to achieve statistical independence. Each bird was weighed (± 0.01g) before and after each experiment and the mean of the two weights was used for the calculation of mass-specific metabolic rate. A constant rate of mass loss throughout the test period was assumed. All fairy-wrens survived and remained healthy during the experiments; they were banded and released at their collection site early the following morning.

Temperature step-down experiments

In addition to the above experiments, one additional fairy-wren captured in each of the three seasons was used in a temperature 'step-down' experiment. This was to ensure that sudden exposure of a fairy-wren resting at room temperature to low T_a had a similar effect on oxygen consumption as being slowly acclimatised over several hours. The procedure was identical, except that each bird was subjected to three different temperatures during an experiment, instead of just one. The temperature in the metabolic chamber was 'stepped-down', starting at room temperature, about 21°C, through to 15°C and then 10°C. Measurements were made for one hour at each temperature, with one additional hour being allowed for acclimatising at each temperature.

CALCULATIONS AND DATA ANALYSIS

Steady-state oxygen consumption, $\dot{V}O_2$ in mL $O_2 \cdot hr^{-1}$, was used as a measure of resting metabolic rate. This was calculated using Equation-2 of Hill (1972) for a system with upstream flow monitoring and fractional concentrations of oxygen measured in dry, CO_2-free air (see also Withers, 2001).

$$\dot{V}O_2 = \dot{V}'I \; \frac{F'IO_2 - F'EO_2}{1 - F'EO_2}$$

Where:

$\dot{V}O_2$ = the volume of oxygen consumed ($mLO_2 \cdot hr^{-1}$).

$\dot{V}'I$ = the volume of dry, CO_2-free incurrent air flowing into the metabolic chamber ($mL \cdot hr^{-1}$).

$F'IO_2$ = the fractional concentration of oxygen in dry, CO_2-free incurrent air (%).

$F'EO_2$ = the fractional concentration of oxygen in dry, CO2-free excurrent air (%).

This permitted the calculation of $\dot{V}O_2$ independent of variations in the respiratory quotient, as suggested by Depocas and Hart (1957). The mean temperature, flow rate and fractional oxygen concentration derived from all 30 second measurements taken over the one hour were used in the calculations and hence $\dot{V}O_2$ was calculated as mean steady state $\dot{V}O_2$ for the one hour test period.

Metabolic rate within the thermoneutral zone, as estimated by $\dot{V}O_2$, did not vary significantly as a function of T_a and is alluded to in this study as *BMR*. *BMR* is often used as a baseline for assessing the relative cost of heat or cold defence (Kendeigh *et al.*, 1977). Total and mass-specific *BMR* are presented as the mean ± standard error, with seasonal values being compared using a one-factor *ANOVA* followed by *Post hoc* Tukey's multiple-comparison tests. Below the thermoneutral zone, the metabolic rate of resting fairy-wrens increased with decreasing T_a and is referred to as *RMR* (see Maddocks and Geiser, 1997 and 2000). Linear regression lines that best describe the relationship between T_a and $\dot{V}O_2$ for each season were fitted by the method of least-squares. Comparison of seasonal regression lines was made by analysis of covariance (*ANCOVA*), with T_a being the covariate, as suggested by Zar (1984). The T_a at the intersection of the regression line describing *RMR* and the horizontal line representing *BMR* was defined as the Lower Critical Temperature (T_{LC}) and was calculated for each season. The mass of birds used to calculate values of mass-specific *RMR* was compared among seasons using a one-factor *ANOVA*, followed by *Post hoc* Tukey's multiple-comparison tests.

RESULTS

TEMPERATURE INDUCED CHANGES IN THE METABOLIC RATE OF SEASONALLY-ACCLIMATISED FAIRY-WRENS

Resting metabolic rate

The mass-specific metabolic rates of resting fairy-wrens over a range of T_a in winter, spring and summer are shown in Figure 4.1. The relation of mass-specific resting metabolic rate (*RMR*) (mL $O_2 \cdot g^{-1} \cdot hr^{-1}$) to T_a (°C) below the thermoneutral zone was best described by the following linear regression equations:

$RMR = 7.88 - 0.18 \cdot T_a$ ($n = 19$; $r^2 = 0.71$) for winter,
$RMR = 7.98 - 0.19 \cdot T_a$ ($n = 11$; $r^2 = 0.73$) for spring, and
$RMR = 8.98 - 0.22 \cdot T_a$ ($n = 22$; $r^2 = 0.52$) for summer.

A comparison of the above regression equations by *ANCOVA* indicated that they are not significantly different ($F_{(2,47)} = 0.725$, $P = 0.489$). Extrapolation of the three regression lines to zero metabolic rate gives values of 43, 42 and 41°C for winter, spring and summer, respectively; these values are close to nocturnal body temperature, which is approximately 41-42°C in small passerines (Withers, 1982). This indicates close conformity to the Newton-Scholander cooling model of heat exchange (Scholander *et al.*, 1950). Consequently, thermal conductance (mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot °C^{-1}$), which is equivalent to the slope of the regression line of *RMR* on T_a below thermoneutrality (assuming that this line extrapolates to close to core body temperature at zero metabolism) is 0.18, 0.19 and 0.22 for fairy-wrens in winter, spring and summer, respectively. These values do not vary significantly ($F_{(2,47)} = 0.725$, $P = 0.489$).

The sex ratio (male : female) of the sampled birds was similar in summer (0.92 : 1) and winter (0.90 : 1). However, the mean mass of birds in summer

used in the calculation of *RMR* (9.53g, n = 23) was significantly greater than mean winter mass (8.84g, n = 19) (F =9.068, $P < 0.005$). As $\dot{V}O_2$ is influenced by body mass, the regression equations given above are reported on a mass-specific basis. However, Swanson (1990a) suggested that seasonal comparisons might be more realistic using per bird $\dot{V}O_2$ to allow for mass gain due to metabolically inert tissues, such as fat. Given that feathers are also metabolically inert and mass was significantly greater in winter than in summer, $\dot{V}O_2$ is also expressed here in per bird terms. Thus the relation of total *RMR* (mL $O_2 \cdot bird^{-1} \cdot hr^{-1}$) to T_a (°C) below thermoneutrality is best described by the following regression equations:

$RMR = 69.7 - 1.55 \cdot T_a$ (n = 19; r^2 = 0.60) for winter,
$RMR = 78.0 - 1.91 \cdot T_a$ (n = 11; r^2 = 0.61) for spring, and
$RMR = 86.9 - 2.24 \cdot T_a$ (n = 22; r^2 = 0.55) for summer.

The linear regression equations describing winter and summer are significantly different ($F_{(1,38)}$ = 5.249, P = 0.028). The slope of the linear regression equation for spring was not significantly different to either winter or summer ($F_{(1,26)}$ = 1.216, P = 0.280 and $F_{(1,29)}$ = 0.845, P = 0.365, respectively). Thermal conductance derived from the above equations was 1.55, 1.91 and 2.24 mL $O_2 \cdot bird^{-1} \cdot hr^{-1} \cdot °C$ for winter, spring and summer, respectively. This indicates that fairy-wrens had a significant 45% reduction in loss of body heat to their surrounds in winter compared to summer. Figure 4.2 shows the linear regression of *RMR* on T_a below the thermoneutral zone in both total and mass-specific terms for all three seasons.

Basal metabolic rate

The average metabolic rate for the temperature range 25-35°C was designated as the basal metabolic rate (*BMR*) of fairy-wrens in all three seasons. The mean (± standard errors) values were 2.96 ± 0.19 (n = 10), 3.00 ± 0.20 (n = 4) and 2.65 ± 0.05 (n = 4) mL $O_2 \cdot g^{-1} \cdot hr^{-1}$ in winter, spring and summer,

respectively. These rates did not differ significantly (F = 0.662, P = 0.53) although the sample sizes are rather small. Using the allometric equation of Bennett and Harvey (1987), appropriate for passerines ($BMR = 0.778 \times (\text{body mass})^{0.67}$), and the mean seasonal mass of birds used in the current metabolic experiments, *BMR*s of fairy-wrens were calculated to be 83, 90 and 94% of the predicted values in summer, winter and spring, respectively. Values for total basal metabolic rate (mL $O_2 \cdot bird^{-1} \cdot hr^{-1}$) were 27.3 ± 1.8, 28.6 ± 2.6 and 25.3 ± 1.6 for winter, spring and summer, respectively; these were also not significantly different (F = 0.420, P = 0.66). The total and mass-specific metabolic rate of fairy-wrens increased sharply at T_a above thermoneutrality (approximately > 35°C), for all three seasons (see Figure 4.1).

Lower critical temperature

In winter, spring and summer, T_{LC} was 28, 26 and 28°C, respectively. These values were almost the same when mass-specific rather than total *RMR* was used in the calculations.

THE EFFECT OF TEMPERATURE STEP-DOWN ON *RMR*

There were no apparent differences in mass-specific *RMR* at a given temperature between the three fairy-wrens tested at multiple temperatures and those measured at a single temperature. After approximately 5-6 hours of temperature step-down to 10°C, fairy-wrens in winter, spring and summer had a mean $\dot{V}O_2$ of 6.84, 6.63 and 7.50 mL $O_2 \cdot g^{-1} \cdot hr^{-1}$. These values were very similar to those calculated from the linear regression equations describing the relation of *RMR* to T_a in winter, spring and summer, which yielded values of 6.08, 6.08 and 6.78 mL $O_2 \cdot g^{-1} \cdot hr^{-1}$ at 10°C, respectively.

Table 4.1 A summer versus winter comparison of *BMR* (mass-specific and total) and the relationship of mass-specific *RMR* to ambient temperature below thermoneutrality of fairy-wrens and four other small passerine species.

BMR values are mean ± standard error, with the sample size in parentheses.
T_{LC} = Lower Critical Temperature (estimated), T_a = ambient temperature
Sum = summer, Win = winter, with mean body mass (g) of bird given in parentheses
Significant differences between seasons ($p < 0.05$) indicated by: < or >.
= means no seasonal difference.

Species Source	Season (Mass)	*BMR* Mass specific (mL O_2 g^{-1} hr^{-1})	 Total (mL O_2 hr^{-1})	Linear Regression Equation	T_{LC} (°C)
Superb fairy-wren	**Sum**	**2.65 ± 0.05**	**25.3 ± 1.6**	***RMR*** **= 8.98 - 0.22 * T_a**	**28**
Malurus cyaneus	**(9.5)**	**(4)**	**(4)**	**r^2 = 0.38 (22)**	
	Win	**2.96 ± 0.19**	**27.3 ± 1.8**	***RMR*** **= 7.88 - 0.18 * T_a**	**28**
	(8.8)	**(10)**	**(10)**	**r^2 = 0.71 (19)**	
Current study		**Sum = Win**	**Sum = Win**		
Australian silvereye	Sum	2.88 ± 0.14	31.4 ± 1.6	*RMR* = 8.50 - 0.22 * T_a	25
Zosterops lateralis	(10.9)	(9)	(9)	r^2 = 0.87 (24)	
	Win	2.30 ± 0.10	25.8 ± 1.2	*RMR* = 6.62 - 0.16 * T_a	27
	(11.2)	(8)	(8)	r^2 = 0.92 (25)	
Maddocks and Geiser (2000)		Sum > Win	Sum > Win		
Black-capped chickadee	Sum	3.54 ± 0.14	43.8 ± 1.7	*RMR* = 9.12 - 0.28 * T_a	20
Parus atricapillus	(13.1)	(10)	(10)	r^2 = 0.87 (12)	
	Win	4.05 ± 0.08	51.6 ± 1.7	*RMR* = 7.88 - 0.24 * T_a	16
	(13.0)	(10)	(8)	r^2 = 0.79 (14)	
Cooper and Swanson (1994)		Sum < Win	Sum < Win		
American goldfinch	Sum	4.24 ± 0.14	54.0 ± 1.8	*RMR* = 8.37 - 0.17 * T_a	24
Spinus tristus	(12.8)	(18)	(18)	r^2 = 0.96 (29)	
	Win	4.65 ± 0.09	67.2 ± 1.1	*RMR* = 7.73 - 0.16 * T_a	19
	(14.5)	(16)	(18)	r^2 = 0.94 (67)	
Dawson and Carey (1976)		Sum = Win	Sum = Win		
Dark-eyed junco	Sum	3.16 ± 0.07	52.2 ± 1.2	*RMR* = 7.32 - 0.19 * T_a	22
Junco hyemalis	(17.8)	(11)	(11)	r^2 = 0.93 (23)	
	Win	3.45 ± 0.13	61.2 ± 2.4	*RMR* = 6.80 - 0.13 * T_a	20
	(19.4)	(8)	(8)	r^2 = 0.80 (21)	
Swanson (1991)		Sum < Win	Sum < Win		

Table 4.2 A comparison of oxygen consumption ($\dot{V}O_2$) of fairy-wrens at estimated mean nocturnal ambient temperature in winter, spring and summer.

T_a = ambient temperature

T_{LC} = Lower Critical Temperature (estimated); these values were calculated on a per bird basis.

T_{diff} = the temperature difference between T_{LC} and T_a.

	WINTER	SPRING	SUMMER
Mean Seasonal Daily Minimum T_a (°C)	**6.4**	**9.4**	**13.5**
T_{LC} (°C)	28	26	28
T_{diff} (°C)	21.6	16.6	14.5
Thermal Conductance below T_{LC} (mL O_2 bird^{-1} hr^{-1} °C^{-1})	1.55	1.91	2.24
$\dot{V}O_2$ (mL O_2 bird^{-1} hr^{-1}) at Mean Seasonal Minimum T_a	33.5	31.7	32.5
$\dot{V}O_2$ (mL O_2 bird^{-1} hr^{-1}) at T_{LC} *(*to maintain *BMR)*	27.3	28.6	25.3
Total Oxygen Consumption (mL O_2 bird^{-1} hr^{-1}) at Mean Seasonal Minimum T_a	**60.8**	**60.3**	**57.8**

Figure 4.1 The relation between ambient temperature and mass-specific metabolic rate ($\dot{V}O_2$) of seasonally-acclimatised fairy-wrens in winter, spring and summer.

Each point is for one bird resting at night.

The horizontal line in the thermoneutral zone represents *BMR*.

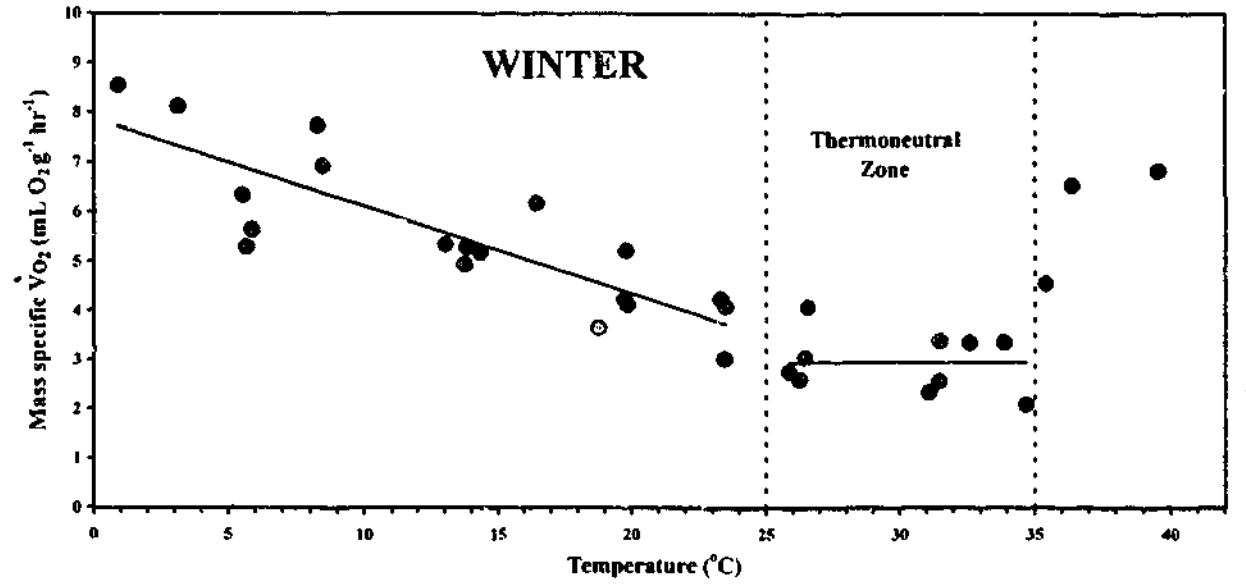
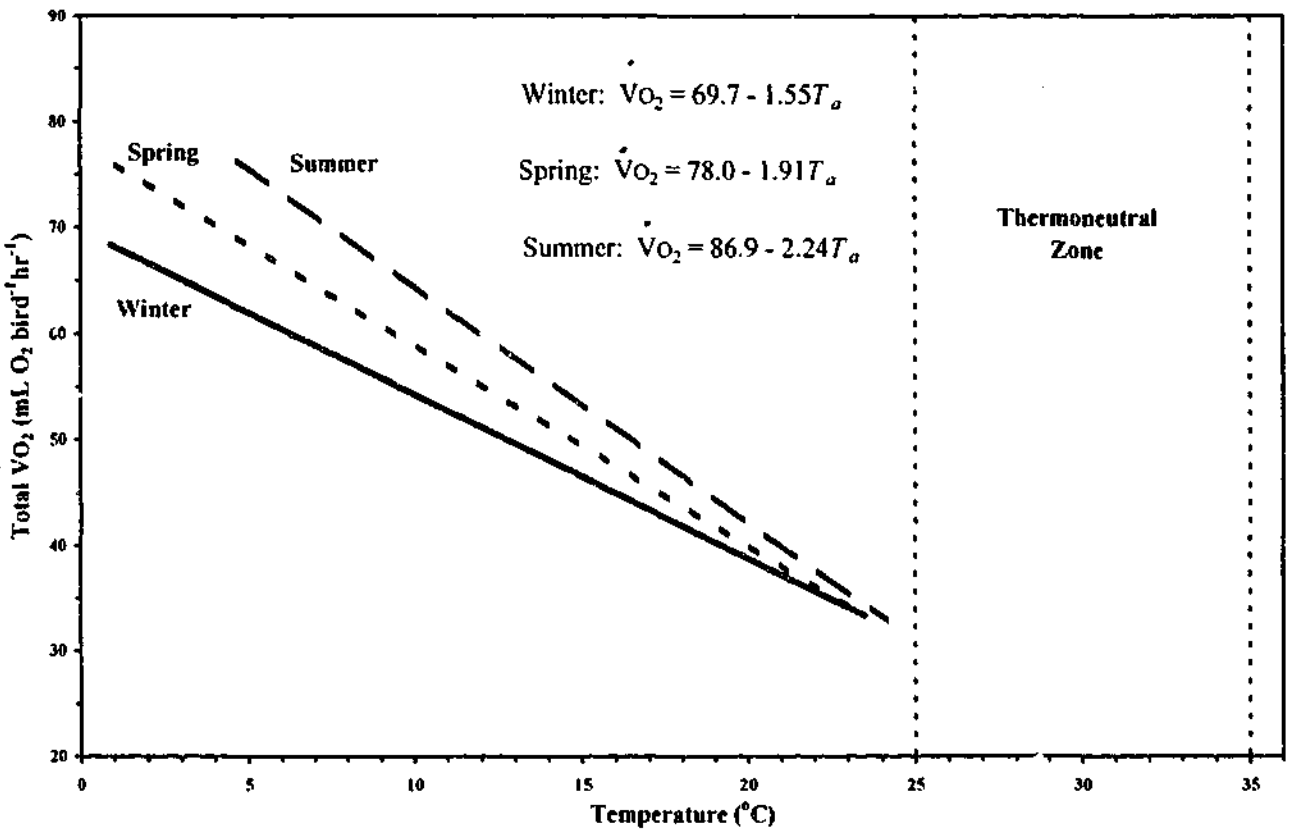
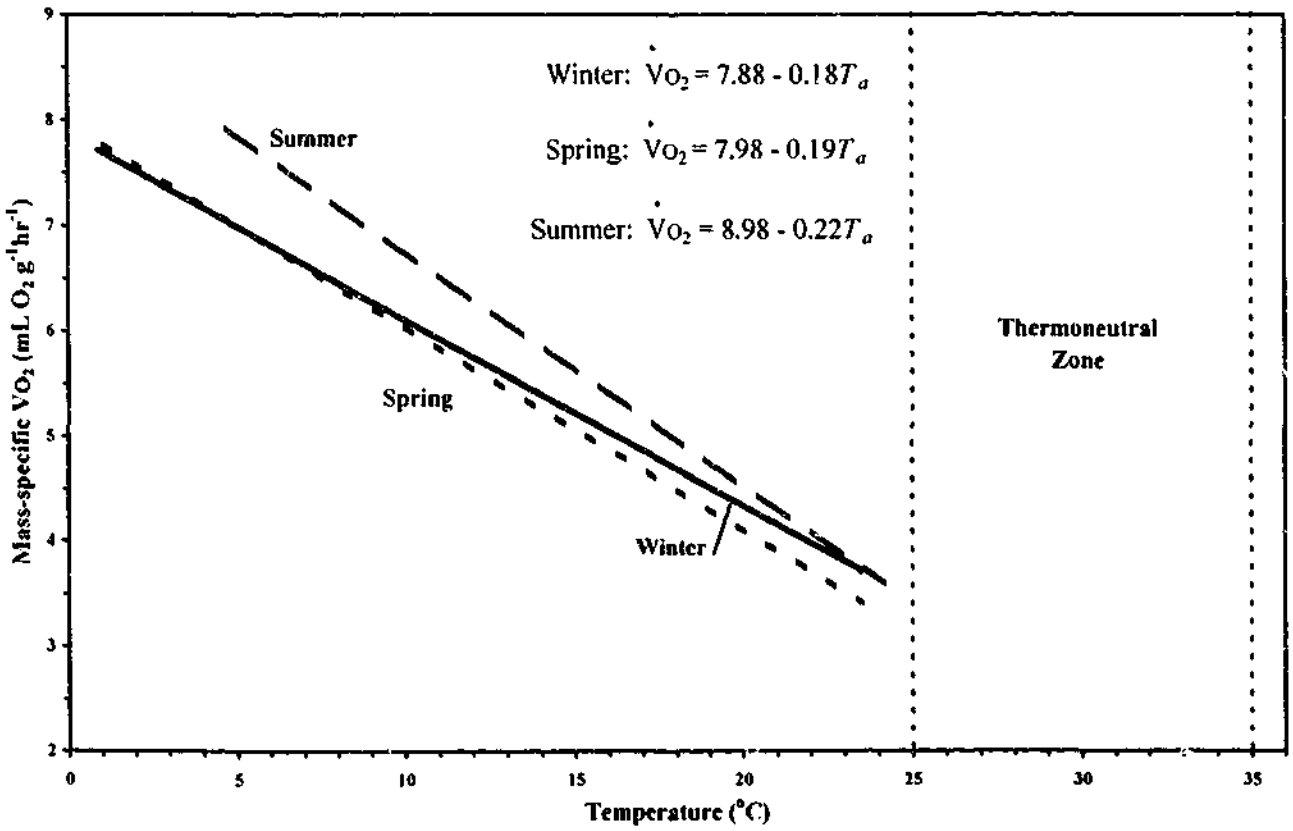


Figure 4.2 Seasonal linear regression equations expressing the relation of total and mass-specific $\dot{V}O_2$ to ambient temperature below thermo-neutrality in winter, spring and summer.

Linear regression equations are shown for each season.





DISCUSSION

BASAL METABOLIC RATE OF FAIRY-WRENS AND OTHER SMALL PASSERINES

The influence of body mass on BMR

Avian *BMR* is highly correlated with body mass (Aschoff and Pohl, 1970; Bennett and Harvey, 1987). Passerines tend to have higher *BMR*s than birds of other orders when appropriate mass scaling is conducted (Dawson and O'Connor, 1996). Summer and winter *BMR*s of fairy-wrens were similar to those for silvereyes, a comparably-sized bird of temperate south-east Australia (Table 4.1), and also with those predicted using the allometric equation for passerines, of Bennett and Harvey (1987). However, *BMR*s for these two south temperate species appear to be somewhat lower than the admittedly slightly heavier North Temperate Zone species listed in Table 4.1. Other variables that influence *BMR* include diet, flight pattern, feeding behaviour and climate (see Dawson and O'Connor, 1996).

The influence of climate and season on BMR

Intraspecific correlation between *BMR* and climate is evident in numerous North Temperate Zone passerine species. In fact, Weathers (1979) suggests that, in general, *BMR* is broadly correlated with climatic variation in that it changes by about 1% per degree change in latitude. Thus House finches (*Carduelis mexicanus*) exposed to the winter cold of Colorado, Michigan and Ohio have significantly higher *BMR*s than conspecifics resident in southern California, where winter is milder (Root *et al.*, 1991). Similarly, *BMR* in House sparrows (*Passer domesticus*) from warmer climates in North America was found to be lower than in their counterparts further north (Hudson and Kimzey, 1966).

Seasonal variation in *BMR* within resident populations of small passerine species at particular locations is also apparent. Table 4.1, which summarises data

available on some small, resident passerines for which summer and winter *BMR*s are known, shows that Black-capped chickadees (*Parus atricapillus*) and Dark-eyed juncos (*Junco hyemalis*) undergo significant seasonal shifts in *BMR*. For both species, *BMR* in winter is significantly higher than in summer. However, the evidence for an influence of season on *BMR* in other small passerines is conflicting. Table 4.1 shows that, like fairy-wrens in this study, American goldfinches and House finches in Michigan have a seasonally invariant *BMR* (O'Connor, 1995a). In contrast, a species from the South Temperate Zone, the silvereye, has a higher *BMR* in summer than in winter. In all five species listed in Table 4.1, *BMR* changes / remains the same across seasons, irrespective of whether it is expressed in mass-specific or on a per bird basis.

Birds exhibiting seasonal changes in BMR

Seasonal changes in *BMR* in which metabolic rates are higher in winter-acclimatised birds have been recorded in a number of studies. In addition to those of Swanson (1991) and Cooper and Swanson (1994) listed in Table 4.1, a similar seasonal change in *BMR* has been recorded in free-living 16g Common redpolls (*Acanthis flammea*) (Pohl and West, 1973) and Monk parakeets (*Myiopsitta monachus*) (Weathers and Caccamise, 1978). One of the largest percentage changes in *BMR* was found in Pine siskins (*Carduelis spinus*) held captive at 29 - 32°C, whose *BMR* increased 85% when conditioned to T_a of –2 - 8°C (Gelineo, 1955 – cited by Dawson and Marsh, 1989). Cold-induced increases in avian *BMR* are often accompanied by an increase in thermogenic capacity, as determined by increased shivering endurance and increased $\dot{V}O_{2\text{-max}}$, which is a measure of maximum oxygen consumption maintained over a 10 minute period (Swanson, 1990a). It is believed that these increases are associated with increased cold tolerance, although the adaptive significance is unclear. It has been suggested that a high winter *BMR* might lower the T_a threshold for shivering or perhaps represent an emergency response linked with protection of peripheral tissues from cold injury, particularly in birds experimentally exposed to cold (Dawson and O'Connor, 1996). Alternatively,

Cooper and Swanson (1994) suggest that an elevated BMR might maintain the increased metabolic machinery needed for increased thermogenic capacity.

While an increase in *BMR* in winter is generally associated with improved cold tolerance, Ambrose and Bradshaw (1988) argued that White-browed scrubwrens (*Sericornis frontalis*) in the arid regions of Western Australia actually had a reduced *BMR* in summer. The authors suggested that this was an adaptation that helped to reduce energy expenditure and water loss at the hottest, driest time of the year, because the same species lacked such a seasonal shift in metabolic rate in more mesic areas. Similarly, four species of Australian chats (*Epthianura*) from arid and semi-arid regions had a lower *BMR* in summer than in winter, especially the more xeric species (Williams and Main, 1976). A review of the adjustment of avian metabolic rate to desert environments concluded that the combination of high temperatures, low primary productivity and water scarcity have resulted in lower metabolic and energy expenditure rates (Tieleman and Williams, 2000). Interestingly, silvereyes resident in the mild mesic climates of south-east temperate Australia were also reported to have a higher *BMR* in summer than winter (Maddocks and Geiser, 2000). A shift in *BMR* in this direction is unusual in such environments and the authors suggested that it was related to the increased energy demands associated with seasonal gonadogenesis. Further, the relatively low winter *BMR*s recorded were believed to lower the energy requirements for body maintenance, thereby enhancing survival over winter. This is also known to occur in some mammals (see Feist and White, 1989) and has been previously been proposed by King and Farner (1961) to occur more widely in birds.

Birds with a seasonally stable BMR

Although seasonal changes in *BMR* often occur in parallel with seasonal-acclimatisation, authors are generally unsure whether the changes contribute to acclimatisation or are merely a by-product of it (Dawson and O'Connor, 1996). Winter-acclimatised American goldfinches in Michigan (U.S.A.) have been shown to augment heat production sufficiently to maintain a differential of nearly

100°C between body core and the environment for up to 6-8 hours, whilst in summer their peak metabolic rate is lower and can only be maintained for up to one hour (Dawson and Carey, 1976). However, the *BMR* of these birds does not change seasonally, with values ranging only between 4.24 and 4.65 mL $O_2 \cdot g^{-1} \cdot hr^{-1}$. House finches in southern California, Colorado and Michigan (U.S.A.) have also been recorded in several different studies with *BMR*s that did not vary seasonally despite seasonal-acclimatisation clearly occurring (Dawson *et al.*, 1985; Root *et al.*, 1991; O'Connor, 1995a). The *BMR* of captive Greenfinches (*Carduelis chloris*) and Pine siskins in West Germany also remained unchanged between winter and summer, suggesting that their tolerance to cold was independent of their thermogenic capacity (Saarela *et al.*, 1989).

A *BMR* that does not change seasonally, such as occurs in wild-trapped fairy-wrens, is not necessarily an indicator of the bird's ability to maintain homoeothermy at low T_a and as such, changes in *BMR* are not an obligatory component of seasonal-acclimatisation in birds. (See also Dawson and Marsh 1989, Dawson and O'Connor, 1996 and Saarela *et al.*, 1989). Other aspects of metabolism may be changed and assist in seasonal-acclimatisation.

SEASONAL CHANGES IN LOWER CRITICAL TEMPERATURE

Below T_{LC}, the rate at which higher vertebrates produce heat is dependent on their rate of thermal conductance, which is controlled by insulatory factors. In general, the mammals and birds that are most cold tolerant have their T_{LC} well below core body temperature; for example, the tropical African vidua (*Vidua paradisea*) has a T_{LC} of approximately +30°C, while the T_{LC} of the Arctic gull (*Larus hyperboreus*) is below –30°C (Scholander *et al.*, 1950). This essentially means that the gull has no need to increase metabolic heat production above that produced by *BMR* until T_a fall below this critical temperature.

T_{LC} varied little for fairy-wrens over the three seasons studied, ranging only between 26 and 28°C in total per bird terms. Values for winter and summer

were both similar to those reported for silvereyes, but generally higher than that recorded in passerines resident in the North Temperate Zone (Table 4.1). While T_{LC} of fairy-wrens was the same in summer and winter, the three North Temperate Zone bird species listed in Table 4.1 have a marginally lower T_{LC} in these two seasons. A similar pattern was also recorded in Greenfinches and Pine siskins (Saarela *et al.*, 1989). Seasonal increases in *BMR* are accompanied by a change in T_{LC}, which is usually one to several degrees lower, in birds artificially maintained at cooler T_a than in those maintained at warm ones (see Dawson and Marsh, 1989). The generally lower T_{LC} values found in the northern bird species listed in Table 4.1 reflect the fact that these birds live in much cooler areas than both the fairy-wren and silvereye.

SEASONAL CHANGES IN *RMR* OF FAIRY-WRENS AND OTHER SMALL PASSERINES

Below T_{LC}, the mass-specific *RMR* of fairy-wrens in each of the three seasons investigated increased at a similar rate. The linear equations best describing the relationship between T_a and *RMR* below thermoneutrality in each season extrapolate to close to resting nocturnal body temperature when *RMR* is zero. Consequently, the gradient of these seasonal equations indicates a range of thermal conductance values of 0.18-0.19 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$ amongst the three seasons. This indicates that in spring and summer oxygen consumption increased by approximately 0.19 $mL \cdot g^{-1} \cdot hr^{-1}$ for every 1°C drop in temperature below T_{LC} and by 0.18 $mL \cdot g^{-1} \cdot hr^{-1}$ in winter. These values are similar to that allometrically predicted by Aschoff (1981) for a 9.5g passerine resting at night (0.20 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$).

Similarly, thermal conductance in chickadees and goldfinches did not differ between winter and summer, although values for chickadees (0.24 and 0.28 mL $O_2 \cdot g^{-1} \cdot hr^{-1} \cdot {}^oC^{-1}$ in winter and summer, respectively) appeared somewhat higher than that recorded for other species in Table 4.1 (Cooper and Swanson,

1994; Dawson and Carey, 1976). In contrast, juncos, greenfinches and silvereyes had significantly lower thermal conductance values in winter than in summer (Swanson, 1991; Saarela *et al.*, 1989; Maddocks and Geiser, 2000). Thermal conductance describes the ease of heat transfer from the body core to the environment by radiation, conductance, convection and evaporation (Aschoff, 1981). Thus, it was argued that the lower winter thermal conductance in these three species was probably due to the better plumage insulation in that season.

Winter-acclimatisation in fairy-wrens

Given the highly significant increase in plumage mass of fairy-wrens in winter (see Chapter 3), it is surprising that thermal conductance, determined on a mass-specific basis, was not lower at this time. However, Dawson and Smith (1986) and Swanson (1990a) suggested that using total $\dot{V}O_2$ to estimate total *RMR* in seasonal comparisons is more appropriate than using mass-specific $\dot{V}O_2$. This takes into consideration body mass gains stemming from storage of metabolically inert tissues such as fat and, more importantly in this study, feathers. Interestingly, if the linear regression equations are expressed in terms of total *RMR*, there are distinct and significant differences between the winter and summer equations, with the slope of the winter equation being shallower than that of the summer one. The slopes of the regression equations indicate that thermal conductance was significantly higher in fairy-wrens in summer than in winter, a season when the birds had significantly heavier plumage mass and hence were better insulated. In fact, if we consider thermal conductance and the temperature difference between T_{LC} and the mean minimum T_a for each season, the total oxygen consumed per bird is very similar among seasons (Table 4.2). Calculations indicate that fairy-wrens use approximately 32.5 ± 1.0 mL $O_2 \cdot bird^{-1} \cdot hr^{-1}$ in each season at an T_a equivalent to the overnight minimum for that season, ie. at 6.4, 9.4 and 13.5°C for winter, spring and summer, respectively. This rate of oxygen consumption is in addition to oxygen already required for *BMR*, which ranged between 25.3 and 28.6-mL$\cdot bird^{-1} \cdot hr^{-1}$ over the three seasons. The estimated total $\dot{V}O_2$ of fairy-wrens exposed to the mean seasonal overnight

T_a was 61, 60 and 58 mL $O_2 \cdot bird^{-1} \cdot hr^{-1}$ in winter, spring and summer, respectively. Therefore, it would seem that the reduction in thermal conductance in fairy-wrens in winter through improved insulation played a major role in reducing heat loss to the environment, and reduced the need for a higher metabolic rate, effectively reducing the energy required to maintain homoeothermy.

Improved insulation through increased plumage mass in small birds, particularly those weighing less than 10-15g, is reported to have little thermal benefit, due to their relatively large surface area-to-volume ratio (Dawson *et al.*, 1983a). This has been highlighted by several authors, notably for birds studied in the North Temperate Zone (Swanson, 1991; Dawson *et al.*, 1983; Barnett, 1970). These authors have reported winter increases in plumage mass of the various small passerines under study, but have discounted the importance of this seasonal change due to: (a) the timing of the increase in feather mass and (b) the constraints imposed by the small size and aerodynamic requirements of these birds. Given the more moderate winter climate in south-east Australia than in the parts of North Temperate Zone where these measurements were taken, perhaps the significantly greater insulation carried by fairy-wrens in the colder months allowed them to reduce their metabolic rate at temperatures below the thermoneutral zone relative to that in other seasons. This would have enabled fairy-wrens to cope with lower T_a without significantly increasing energy usage and thus reduced the need to store large amounts of body lipids (see Chapter 3).

EXPERIMENTAL DESIGN AND CALCULATION OF $\dot{V}O_2$

Experimental conditions

Several experimental studies on small passerines of the North Temperate Zone have involved exposing the birds to extremely cold conditions, using T_a that some of the birds would most likely not encounter or encounter very rarely in the wild. For example, Dawson and Carey (1976) exposed American

goldfinches from Michigan (U.S.A.) to temperatures as low as –70°C. These experiments certainly allow the estimation of the maximum $\dot{V}O_2$ and metabolic endurance of the bird, but the significance of the metabolic rates observed after such experimental manipulation of temperature is difficult to gauge, given the artificial conditions to which the birds are exposed. Further complications are introduced by the interpretation of observations on captive birds as used, for example, by Maddocks and Geiser (2000) or Saarela *et al.* (1989). The findings create uncertainty as to whether the observed metabolic changes are a natural response of the species or an artificial one, possibly induced by loss or gain of 'condition' in captivity. In fact, estimates of energy metabolism using measurements of 'existence metabolism' and 'daily energy expenditure' in captive birds can differ by as much as ± 50% from metabolic rates measured directly in free-living birds (Nagy, 1987). In my study, all metabolic rate experiments were performed on wild-trapped fairy-wrens that were exposed to T_a normally encountered by the birds at least at some time in the year.

The effects of temperature step-down on the resting metabolic rate of fairy-wrens

Individual Black-capped chickadees (*Parus atricapillus*) showed no apparent differences in $\dot{V}O_2$ when tested at two or three different temperatures in summer and winter, respectively, and at a single temperature (Cooper and Swanson, 1994). Similarly, $\dot{V}O_2$ in resting fairy-wrens at 10°C (the mean overnight temperature for all seasons at Braeside Park) was similar in winter, spring and summer, regardless of whether the bird was left to acclimate in the metabolic chamber for one hour or the temperature was stepped-down to 10°C gradually over six hours. Thus the $\dot{V}O_2$ of a seasonally-acclimatised fairy-wrens resting in a metabolic chamber was comparable whether the bird was instantly exposed to cold or gradually acclimatised to it.

The calculation of $\dot{V}O_2$ in fairy-wrens

In this study, $\dot{V}O_2$, and hence metabolic rates, have been determined from mean fractional oxygen concentrations. This means that $\dot{V}O_2$ was calculated using the mean volume of oxygen consumed by a fairy-wren over the entire one-hour experiment. This is a time period when the fairy-wren was presumed to have reached a minimum and stable metabolic rate. Often though, a typical oxygen consumption record includes a few quiescent periods interspersed with more variable periods. Consequently, $\dot{V}O_2$ could also be calculated from a subset of experimental values which represent the lowest steady oxygen consumption rate (Withers, 2001). However, the subset of values chosen that best represent the lowest steady oxygen consumption levels is often difficult to determine and is open to subjectivity.

Since the main aim of this study was to compare seasonal differences in metabolic rate, the method used for calculation of $\dot{V}O_2$ should make little difference, provided that the same method of calculation was used for all three seasons. Further, the studies on North Temperate Zone birds summarised in Table 4.1 all used mean oxygen consumption over a 45-60 minute test period. Therefore, it seemed appropriate that all analyses in the present study on fairy-wrens should be performed on mean fractional oxygen concentrations over the full one-hour experiment, thus facilitating realistic interspecies comparisons.

FURTHER STUDY

Many bird species have been reported to exhibit nocturnal hypothermia or torpor in cold regions (Reinertsen, 1996). This effectively allows the birds to reduce their body temperature by 5-20°C and metabolic rate by 30-40%, thus reducing their energy expenditure. This is particular useful for small birds, as the rate of rewarming during arousal is known to be inversely related to their mass (Reinertsen and Haftorn, 1984). In particular, nocturnal hypothermia has been recorded in Willow tits (*Parus montanos*) (Reinertsen and Haftorn, 1983) and

some finches (Saarela *et al.*, 1995), species from the North Temperate Zone that are naturally acclimatised to T_a well below zero. In temperate Australia, silvereyes are known to drop their body temperature 3-5°C overnight, reducing their *RMR* and daily energy expenditure. Two widespread Australian honeyeater species (*Meliphagidae*) found in a variety of habitats also employ mild hypothermia at T_a below 12°C (Collins *et al.*, 1980). Although these species do not commonly engage in group-huddling when roosting overnight as do fairy-wrens, they are of similar size. It would be interesting to determine whether mild nocturnal hypothermia exists in fairy-wrens and reduce their daily energy requirements.

CHAPTER 5

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The aim of this study was to determine the physiological responses to energy challenges that are imposed on free-living Superb fairy-wrens in south-east Australia during their annual cycle. Two seasons were highlighted, spring and winter, in which significant changes in the physiology of fairy-wrens probably contribute to their breeding success and survival at low temperatures, respectively. There were no apparent peaks in any measured physiological parameter that coincided with the time when the majority of birds were moulting. Most of the seasonal changes observed and the conclusions drawn from this study contrast with those reported for similarly-sized passerines in the North Temperate Zone.

Physiological adaptations for breeding in fairy-wrens

Breeding in small birds is thought to involve high energy expenditures for pair-bonding, egg-laying, incubation (Carey, 1996; Williams, 1996), feeding nestlings and fledglings (Weathers, 1996) and territory defence. Of particular importance is that several of these breeding activities require a marked increase in flight activity, which is often reflected in an increase in field metabolic rate and oxygen consumption. Physiological changes stemming from the increased demands of breeding are relatively poorly documented in passerines, but observations in migratory birds can perhaps give us an insight into the likely changes required to meet the increased demands of flight during breeding. Migration in both passerines and non-passerines is commonly preceeded by a period of significant fattening to fuel long distance flight (see Dawson *et al.*, 1983a). Often blood *OCC* also significantly increases to help meet the higher oxygen demands of the flight muscles (Morton, 1994; Piersma and Everaarts, 1996; Davey *et al.*, 2000). During migration, the total aerobic capacity of the pectoralis muscles, as indicated by *CS* activity, is increased (Marsh, 1981). Glycogen also plays an important role during take-off, burst flight and to fuel

glucose-dependent tissues, such as the central nervous system (Rothe *et al.*, 1987; Schwilch *et al.*, 1996).

The breeding season of fairy-wrens extends throughout spring and early summer and often involves several breeding attempts (Rowley and Russell, 1997). The measured activity of *CS* from flight muscle indicated peak aerobic activity, and hence increased flight capacity, in this season compared to the other three seasons. Given the nature of fairy-wren's flight, which typically involves burst flying (Higgins *et al.*, 2001), increased activities associated with breeding are likely to be fuelled by glycogen, which occurred at concentrations twice those found in other seasons. This interpretation is supported by the observed high activities of *PHOS* and *HK*, the enzymes responsible for glucose / glycogen catabolism, which indicate an increased turnover of glycogen in spring compared to winter, summer and autumn. In addition, the increase in flight activity of fairy-wrens in spring would probably result in a higher oxygen demand in the flight tissues, leading to the observed significant increase in blood *OCC*.

Winter acclimatisation in fairy-wrens

Thermoregulatory responses of normothermic birds resident in cold climate areas in the North Temperate Zone include increasing insulation, behavioural thermoregulation and augmented heat production, which generally involve a combination of physiological and morphological adaptations (Dawson and O'Connor, 1996). Typically, insulative changes are observed in small birds in winter, but are found not to be sufficient to ensure survival at the extremely cold overnight temperatures encountered (Swanson, 1991; Dawson *et al.*, 1983). Consequently, the maintenance of a constant body temperature requires heat to be generated via shivering thermogenesis in the pectoralis and supracoracoideus muscles (Connolly *et al.*, 1989). This process often involves asynchronous muscle contraction over extended periods of time (Calder and King, 1974), necessitating the delivery of more oxygen and larger energy supplies to fuel the shivering muscles. Thus seasonal acclimatisation in North Temperate Zone birds mostly involves adjustments in winter of one or several of the following

parameters: plumage mass, blood *OCC*, body weight (due to stored lipid levels), muscle and / or liver glycogen levels and activities of catabolic enzymes involved in the release of chemical energy from lipids and glycogen.

To maintain a stable body temperature, fairy-wrens were required to generate additional heat at any ambient temperature below their T_{LC}, which was approximately 28°C. This T_{LC} is above the mean daily maximum ambient temperatures in all four seasons at Braeside Park, necessitating heat production in fairy-wrens during the day. Activity in birds while foraging, flying and breeding etc., is associated with an elevation in field metabolic rate by a factor of 5-10× the resting rate and substitutes for regulatory thermogenesis in the maintenance of body temperature (Brackenbury, 1984; Dawson and O'Connor, 1996). Overnight, resting fairy-wrens typically encounter cool temperatures, particularly in winter, and therefore must presumably rely on shivering thermogenesis to maintain their core temperature while sleeping throughout the year. A summer versus winter comparison of the physiological parameters commonly associated with seasonal acclimatisation identified only a few differences in fairy-wrens. Their blood *OCC* remained stable. Stored energy reserves in the form of body lipids and glycogen and the activity levels of the enzymes responsible for their catabolism also remained constant between winter and summer. In contrast, plumage mass, and hence the fairy-wren's insulation, increased significantly in winter. This caused a decrease in thermal conductance, leading to a relatively lower *RMR* than in summer. Consequently, the overnight *RMR* of winter-acclimatised fairy-wrens at low ambient temperatures (≅ 6°C) was similar to that of summer-acclimatised birds at relatively warmer temperatures (≅ 14°C). Since a significant increase in *RMR* was apparently not necessary for overnight survival during the colder months, enhanced blood-oxygen delivery and larger stored energy reserves beyond what was utilised in summer were presumably not required. Moreover, behavioural adaptations, such as group huddling and the use of protected roost sites, may assist in fairy-wren survival in cold weather all year round (Rowley and Russell, 1997; Walsberg, 1990).

The marked disparity in winter survival adaptations found between fairy-wrens and many small birds in the North Temperate Zone may be explained by the severity of winter conditions. While fairy-wrens at the study site were rarely exposed to ambient temperatures below 2°C even during the coldest winter nights, many low-altitude north temperate locations are snow-covered and have mean daily ambient temperatures below 0°C in winter (see, for example, Cooper and Swanson, 1994). Further, winter fattening, commonly reported in northern passerines, entails appreciable costs, notably reduced flying efficiency and increased predation risk (Rogers and Smith, 1993). It is possible that the active foraging technique of fairy-wrens (involving mainly gleaning for insects over open ground and fallen branches), the constant threat of predation and the relatively mild austral winter greatly reduce the relative advantage of costly fat storage in fairy-wrens.

The apparent lack of physiological adaptations to moulting in fairy-wrens

The energy costs associated with moulting are believed to be relatively low and are possibly compensated for through decreased locomotor activity (Murphy, 1996). The majority of fairy-wrens captured in this study were found to be in heavy moult in autumn, a time that did not coincide with any significant changes in physiological parameters measured. Conversely, seasonal peaks in blood *OCC*, glycogen levels and catabolic enzymes occurred when moulting was not so pronounced.

FURTHER STUDIES

The observations reported in this study concerning winter-acclimatisation of fairy-wrens are quite different to those found in many of the studies on similarly sized birds resident in the North Temperate Zone. Whilst at low altitude the two regions contrast quite dramatically in winter conditions, it would

be premature to conclude that this fully explains the apparent north - south difference. Additional work supporting these findings is required, preferably on other small Australian bird species. In particular, seedeaters such as finches show the largest seasonal changes in fat deposition amongst northern birds and Australian granivores and would be especially interesting to study in this context. Further study on fairy-wrens resident at higher altitudes (they are found up to at least 1400m above sea level) which experience colder winter temperatures would also be of great value in understanding north - south differences.

The physiological adaptations evident in fairy-wrens in the spring breeding season are most likely to be related to higher flight activity requirements at this time. Seasonal measurements of field metabolic rate could help to confirm this conjecture for fairy-wrens, because a substantial increase in energy demand should be reflected in a higher field metabolic rate. Although there is much research on migration-related flight activity and on energy consumption at each stage of the breeding cycle for north temperate birds, seasonal physiological changes related to increased activity during the breeding season is sparse for small passerines living in both temperate zones. Clearly, additional work on the breeding energetics of small passerines in both the South and North Temperate Zones is necessary to validate the findings of this study.

APPENDICES

Table I. List of abbreviations, symbols and units.

µL	microlitre
µmole	micromole
ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-monophosphate
ANOVA	analysis of variance
ANCOVA	analysis of covariance
ATP	adenosine 5′-triphosphate
BMR	basal metabolic rate
cm	centimetre
CP	creatine phosphate
CPK	creatine phosphokinase
CS	citrate synthetase
dL	decilitre
DTNB	5, 5′-dithiobis-[2-nitrobenzoic acid]
E	east
EDTA	ethylenediaminetetraacetic
fL	femptolitres
g	gram
g	gravitational force
G 6-PDH	glucose 6-phosphate dehydrogenase
G-1,6-diP	glucose-1,6-diphosphate
ha	hectare
Hb	whole-blood haemoglobin
HCl	hydrochloric acid
Hct	haematocrit
HK	hexokinase
HOAD	ß-hydroxyacyl-CoA dehydrogenase
KCl	potassium chloride
$KHCO_3$	potassium hydrogen carbonate

kJ	kilojoule
km	kilometre
L	litre
M	molar concentration
m	metre
mg	milligrams
$MgCl_2$	magnesium chloride
min	minute
mL	millilitre
mm	millimetre
mM	millimole
NaCl	sodium chloride
NAD	Nicotinamide-adenine dinucleotide
NADH	reduced form of NAD
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	reduced form of NADP
nm	nanometre
°C	degrees Celsius
OCC	oxygen carrying capacity
OD	optical density
PCV	packed cell volume
pg	picograms
PGM	phosphoglucomutase
pH	-Log H^+ concentration
PHOS	phosphorylase
Rbc	red blood cell
RMR	resting metabolic rate
S	south
T_a	ambient temperature
T_{diff}	temperature difference
T_{LC}	lower critical temperature

Table II. Original paper published in the *Australian Journal of Zoology*.

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Seasonal variation in body mass and blood oxygen carrying capacity of the superb fairy-wren (*Malurus cyaneus*)

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Abstract

The responses of small birds to many seasonal energy challenges include enhancement of aspects of aerobic metabolism, sometimes involving an increase in the rate of oxygen delivery to the metabolising tissues. One such mechanism that enhances oxygen delivery seasonally is an increase in blood oxygen carrying capacity. This response is enhanced in birds because of their rapid erythrocyte turnover rate. Some small birds have also evolved winter fattening, which helps them to meet the energy challenge presented by winter conditions. Such adaptations, while well documented for North Temperate birds, have received little attention in birds inhabiting temperate Australia.

Over a two-year period, we examined seasonal changes in mass, an approximate indicator of fattening, and the parameters determining blood oxygen carrying capacity in a population of superb fairy-wrens (*Malurus cyaneus*) in outer Melbourne, Australia. Body mass did not vary significantly seasonally, but haematocrit and whole blood haemoglobin were significantly higher in the breeding season than at other times of year and the erythrocyte count was significantly higher in spring than in autumn. We conclude that the failure of the fairy-wrens to increase mass in winter (i.e. show marked winter fattening) was probably due to the comparative mildness of the climate and to the known fitness costs of fat storage. The significant 18% increase in blood oxygen carrying capacity in spring probably helped the birds to meet the additional energy requirements of breeding, particularly the likely increase in flight activity. However, given the magnitude of the increase, other mechanisms must have been involved in meeting breeding costs. The seasonal peak in blood oxygen carrying capacity did not coincide with the time when moulting was most pronounced.

Introduction

Most adult birds face some significant energy challenges during their annual cycle, the ubiquitous ones being those associated with moulting and breeding. Moulting entails an estimated energy cost of 90–1225 kJ g^{-1} feathers produced, although reduced locomotor activity may compensate for this expenditure in some species (Murphy 1996). The estimated cost of producing a single egg is equivalent to 13–41% of the female's daily basal metabolic rate (BMR) in passerines (Carey 1996). Incubation probably involves a significant additional energy cost at temperatures below thermal neutrality too, possibly on the order of 30–60% BMR in small passerines (Williams 1996). Weathers (1992, 1996) showed for a suite of species with various developmental modes that the mass-specific energy cost of producing a 'fledgling' ranged from 15.7–61.8 kJ g^{-1} fledging mass. For 30 mostly altricial species he also showed that the estimated peak daily cost averaged about 1.5× adult passerine rest-phase BMR.

Some passerines that breed in the North Temperate Zone also incur the energy costs of seasonal return migration, which are reflected in pre-migratory fat stores that average 24% of lean body mass, but increase to 40–70% prior to crossing major ecological barriers (Alerstram and Lindstrom 1990). Resident species, on the other hand, must maintain homeothermy at reduced ambient temperatures (T_a) in winter, when food

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resources are less abundant and the photoperiod is minimal. Small passerines overwintering in the North Temperate Zone exhibit enhanced regulatory thermogenesis in winter (Dawson and O'Connor 1996). Some of these species have a higher field metabolic rate during winter than in the breeding season (Weathers *et al.* 1999; Cooper 2000; Doherty *et al.* 2001).

Meeting many of these seasonal energy challenges involves enhancement of various facets of aerobic metabolism (Dawson and Marsh 1989; Dawson *et al.* 1983; Swanson 1991; Murphy 1996). It can sometimes also involve an increase in the oxygen delivery rate to the metabolising tissues, particularly if additional expensive flight activity (Norberg 1996) is involved. This can potentially be achieved through reducing blood oxygen affinity and/or enhancing cardiac output and/or increasing blood oxygen carrying capacity (OCC) (Swanson 1990). This latter mechanism is enhanced in birds by a relatively rapid erythrocyte turnover rate (Rodnan *et al.* 1957). Moulting and long-distance migration are known to be accompanied by an increase in OCC in some sparrows and godwits, respectively (Morton 1994; Murphy 1996; Piersma *et al.* 1996). Some small birds overwintering in the harsh climate of the North Temperate Zone also exhibit a winter increase in the blood parameters determining OCC (Carey and Morton 1976; Clemens 1990; Swanson 1990). However, there have only been a few investigations of seasonal variation in OCC of birds living at low altitudes in temperate Australia, where winter is less harsh, migration less common and the breeding season more protracted than in many of the north temperate locations in which the investigations cited above were conducted (Ford 1989). It is therefore interesting to examine the occurrence and magnitude of seasonal changes in OCC in small birds inhabiting this region of Australia.

Another common overwintering adaptation in small birds in the North Temperate Zone is the accumulation of substantial fat stores in winter (Blem 1990). Stores accumulated daily are crucial in many resident birds in sustaining them during the cold winter night or through the following morning if early foraging conditions are unfavourable. Seasonal fat stores can sustain some birds for a few days when poor foraging conditions prevail (King 1972), although in most species fat stores do not last this long. Again, it is pertinent to determine whether winter fattening has evolved in small birds that overwinter at low altitude in the much milder climate of temperate Australia, particularly in view of the likely costs associated with this adaptation (Witter and Cuthill 1993).

The insectivorous superb fairy-wren (*Malurus cyaneus*) occurs in subtropical and temperate eastern Australia up to approximately 1400 m above sea level in a range of natural and modified habitats that combine dense, low vegetation cover and open areas (Higgins *et al.* 2001). Our aim was to determine whether there were annual patterns of variation in OCC and body mass (an approximate indicator of fat deposition) of resident superb fairy-wrens that corresponded with the timing of the energy challenges posed by winter conditions, breeding and possibly moulting. At low altitude in this species' range, winter occurs from June to August. The pre-breeding moult, involving just body feather replacement and in which males assume nuptial plumage, occurs mainly in winter and early spring. Up to eight breeding attempts may be made in the peak breeding season (September to January) and the mean clutch size is 3.3 eggs. Adults undergo a complete (pre-basic) moult, in which males assume non-breeding plumage, in the period from January to April (Higgins *et al.* 2001). We examined seasonal patterns in body mass and OCC by measuring mass and three key blood parameters (haematocrit, whole blood haemoglobin and red blood cell count) in samples of wild fairy-wrens in all four seasons of the year over two successive years.

Methods

Study species

Superb fairy-wrens weigh 8–12 g. They are territorial year-round and live in heterosexual pairs or groups of up to nine birds, in which the additional individuals are adults, usually males, that fledged on the territory in previous years. These supernumeraries act as helpers to the breeding pair, provisioning the nestlings and defending the territory. Extra-pair paternity occurs at high frequencies in some populations (Rowley 1957; Mulder 1995, 1997; Higgins *et al.* 2001).

Study area

The study lasted from mid-1995 to mid-1997; we refer to the period June 1995 – May 1996 as 'Year 1' and the remainder of the study period as 'Year 2'. The research was conducted in the conservation reserve of Braeside Park (38°00′S, 145°11′E) in suburban Melbourne. The 22-ha native bushland remnant is <50 m above sea level. The vegetation is woody heathland, in which the main canopy tree species are *Eucalyptus camaldulensis*, *E. pryoriana* and *E. radiata* and various acacias. The shrub layer contains tea-trees (*Kunzea* spp.) and banksias (notably *Banksia integrifolia* and *B. marginata*) and the ground cover comprises austral bracken (*Pteridium esculentum*) and various sedges (family Cyperaceae). Mean monthly precipitation ranged from 197 mm in winter to 151 mm in summer. Average daily maximal T_a ranged from 25°C in summer to 14°C in winter and average daily minimal T_a from 13.5°C in summer to 6.4°C in winter.

Data collection

Fairy-wrens were caught in mist nets. In each season, spring (September–November), summer (December–February), autumn (March–May) and winter (June–August), trapping was conducted on several days spread across the season and at several sites. In total, 228 wrens were caught in approximately 265 h of trapping, mainly from sunrise to midday, but sometimes in the afternoon.

Captured birds were banded, sexed from plumage characteristics, weighed (±0.5 g) and examined for moult. Moult was scored by examining the head, neck, body, primaries and rectrices and categorised as 'light' if new feathers were present in 1 or 2 of these areas, or 'heavy' if they occurred in 3 or more areas. A 75-μL blood sample was obtained from each bird by piercing a brachial artery with a 27.5-gauge hypodermic needle and withdrawing the blood by capillarity into heparinised micro-haematocrit tubes. The samples were stored at 0°C until processed in the laboratory within 4 h of collection. Individual birds were sampled only once in order to achieve statistical independence of the data.

Blood variables were measured with standard techniques developed for human blood (Lewis *et al.* 2001). The accuracy of the methods was checked regularly using a whole-blood quality control that had been analysed on a Technicon H2 autoanalyser. Haematocrit (Hct) (%) was determined by centrifugation at 3000 rpm for 5 min. Whole-blood haemoglobin content (Hb) (g per 100 mL blood) was measured by cyanomethaemoglobin spectrophotometry (using an extinction coefficient of 44.0 $mM^{-1} cm^{-1}$ with respect to Hb tetramer) after centrifugation at 13000 rpm for 5 min. The erythrocyte count (RBC) (cells × 10^6 μL^{-1}) was determined with an improved Neubauer haemocytometer. This is the least accurate of the techniques used (Lewis *et al.* 2001) and so each count was repeated three times and the mean value used in analysis. Moreover, to check the repeatability of red blood cell counts, five replicate samples taken from a single fairy-wren in each of the eight study seasons were counted in triplicate. The method used for counting erythrocytes proved to be consistent in that the cell counts of each of the five batches of diluted blood taken from a particular bird were statistically indistinguishable in all eight seasonal samples ($F = 0.315–2.353$, $P > 0.05$ in all cases, single-factor ANOVAs).

From these basic measurements, and with the appropriate corrections to common units, we calculated mean erythrocyte volume (MCV) (in femtolitres, fL) (Hct ÷ RBC) and mean erythrocyte haemoglobin (MCH) (in picograms, pg) (Hb ÷ RBC). Blood oxygen carrying capacity (OCC) (in mL O_2 per 100 mL blood) was calculated as Hb × 1.34, assuming that in birds 1 g of haemoglobin binds 1.34 mL of oxygen (Withers 1992).

Data analysis

Power analysis indicated that a sample size of at least 18 was required to achieve 80% confidence of detecting a 20% seasonal change in any blood parameter. Time constraints precluded sampling 18 birds of each sex in every season and we had to combine measurements of males and females when analysing seasonal variation in the blood parameters. Thus our samples varied from 24 to 35 birds per season, but had

male : female sex ratios ranging from 3 to 0.4. However, seasonal trends in the measured blood parameters were similar in males and females, so these differing sex ratios were unlikely to have influenced seasonal comparisons of blood parameters.

Body mass and blood data were normally distributed, so untransformed values were used in all analyses. Analyses of variance (ANOVA) were used to explore variation in mass and blood parameters between the sexes, among seasons and between years. *Post hoc* Tukey's multiple-comparison tests were used in conjunction with ANOVA to determine the precise nature of significant seasonal variation in blood parameters. Least-squares linear regression analysis was employed to examine the relationship between body mass and the measured blood parameters. A Chi-square Goodness of Fit test was used to determine whether the frequency of moulting differed seasonally.

Results

Seasonal changes in mass and moult

Body mass did not vary significantly among the eight seasons or between the two 'years' of the investigation. Mean seasonal values ranged from 9.8 to 10.1 g for adult males and from 9.1 to 9.6 g for adult females (Table 1). The degree of moult predictably varied significantly among seasons ($\chi^2_{(6)} = 72.9$, $P < 0.01$). In winter, 28% of trapped wrens were in moult; this proportion increased through spring (58%) and summer (75%) and peaked in autumn (87%) (Table 2). The proportion of birds in 'heavy' moult was very low in winter and highest in autumn, whereas the proportion of birds in 'light' moult was lowest in autumn and winter and highest in summer.

Table 1. Seasonal variation in body mass of adult superb fairy-wrens

Values (in grams) are mean ± s.e., with sample size given in brackets. F ratios from three-factor ANOVA examining seasonal mass variation in both sexes over the two-year study period: Main effects – Year ($F = 2.564$, $P > 0.05$), Season ($F = 0.676$, $P > 0.05$), Sex ($F = 35.19$, $P < 0.001$). Interactions – Year × Season ($F = 1.034$, $P > 0.05$), Year × Sex ($F = 0.451$, $P > 0.05$), Season × Sex ($F = 0.859$, ($P > 0.05$), Year × Season × Sex ($F = 0.019$, $P > 0.05$)

Season and year		Mass of male		Mass of female	
Winter	1995	9.9 ± 0.1	[22]	9.3 ± 0.3	[9]
	1996	9.9 ± 0.1	[16]	9.3 ± 0.1	[16]
	Pooled	9.9 ± 0.1	[38]	9.3 ± 0.1	[25]
Spring	1995	10.0 ± 0.2	[16]	9.3 ± 0.3	[8]
	1996	10.1 ± 0.1	[21]	9.6 ± 0.3	[14]
	Pooled	10.1 ± 0.1	[37]	9.5 ± 0.2	[22]
Summer	1995	9.8 ± 0.1	[14]	9.1 ± 0.2	[13]
	1996	9.9 ± 0.2	[10]	9.5 ± 0.1	[17]
	Pooled	9.9 ± 0.1	[24]	9.3 ± 0.1	[30]
Autumn	1995	9.9 ± 0.3	[8]	9.6 ± 0.1	[16]
	1996	9.9 ± 0.2	[12]	9.6 ± 0.1	[14]
	Pooled	9.9 ± 0.2	[20]	9.6 ± 0.1	[30]

Table 2. Seasonality of moult in superb fairy-wrens

Data are numbers of birds, with percentage of total birds for the season shown in parentheses. Light and heavy moult are defined in 'Methods'

Season	No moult		Light moult		Heavy moult	
Winter	44	(72%)	14	(23%)	3	(5%)
Spring	25	(42%)	20	(34%)	14	(24%)
Summer	13	(25%)	24	(45%)	16	(30%)
Autumn	7	(13%)	12	(22%)	36	(65%)

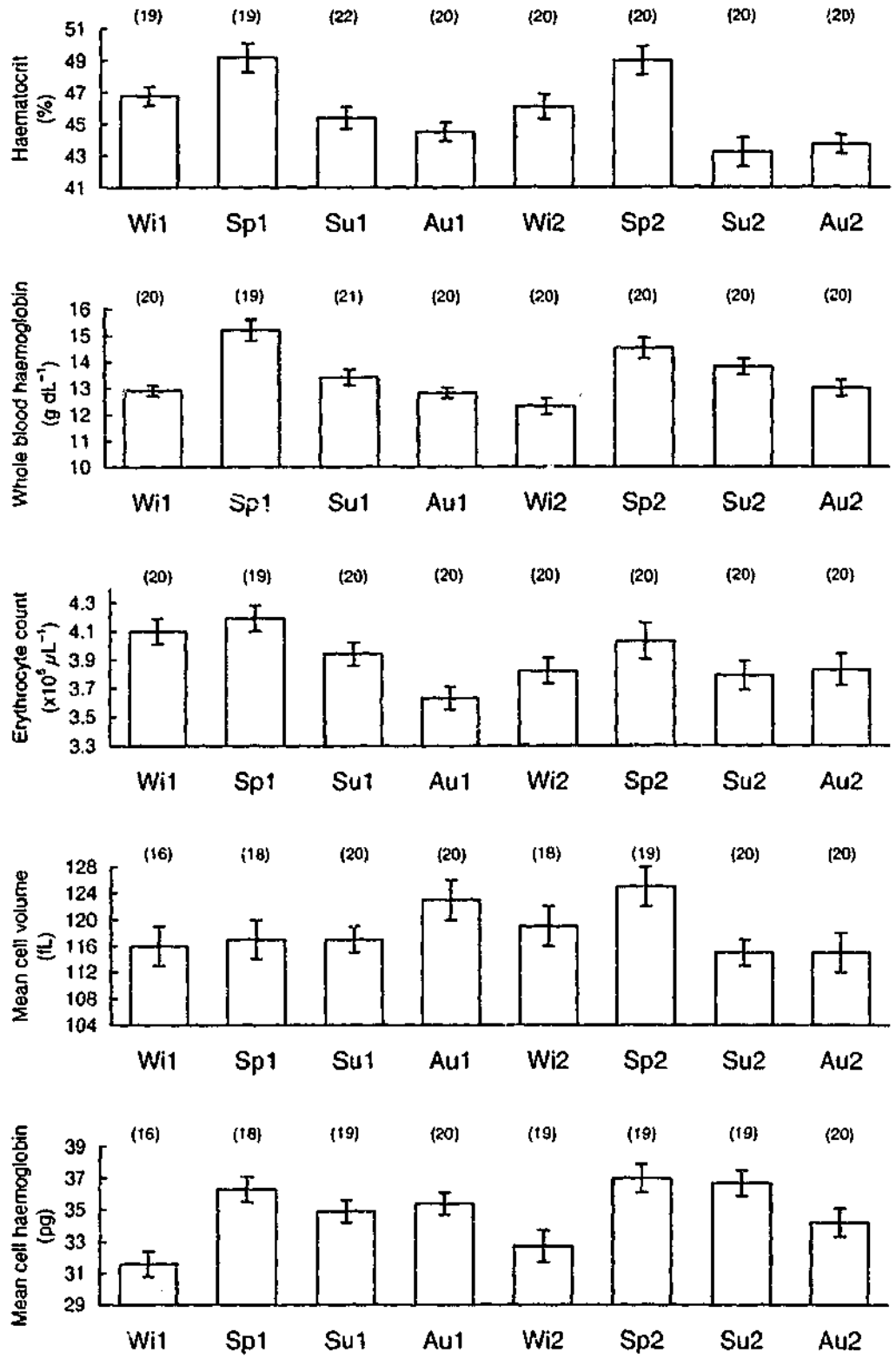

Fig. 1. Seasonal variation in mean (±s.e.) values of blood parameters of superb fairy-wrens. Season labels 1 and 2 refer to 'Year 1' and 'Year 2'. Sample sizes are given in parentheses above each column. Wi = winter, Sp = spring, Su = summer, Au = autumn.

Seasonal changes in blood parameters

Two-factor ANOVA indicated that none of the measured or calculated blood parameters exhibited significant variation between 'years' (Fig. 1) (F = 0.008–3.720 for 'Year' term, $P > 0.05$ in all cases). Hct, Hb, RBC and MCH all varied significantly seasonally (Hct: $F = 20.353$, $P < 0.001$; Hb: $F = 20.389$, $P < 0.001$; RBC: $F = 5.389$, $P < 0.01$; MCH: $F = 11.15$, $P < 0.001$), but MCV did not ($F = 1.565$, $P > 0.05$). There was a significant interaction between 'Season' and 'Year' for MCV ($F = 3.207$, $P < 0.05$), but not for any other parameter (F = 0.733–2.331, $P > 0.05$ in all cases). *Post hoc* Tukey HSD tests ($\alpha = 0.05$) showed that Hct was significantly higher (by 6–11%) in spring than in the other three seasons. Hb exhibited a similar seasonal pattern, but additionally was significantly higher in summer (mean 13.6 ± 0.2 g per 100 mL) than in winter (mean 12.6 ± 0.2). Significant seasonal variation in RBC was restricted to a difference between the spring and autumn means (4.11 and 3.73 × 10^6 cells μL^{-1}, respectively). MCH was significantly (2.6–4.5 pg) lower in winter than in the other seasons. The pattern of seasonal variation in MCV differed significantly in the two study 'years'. The most striking disparity was that the highest seasonal mean occurred in autumn in 'Year 1', but in spring in 'Year 2'. The winter mean was also higher relative to the spring and autumn values in 'Year 2'.

Sex differences in mass and blood parameters

The sex ratio (male : female) of the sampled birds was consistently male-biased in winter (mean 2.4) and spring (1.5) and female-biased in summer and autumn (0.5). Had the blood parameters varied systematically as a function of body mass or sex, any overall seasonal variation in them could potentially have been confounded by this sex-ratio variation among samples. Males were heavier (by 3.1–7.7%) than females in all seasons ($P < 0.001$ in all cases) (Table 1), as has been observed for other fairy-wren species (Rowley and Russell 1997). However, regression analysis indicated that body mass, at the resolution at which we measured it, did not significantly influence any of the blood variables. The linear regression equations (where M = mass, in grams) were as follows: Hct: $y = 0.392M + 42.174$, $r^2 = 0.005$, $F = 0.835$, $n = 160$; Hb: $y = 0.255M + 11.008$, $r^2 = 0.013$, $F = 1.997$, $n = 160$; RBC: $y = 0.07M + 3.233$, $r^2 = 0.012$, $F = 1.848$, $n = 159$; MCV: $y = 0.436M + 122.02$, $r^2 < 0.001$, $F = 0.111$, $n = 148$; MCH: $y = 0.003M + 34.871$, $r^2 < 0.001$, $F = 0.994$, $n = 150$; F tests examined whether the slope differed from zero, $P > 0.05$ in all cases.

Males had a higher Hct than females in summer and a higher RBC in winter ($P < 0.05$ in both cases), but there were no other significant seasonal sex-related differences in the three measured blood parameters (Table 3). Male-biased sampling could thus potentially have inflated the population's mean RBC in winter because males had a higher erythrocyte count at that time, but the only seasonal population difference observed in RBC was a disparity between spring and summer. Female-biased sampling could also potentially have reduced the population's mean Hct in summer because of the females' lower Hct at that time, but it could not have accounted for the similarity in the population's mean winter, summer and autumn Hcts. Thus the sex-biased sampling regime was not responsible for the observed seasonal population trends in the blood parameters.

Discussion

Seasonal constancy in body mass

Change in body mass can be used as an approximate indicator of seasonal fat storage patterns in birds, although without calibration it is not an infallible guide, because of

Table 3. Comparison of blood parameters of male and female superb fairy-wrens
Symbols for blood parameters given in 'Methods'. Values are mean ± s.e., with sample size given in brackets. Significance levels are as follows: **, $P < 0.01$; *, $P < 0.05$

Season	Blood parameter	Males		Females		F
Winter	Hct	46.7 ± 0.6	[26]	45.9 ± 0.8	[13]	0.872
	Hb	12.8 ± 0.2	[28]	12.3 ± 0.3	[12]	1.140
	RBC	4.07 ± 0.07	[26]	3.75 ± 0.11	[14]	8.596**
Spring	Hct	49.6 ± 0.6	[23]	48.4 ± 1.11	[16]	1.087
	Hb	15.2 ± 0.4	[23]	14.3 ± 0.41	[16]	2.711
	RBC	4.19 ± 0.11	[24]	3.97 ± 0.10	[15]	1.924
Summer	Hct	46.4 ± 0.7	[13]	43.5 ± 0.7	[29]	5.918*
	Hb	13.9 ± 0.3	[13]	13.4 ± 0.3	[28]	1.199
	RBC	3.96 ± 0.10	[13]	3.82 ± 0.08	[27]	0.994
Autumn	Hct	44.5 ± 0.6	[13]	43.9 ± 0.5	[27]	0.410
	Hb	12.8 ± 0.4	[13]	12.9 ± 0.2	[27]	0.062
	RBC	3.74 ± 0.09	[13]	3.73 ± 0.09	[27]	0.009

possible changes in body water or protein content (Biebach 1996). Many small passerines resident in the North Temperate Zone increase in body mass in winter, partly because of fat deposition (Carey *et al.* 1978; Blem 1990; Swanson 1991). These fat stores constitute a major energy reserve that aids survival during nocturnal fasting at low T_a and in daytime conditions unfavourable to foraging (King 1972). In contrast, the relatively few systematic, published studies of seasonal mass trends in small passerines in temperate Australia indicate that few of them exhibit mass gains in winter (Rooke *et al.* 1986; Breuer *et al.* 1995; Maddocks and Geiser 2000), although Chan (1995) demonstrated a diurnal increase in fat content in silvereyes (*Zosterops lateralis*). Superb fairy-wrens at Braeside showed no significant seasonal mass changes. Admittedly, many of our mass measurements were taken in the morning after nocturnal fasting, which could potentially somewhat mask seasonal differences that should be most apparent in measurements taken before roosting (Chan 1995). Also, the accuracy of our measurements would only have detected average variations of at least 5%. This would not have detected the magnitude of increase in fat content of silvereyes reported by Chan (1995), although winter mass increases in north temperate passerines are often substantially greater (Carey *et al.* 1976; Swanson 1991).

The magnitude of winter fat stores in north temperate passerines is negatively correlated with T_a (Blem and Shelor 1986; Rogers 1995). Mean minimum and maximum daily T_a at Braeside in winter were approximately 6°C and 14°C, respectively. There were only 10 winter nights when T_a fell below 2°C and there was no snowfall. This contrasts dramatically with winter conditions in many low-altitude north temperate locations, where maximum daily winter T_a is often below 4°C, nocturnal T_a is commonly well below 0°C and thick snow cover often impairs foraging (Dawson and Marsh 1983; Swanson 1990; Heinrich 1993). Nonetheless, superb fairy-wrens at Braeside spent most of winter at T_a significantly below their estimated Lower Critical Temperature of 22–23°C (estimated from Kendeigh *et al.* 1977) and so had a significant additional thermoregulatory energy requirement. However, fat storage also entails appreciable costs, notably reduced flying efficiency and increased predation risk (Rogers and Smith 1993; Wittmer and Cuthill 1993). It seems likely that the relatively mild austral winter conditions at Braeside, and perhaps at low altitude generally in temperate Australia, greatly reduce the advantage of such costly fat storage.

Seasonal changes in blood parameters

The minimum and maximum mean seasonal values recorded for the blood parameters measured in superb fairy-wrens were: Hct, 44–49%; Hb, 12.6–14.8 g $(100\ mL)^{-1}$; and RBC, 3.73–4.11 × 10^6 cells μL^{-1}. These values are broadly similar to means reported for a phylogenetically diverse range of other small (6.5–13.5 g) passerines living at low altitudes in the North and South Temperate Zones: Hct, 43–59%; Hb, 12.6–17.6 g $(100\ mL)^{-1}$; and RBC, 2.32–5.37 × 10^6 cells μL^{-1} (Carey and Morton 1976; Palomeque *et al.* 1980; Clemens 1990; Peurta *et al.* 1995; Breuer *et al.* 1995).

Breuer *et al.* (1995) reported similar mean summer and winter values to those obtained in the present study for Hct and Hb of superb fairy-wrens at Healesville, about 50 km north-east of Braeside Park. However, they reported a much lower RBC in summer than that at Braeside (2.11 v. 3.87 cells per 100 mL blood) and the reason for the difference is unclear. The main seasonal changes in the blood parameters of the Braeside fairy-wrens were the elevated Hct in spring and Hb in spring and summer. The breeding season of this species in southern Victoria is from September to December (i.e. spring and early summer). The typical breeding unit comprises either just a breeding pair or a pair plus male offspring from earlier broods acting as 'helpers' and it may undertake several breeding attempts per season (Higgins *et al.* 2001). Breeding is an energetically expensive activity in altricial birds (Williams 1996; Weathers 1996), especially when it involves a marked increase in flight activity when feeding nestlings. Sustained flight involves a 5–10-fold increase in oxygen consumption above resting level (Norberg 1996). Male superb fairy-wrens probably also incur significant additional energy costs in both seeking out and precluding extra-pair copulations (Mulder 1997). Thus it is likely that the observed increase in OCC in the breeding season significantly helped fairy-wrens to meet the additional oxygen demand associated with breeding. However, other mechanisms enhancing blood oxygen transport must also be involved in breeding superb fairy-wrens, because the 18% increase in OCC from the winter mean would be insufficient to completely meet the estimated additional oxygen-delivery demands of breeding. It would therefore be pertinent to determine whether the oxygen affinity of haemoglobin also changes seasonally in this species.

The 'increased-demand' energetic hypothesis (Masman *et al.* 1989) proposes that breeding results in a substantial increase in the energy demand on adult birds. In contrast, the 'reallocation' hypothesis proposes that favourable environmental conditions in the breeding season reduce thermoregulatory and foraging costs, allowing energy to be reallocated to breeding (Weathers *et al.* 1999). The former hypothesis predicts that the field metabolic rate will increase during breeding, whilst the latter predicts that field metabolic rate will remain constant seasonally. That OCC of superb fairy-wrens increased significantly only in the breeding season is consistent with the 'increased-demand' hypothesis, but seasonal comparisons of field metabolic rate are needed to establish with certainty the validity of this hypothesis for fairy-wrens.

Changes in Hct and Hb can also occur indirectly as a consequence of changes in plasma volume. Thus these parameters decrease in some species as a result of an increase in plasma osmotic pressure mediated by hormonal events associated with moulting (Chilgren and deGraw 1977) and increase as a result of dehydration brought about by, for example, sustained migratory flight (Morton 1994) or high T_a. It is possible that the former process contributed to the lower Hct and Hb of fairy-wrens in autumn, although moult was far less pronounced in winter (Table 2) and no further change in these parameters occurred. Dehydration was very unlikely at Braeside in spring when the weather was mild,

fairy-wrens were strongly territorial and most groups had access to a permanent water body. Stress resulting from handling can also lead to rapid, hormonally mediated changes in blood parameters influencing oxygen transport (LeMaho *et al.* 1992). However, our handling regime was constant throughout the investigation and so this could not account for the observed seasonal variation. Changes in circulating levels of testosterone and estrogen during breeding can affect erythropoiesis, although the evidence on the precise nature of these effects is mixed (Sturkie and Griminger 1976; Kern *et al.* 1992). We cannot totally exclude this as a possible factor in the observed spring increase in OCC.

Given the apparent lack of marked winter fattening in the fairy-wrens (Table 1), it is perhaps not surprising that there was no increase in OCC in that season. Breuer *et al.* (1995) also found no difference in the summer and winter Hct and Hb of superb fairy-wrens and three other small to medium-sized passerines in southern Victoria. Rooke *et al.* (1986) found no winter increase in Hct in silvereyes in temperate Western Australia. In contrast, several small passerines overwintering in the North Temperate Zone exhibit seasonally enhanced OCC (Carey and Morton 1976; Clemens 1990; Swanson 1991). Presumably this difference reflects the marked disparity in the winter climates prevailing in temperate North America and temperate Australia. Documenting seasonal variation in FMR in small, resident passerines in temperate Australia would help in assessing the validity of this inference.

Assuming that the observed seasonal variation in Hct and Hb was not mainly the result of variation in plasma volume, the other measured and calculated blood variables do not provide a very clear-cut picture of the underlying causation. RBC was only significantly higher in spring than in autumn and MCV was only significantly higher in spring than in the other seasons in 'Year 2'. However, although we demonstrated quite good replicability of our RBC measurements, Lewis *et al.* (2002) point out that manual measurements of this parameter are much less accurate than the measurements of Hct and Hb that we used.

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Table III. Table showing the reliability of manually counting red blood cells using an improved Neubauer haemocytometer.
Each batch in each of the eight seasons were counted in triplicate, and the average cell count shown. Each season the cell counts were analysed using a series of single-factor *ANOVA* s, and the f-ratio and probability level were calculated.

Season	Batch	Average Cell Count	f-ratio (probability)	degrees of freedom
Winter 1	1	354	2.353 (0.179)	4
	2	363		
	3	402		
	4	415		
	5	368		
Spring 1	1	385	0.663 (0.660)	4
	2	387		
	3	359		
	4	401		
	5	362		
Summer 1	1	389	1.341 (0.351)	4
	2	407		
	3	360		
	4	336		
	5	380		
Autumn 1	1	390	0.789 (0.615)	4
	2	374		
	3	395		
	4	360		
	5	397		
Winter 2	1	424	1.035 (0.437)	4
	2	376		
	3	395		
	4	384		
	5	423		
Spring 2	1	409	1.167 (0.402)	4
	2	388		
	3	420		
	4	395		
	5	379		
Summer 2	1	400	0.315 (0.866)	4
	2	391		
	3	380		
	4	408		
	5	376		
Autumn 2	1	348	0.812 (0.585)	4
	2	387		
	3	382		
	4	394		
	5	376		

Table IV. Haematology of fairy-wrens as a function of sex and season.
Values are mean ± standard error, and sample size is given in parentheses.
M, males; F, females.

Season	Sex	Haematocrit (%)	Haemoglobin concentration (g / dL)	Erythrocyte count ($\times 10^6$ / µL)	Mean cell volume (fL)	Mean cell haemoglobin (pg)	O_2-carrying capacity (ml O_2 / 100mL)
Winter 1	**M**	46.8 ± 0.8 [12]	13.0 ± 0.2 [15]	4.18 ± 0.09 [13]	113 ± 4 [10]	31.4 ± 0.9 [12]	17.4 ± 0.3 [15]
	F	47.0 ± 0.9 [7]	12.6 ± 0.3 [5]	3.94 ± 0.17 [7]	120 ± 4 [6]	31.9 ± 1.5 [4]	16.8 ± 0.4 [5]
Winter 2	**M**	46.7 ± 0.9 [14]	12.5 ± 0.4 [13]	3.95 ± 0.11 [13]	115 ± 3 [12]	31.7 ± 1.0 [13]	16.7 ± 0.6 [13]
	F	44.7 ± 1.3 [6]	12.1 ± 0.4 [7]	3.56 ± 0.09 [7]	127 ± 6 [6]	34.9 ± 2.0 [6]	16.3 ± 0.6 [7]
Winter Pooled		**46.5 ± 0.5** [39]	**12.6 ± 0.2** [40]	**3.96 ± 0.06** [40]	**117 ± 2** [34]	**32.2 ± 0.6** [35]	**16.9 ± 0.2** [40]
Spring 1	**M**	50.1 ± 1.2 [11]	15.5 ± 0.6 [11]	4.28 ± 0.12 [12]	116 ± 3 [11]	36.5 ± 1.2 [11]	20.8 ± 0.8 [11]
	F	48.0 ± 1.1 [8]	14.7 ± 0.5 [8]	4.03 ± 0.13 [7]	119 ± 4 [7]	36.1 ± 0.9 [7]	19.7 ± 0.7 [8]
Spring 2	**M**	49.2 ± 0.6 [12]	14.9 ± 0.4 [12]	4.11 ± 0.18 [12]	125 ± 4 [11]	38.1 ± 1.1 [11]	20.0 ± 0.6 [12]
	F	48.8 ± 2.0 [8]	13.9 ± 0.6 [8]	3.92 ± 0.16 [8]	125 ± 3 [8]	35.6 ± 1.4 [8]	18.6 ± 0.9 [8]
Spring Pooled		**49.1 ± 0.6** [39]	**14.8 ± 0.3** [39]	**4.11 ± 0.08** [39]	**121 ± 2** [37]	**36.7 ± 0.6** [37]	**19.9 ± 0.4** [39]
Summer 1	**M**	46.6 ± 0.9 [9]	13.8 ± 0.3 [9]	4.04 ± 0.11 [9]	116 ± 3 [9]	34.3 ± 1.1 [9]	18.5 ± 0.5 [9]
	F	44.6 ± 0.9 [13]	13.2 ± 0.4 [12]	3.86 ± 0.11 [11]	117 ± 3 [11]	35.5 ± 0.8 [10]	17.6 ± 0.6 [12]
Summer 2	**M**	45.8 ± 0.9 [4]	14.2 ± 0.4 [4]	3.78 ± 0.18 [4]	122 ± 5 [4]	37.9 ± 1.8 [4]	19.1 ± 0.5 [4]
	F	42.6 ± 1.0 [16]	13.6 ± 0.4 [16]	3.80 ± 0.12 [16]	113 ± 2 [16]	36.4 ± 1.0 [15]	18.3 ± 0.5 [16]
Summer Pooled		**44.4 ± 0.6** [42]	**13.6 ± 0.2** [41]	**3.87 ± 0.07** [40]	**116 ± 1** [40]	**35.8 ± 0.5** [38]	**18.2 ± 0.3** [41]
Autumn 1	**M**	45.2 ± 0.7 [5]	13.3 ± 0.5 [5]	3.74 ± 0.22 [5]	122 ± 6 [5]	36.0 ± 2.3 [5]	17.8 ± 0.6 [5]
	F	44.3 ± 1.0 [15]	12.7 ± 0.2 [15]	3.60 ± 0.07 [15]	124 ± 5 [15]	35.3 ± 0.6 [15]	17.0 ± 0.3 [15]
Autumn 2	**M**	44.0 ± 0.8 [8]	12.6 ± 0.6 [8]	3.75 ± 0.07 [8]	118 ± 3 [8]	33.6 ± 1.5 [8]	16.9 ± 0.8 [8]
	F	43.5 ± 0.9 [12]	13.3 ± 0.4 [12]	3.89 ± 0.18 [12]	114 ± 5 [12]	34.5 ± 1.0 [12]	17.8 ± 0.5 [12]
Autumn Pooled		**44.1 ± 0.4** [40]	**12.9 ± 0.2** [40]	**3.73 ± 0.07** [40]	**119 ± 2** [40]	**34.8 ± 0.6** [40]	**17.3 ± 0.3** [40]

Table V. Lipid mass extracted from two fairy-wrens trapped in summer and winter using petroleum ether in a Soxhlet apparatus after 60, 90, 120, 180 and 240 min.

Extraction for 240 min yielded approximately 2% more lipids than extraction for 120 min, for both birds.

Extraction Time (min)	Lipid Mass (g)	
	Winter	Summer
60	0.386 (77%)	0.273 (80%)
90	0.423 (85%)	0.305 (89%)
120	0.488 (98%)	0.335 (98%)
180	0.495 (99%)	0.339 (99%)
240	0.499 (100%)	0.341 (100%)

Table VI. Glucose assay system used for glycogen determination in liver and pectoralis muscle tissue homogenates.

Glucose Assay Procedure		
Solution	**Volume used (mL)**	
	Blank	**Sample**
Triethanolamine buffer	0.50	0.50
Sample solution	—	0.05
Distilled water	1.00	0.95
Mix and read OD_{340} after 3 min (at 25°C).		
Enzyme suspension	0.01	0.01
Mix and read OD_{340} after 15 min (at 25°C).		
Total volume	1.51	1.51

REAGENTS

Triethanolamine buffer, pH 7.6 contained:
NADP (110mg)
ATP (260mg)
magnesium sulphate ($MgSO_{4)}$
Stabilisers

Enzyme suspension (Boehringer Mannheim) contained:
HK (320U)
G 6-PDH (160U)

Table VII. Enzyme assay procedures used for determination of Hexokinase (*HK*), ß-hydroxyacyl-CoA dehydrogenase (*HOAD*), Citrate synthase (*CS*) and Phosphorylase (*PHOS*) activity in pectoralis muscle tissue homogenate.

HK

Solution	Volume Used (µL)	Final Concentration
4mM $NADP^+$	100	0.4mM
25mM ATP	100	2.5mM
100mM CP	100	10mM
1IU CPK	4	
1IU G 6-PDH	4	
HK assay buffer	542	
Warm at 37°C for 7 minutes.		
muscle homogenate	50	
Mix and read OD_{340} for background.*		
10mM glucose	100	1.0mM
Mix and read reaction OD_{340}.*		
Total Volume	1.0mL	

* 25 readings at 5sec intervals were recorded for both the background and reaction and the average calculated.

HOAD

Solution	Volume Used (µL)	Final Concentration
2mM NADH	100	0.2mM
HOAD assay buffer	790	
Warm at 37°C for 7 minutes.		
muscle homogenate	10	
Mix and read OD_{340} for background.*		
1mM acetoacetyl Co-A	100	0.1mM
Mix and read reaction OD_{340}.*		
Total Volume	1.0mL	

* 25 readings at 5sec intervals were recorded for the background and 40 readings for the reaction and the average calculated.

(continued over)

Table VII (cont.).

CS

Solution	Volume Used (µL)	Final Concentration
2mM DTNB	100	0.2mM
10mM oxaloacetate	50	0.5mM
CS assay buffer	820	
Warm at 37°C for 7 minutes.		
muscle homogenate	5	
Mix and read OD_{412} for background.*		
12mM acetyl Co-A	25	0.3mM
Mix and read reaction OD_{412}.*		
Total Volume	1.0mL	

* 25 readings at 2sec intervals were recorded for both the background and reaction and the average calculated.

PHOS

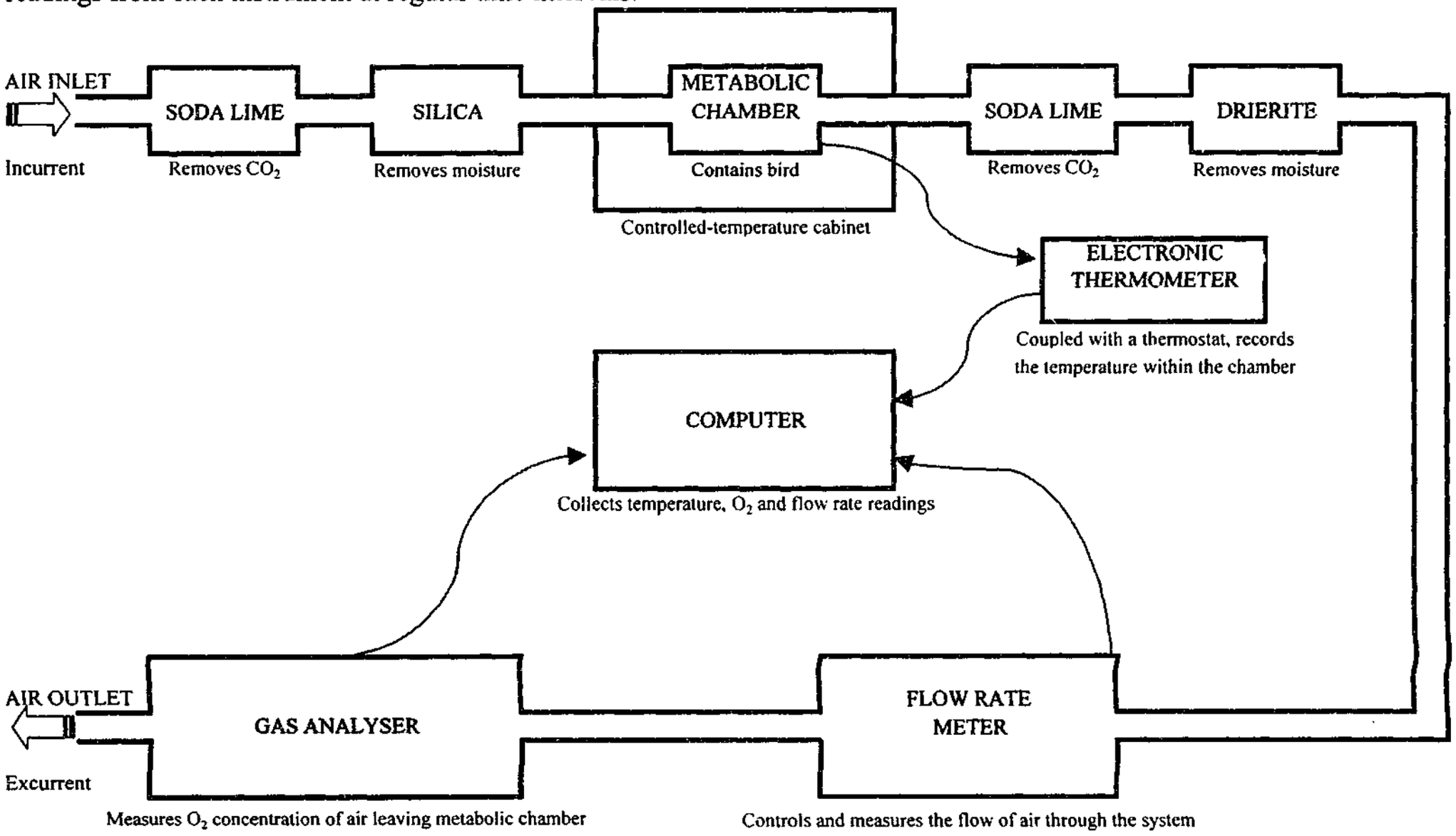
Solution	Volume Used (µL)	Final Concentration
4mM $NADP^+$	100	0.4mM
40µM G-1,6-diP	100	4µM
16mM AMP	100	1.6mM
PGM	3	
G-6-PDH	3	
PHOS assay buffer	584	
Warm at 37°C for 7 minutes.		
muscle homogenate	10	
Mix and read OD_{340} for background.*		
glycogen	100	
Mix and read reaction OD_{340}.*		
Total Volume	1.0mL	

* 25 readings at 3sec intervals were recorded for the background and 40 readings for the reaction and the average calculated.

Table VIII. Mean activity of four catabolic enzymes measured at 37°C from freshly prepared muscle homogenates and frozen muscle homogenates.
Enzyme activity values are mean ± standard error and sample size is six for both fresh and frozen tissue for each enzyme.
f-ratios and probability levels from four one-factor *ANOVA* s which examine variation in enzyme activity from fresh and frozen tissue.

	Muscle Tissue Enzyme Activity (µmoles min.$^{-1}$ g muscle^{-1})		**f-ratio (probability)**
	Fresh	**Frozen**	
HOAD	22.5 ± 2.6	20.6 ± 2.6	0.285 (0.605)
HK	2.3 ± 0.3	1.5 ± 0.3	3.203 (0.104)
CS	115.0 ± 7.4	108.9 ± 15.6	0.125 (0.731)
PHOS	76.3 ± 7.5	35.7 ± 11.4	**8.883 (0.014)**

Table IX. Open-circuit respirometry system used to measure $\dot{V}O_2$ (oxygen consumption) by fairy-wrens in dry, CO_2-free air. A computer with customised software was used to collect the temperature, flow rate and fractional O_2-concentration readings from each instrument at regular time intervals.



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