ECOLOGICAL PHYSIOLOGY OF MALLEEFOWL (*Leipoa ocellata*)

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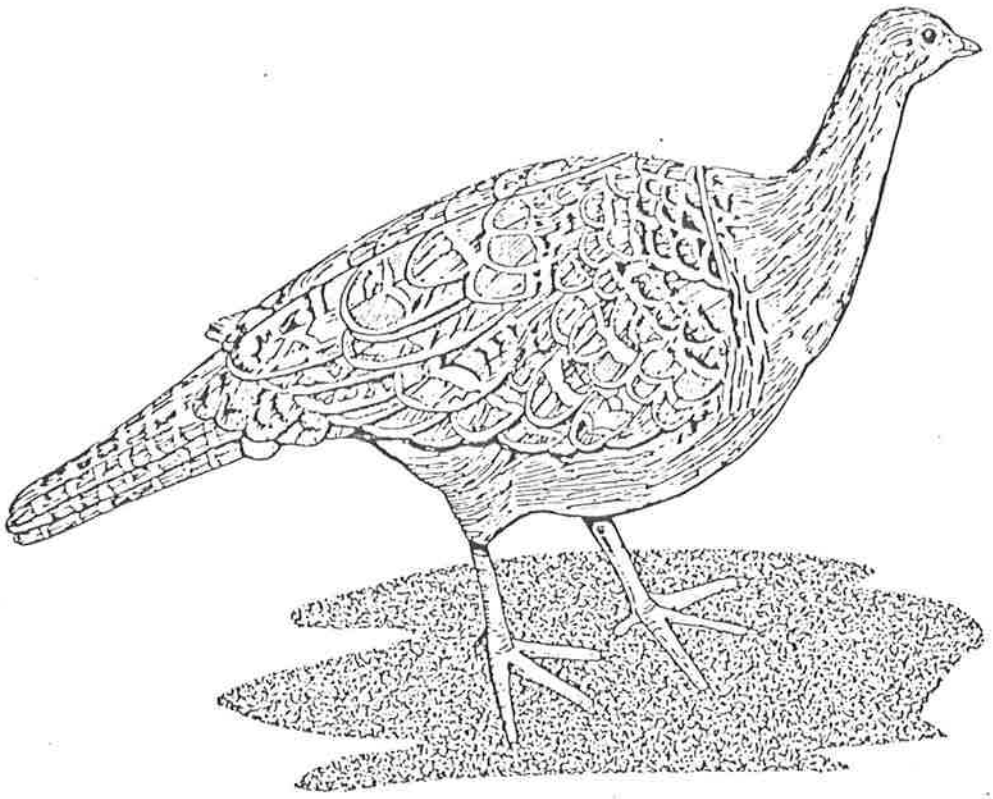

"Malleefowl.....as its trivial name implies, is an inhabitant of the arid dreary mallee scrubs. .....lonely this bird decidedly is, leading a solitary life; for, except at the period of incubation, it is very rarely that two are seen together, and when met while quietly feeding its actions are suggestive of melancholy, for it has none of the liveliness that characterises almost all other birds, but stalks along in a solemn manner as if the dreary nature of its surroundings and its solitary life weighed heavily on its spirits." Bennett (1883).

ABSTRACT

Aspects of the incubation biology and environmental physiology of Malleefowl Leipoa ocellata have been studied in the field near Renmark, South Australia, and in the laboratory.

Malleefowl eggs were incubated artifically at temperatures of 30, 32, 34, 36, and 38 C. The energetics of incubation at these temperatures was determined by monitoring oxygen consumption over the incubation period. Egg water loss and egg surface temperature were also monitored. No eggs developed at 30 C (N=6), 22% (N=9) hatched at 32 C, 80% (N=10) hatched at 34 C, 44% (N=9) hatched at 36 C, and 38% (N=8) hatched at 38 C. The amount of oxygen consumed during incubation varied with incubation temperature. Eggs incubated at 32 C consumed 42% more oxygen than eggs incubated at 34 C, while eggs incubated at 36 and 38 C consumed 19 % less.

Water loss from developing eggs increased with incubation time at all temperatures. The cause of increased water loss in eggs incubated at 34 C was investigated and can be attributed to an increase in eggshell gas conductance.

During incubation a temperature gradient developed between the eggshell and the surrounding sand at all incubation temperatures. This gradient reached 1.5 - 2.0 C in full term eggs. Egg surface and surrounding sand temperatures were monitored continuously throughout incubation in a natural incubation mound, and sand temperature was found to vary from 27 C to 32 C, while egg surface temperature varied from 27 C to 35 C. This experiment demonstrated that Malleefowl eggs can hatch

successfully after exposure to temperatures as low as 28 C for at least a week, and that a temperature gradient of up to 3.0 C develops between the eggshell surface and the surrounding sand.

The ontogeny of thermoregulation was investigated in full term eggs and hatchling chicks. The development of an 'endothermic response' appears during the last week of incubation in most eggs. Hatchlings were found to be competent homeotherms only five hours after hatching, to have low evaporative water loss, and to be tolerant of heat stress.

Water turnover using the tritium labeled water technique was monitored over a twelve month period in both captive birds kept in Adelaide and free-ranging birds at Renmark. Water turnover in captive birds remained relatively constant and was only 33% of the expected rate based on allometric criteria. Water turnover in free-ranging birds was more variable and greater than in captive birds, but was still only about 50% of the predicted rate. These results suggest that Malleefowl have evolved low water requirements in response to their arid environment.

DECLARATION

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, contains no material previously published or written by another person, except where due reference is made in the text. Upon the award of the Doctor of Philosophy Degree, I consent to this thesis being made available for photocopying and loan.

David Terrington Booth.

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Abbreviations used in text

EWL evaporative water loss

F_{O_2} fractional oxygen content

G_{H_2O} water vapour conductance

G_{O_2} oxygen conductance

H heat content of body

H_e Rate of heat loss due to evaporation of water

h_s coefficient of heat strain

HTO tritiated water

K thermal conductance

K_n dry thermal conductance

M mass

M rate of heat production due to metabolic rate

M_{H_2O} rate of water loss

MWP metabolic water production

$P_{H_2O_{in}}$ partial pressure of water vapour in the gas space inside an egg

$P_{H_2O_{out}}$ partial pressure of water vapour outside an egg

P_{O_2} partial pressure of oxygen

P_{O_2c} critical partial pressure of oxygen

P_{O_2in} partial pressure of oxygen in the gas space inside an egg

P_{O_2out} partial pressure of oxygen outside an egg

Q_{10} Rate of increase per 10 C increase in temperature

RE respiratory gas exchange ratio

RQ respiratory quoient

SMR standard metabolic rate

T temperature difference

Abbreviations continued

T_a ambient temperature

T_b body temperature

T_e egg temperature

T_{es} egg surface temperature

TBW total body water

TNZ thermoneutral zone

t time

VCO_2 carbon dioxide production rate

VO_2 oxygen consumption rate

Chapter 1 GENERAL INTRODUCTION

1.1 A summary of Malleefowl biology

Malleefowl (Leipoa ocellata Gould) are galliform birds belonging to the family Megapodiidae, the so-called moundbuilding birds. All 12 species of megapode are confined to the Australasian region (Clark 1964b). From a comparative aspect, megapodes are interesting because of their unusual methods of nidification and the precocial nature of their chicks. Unlike other birds which utilize body heat for incubation, megapodes use heat from solar, geothermal, microbial decay of vegetation, or a combination of these sources to incubate their eggs. Because of the underground nesting habits of megapodes, the physical conditions of the incubation environment are different from other birds (Seymour and Ackerman 1980).

In contrast with all other megapodes which inhabit mesic environments of thick jungle or scrub, Malleefowl are distributed in semi-arid mallee country (200 - 400 mm rainfall annually). Their original range extended throughout southern Australia including western New South Wales, north western Victoria, South Australia, across the Nullarbor plains into Western Australia where they could be found along the west coast as far north as the Tropic of Capricorn (Fig. 1.1). The clearing of land for agricultural purposes, the stocking of uncleared land with sheep and the introduction of rabbits have greatly reduced the present day range of Malleefowl. They can still be found in isolated patches of mallee in south western New South Wales, north western Victoria, southern South Australia and south western West Australia, but the species is listed by Wildlife Authorities as 'rare' in all States.

Almost all information on Malleefowl biology comes from the six year study of Harold Frith (Frith 1955, 1956, 1957, 1959a, 1962a, 1962b). His work was principally concerned with the breeding biology of the species, and included studies on the construction, maintenance, and regulation of incubation mounds, fecundity, and hatching success of eggs. More recent studies have concerned the physiology of incubation mounds and eggs (Seymour and Ackerman 1980, Vleck et al. 1984).

Up to 11 months of the year are spent by Malleefowl on construction and maintenance of incubation mounds (Frith 1955, 1956, 1959, 1962b). Usually an old mound is renovated; rarely is a completely new mound prepared. Renovation usually starts in early April when an old mound is excavated to form a crater 2.5 - 3.5 m in diameter and approximately a meter deep. Over the following months, litter from mallee vegetation is raked with the feet into windrows and moved toward the mound. By June or July litter from windrows is placed in the mound crater, and work does not continue until rain thoroughly wets the litter. After winter and spring rains, birds turn over the material several times and sand becomes mixed in with it. Microbial agents start to decompose the litter, and the mound begins to warm. In drought years the litter fails to decompose, mounds do not heat, and breeding is abandoned (Bennett in Campbell 1901, McLennon 1906, Mattingley 1908, Lewis 1939, Frith 1959a, 1962b). By late August or early September, mounds normally approach incubation temperature, and birds shape an egg chamber about 60 - 80 cm in diameter and 40 cm deep in the top of the litter material. Soon thereafter, they place a layer of sand over the top and prepare to lay eggs. The first eggs normally appear in late September or early October. Up to this stage both birds work on the mound, although the male is predominant. During the period of

egg-laying, the male works the mound almost exclusively and the female concentrates on gathering food for egg formation (Frith 1959a, 1962b). Toward the end of the breeding season when egg-laying has ceased (January - February), but eggs are still being incubated, the female once again joins the male in mound maintenance (Frith 1959, 1962b).

Malleefowl are apparently able to sense the temperature of the mound material with their tongue (Frith 1956). When digging out the mound for egg-laying or temperature regulation, the bird thrusts its open mouth down into the sand of the egg chamber and checks the temperature.

The cue to begin egg-laying is probably the mound reaching suitable incubation temperatures (Frith 1959a). Once egg laying has begun, the mound temperature is regulated fairly precisely, the preferred incubation temperature being 34 C (range 32 - 36 C, Frith 1956, 1959a). At the beginning of the breeding season (September - November), almost all of the heat for incubating eggs is derived from the decaying litter. In fact, too much heat is often generated by this source, and the male visits and opens the mound at dawn every one or two days, releasing excess heat to the cool morning air (Frith 1956). In the middle of the breeding season (December - January), heat from the litter declines, but the higher air temperatures and the thermal inertia of the mound maintain incubation temperature without the bird having to do a great deal of work. The mound is only dug out once or twice a week over this period. At the very end of the breeding season (February - March), the mound litter dries out and microbial heat production ceases. The birds then rely entirely on solar heat for incubation. Every sunny day the mound is dug out down to within 20 - 30 cm of the

incubating eggs during mid-morning when the sun is high in the sky. After being heated by the sun, the sand is slowly piled onto the mound over the next few hours, until by late afternoon the mound is once again built up high. The warmed sand keeps the egg chamber at suitable incubation temperatures throughout the night. On cloudy or rainy days the mound is left built up. When the amount of sunshine decreases to such a level that mounds can no longer maintain incubation temperature, the mound is dug out and abandoned.

Because of the long egg-laying period (2 - 3 months) Malleefowl produce large clutches of eggs. Frith (1959a) found a mean clutch size of 18 (range 5 - 33) for 54 clutches produced over six breeding seasons. He found that at Griffith, NSW, on average 49.5% of eggs laid were successfully incubated, 1.4% were broken by birds while working the mound, 11.9% failed to hatch (either infertile or die during incubation) and 37.2% were eaten by foxes (Frith 1959a).

Malleefowl lay large eggs for their body size (ca 1800 g). Frith (1959a) gave the mean mass of 844 eggs as 187 g, and Seymour and Ackerman (1980) the mean as 170 g, which is 3 times the mass predicted for a 1800 g galliform bird (Lack 1968). The eggs have relatively large yolks, high energy content, and a long incubation period (Vleck et. al. 1984). The large yolk with its high energy content is necessary for the development of the extremely precocious chick over the long incubation period.

Eggs of Malleefowl have several adaptations to underground nesting. Most bird eggs need to be turned during incubation, a failure to do so usually leads to high mortality among embryos before hatching

(Drent 1975). Malleefowl eggs, like those of other megapode species are not moved once they are laid, yet they have a high hatching success. Marshall (1939) concluded megapode eggs must have some physiological and/or anatomical adaptation to allow high incubation success without turning.

Malleefowl produce eggs with thin shells and high eggshell conductances (Seymour and Ackerman 1980). A thin eggshell is not a liability as the eggs are unlikely to be knocked and broken once they have been laid. High eggshell gas conductance facilitates O_2 and CO_2 exchange in an environment where high CO_2 and low O_2 tensions are experienced (Seymour and Ackerman 1980). Excessive water loss from eggs due to the high eggshell conductance is not a problem, as the underground environment has a high humidity (Seymour and Ackerman 1980). Because of the high water vapour pressure, water loss from the incubating egg is relatively low and eggs do not form a fixed air cell or air space at the blunt pole like other bird eggs (Seymour and Ackerman 1980, Vleck et. al. 1984). In most bird eggs the air cell plays an important role in the transition from chorioallantoic to pulmonary respiration during hatching by providing an easily obtainable gas space from which the lungs can be ventilated (Visschedijk 1968). The lack of a fixed air cell in megapode eggs is correlated with a quick hatching process (Vleck et. al. 1984, Seymour 1984).

Megapodes lose their egg tooth before hatching and do not have a functional complexus muscle (Clarke 1964a). The egg tooth and complexus muscle of other hatching birds are used to break and escape from the eggshell. In Malleefowl the eggshell is broken at hatching with a kicking motion of the feet and expanding of the shoulders (Frith 1959a,

1962b). After escaping the eggshell, Malleefowl chicks must struggle up to the mound's surface unassisted. The upward journey is made in a spasmodic manner with periods of 5 to 10 min of active upward pushing separated by rest periods of about an hour (Frith 1959a, 1962b, Vleck et. al. 1984). The whole process from hatching to reaching the surface takes between 2 and 15 h (Frith 1959a, 1962b).

Megapodes produce extremely precocious chicks (Frith 1959, 1962b, Clark 1960, 1964a, 1964b, Nice 1962). At hatching, megapodes are homeothermic and independent of their parents, being able to feed themselves and even fly just 24 h after hatching (Nice 1962, Frith 1962b).

Although adult Malleefowl will drink freely if water is available (Leake in Ashby 1929), they do not need to under normal conditions in their natural habitat (Bennett 1883, Bellchambers 1916, Leake in Ashby 1929, Frith 1962a, 1962b). In fact, during the hot summer months the birds are usually unable to drink as surface water is usually unavailable within their mallee habitat. Bellchambers (1916) reported Malleefowl taking dew found on plants near Adelaide, but dew in the mallee is infrequent at best, and non-existent over the summer period (Bellchambers 1916).

The natural diet of Malleefowl is not well known, with most information comming from Frith's (1962a) study. Foods include any invertebrates disturbed while foraging (chiefly ants, a few beetles, cockroaches and spiders) and seeds, flowers, and fruits of herbs and shrubs which are found on the ground or up to 60 cm above it. The diet varies as a consequence of seasonal availability of food types. The

bulk of the diet over the summmer (December - February) period are seeds of the legumes Cassia sp. and Acacia sp. During winter months (May - July), shrubs are not flowering or setting seeds so herbs become the chief food source. The shrub Beyria opaca is another important food plant, both buds and berries of this species are eaten over the spring period (September - November). Leake (in Ashby 1929) describes Malleefowl as partly insectivorous, while Gray (in Gould 1865) describes them as 'chiefly' insectivorous. Ross (1919) observed Malleefowl actively feeding on ants. These observations suggest insects are a more important part of the diet than Frith (1962a) asserts.

1.2 Investigations of the present study

Work by Frith and others has not covered many important aspects of Malleefowl biology. This study investigates several of these little known areas. The specific aspects studied are as follows: incubation biology, the unusual hatching process, ontogeny of homeothermy, water relations of captive and free-ranging birds, home range size, and the fecundity and breeding success of Malleefowl.

A range of successful incubation temperatures (30 - 38 C) and incubation periods (48 - 99 d) have been reported for Malleefowl (Bellchambers 1917, Frith 1957, 1959a, in Nice 1962). Such a large variation in successful incubation period is unusual in birds (Clark 1964b) and may affect the energy and water requirements of the developing embryo. The effects of incubation temperature on incubation length, water loss, and energetic cost of development in Malleefowl eggs is examined in chapter 3.

Megapodes do not hatch in the same manner as other birds. There

is no internal pipping in megapodes as in other birds, and the eggshell is broken with the feet rather than with an egg tooth. When the shell is broken the chorioallantois is destroyed, so the swicth over from chorioallontoic to pulmonary respiration is quick (Vleck et. al. 1984, Seymour 1984). In other hatching birds a prolonged transition process from chorioallantoic to pulmonary respiration occurs between internal pipping and the time the chick finally breaks free of the egg. To further understanding of the unusal hatching chronology of megapodes, patterns of respiratory gas exchange in hatching Malleefowl are investigated in chapter 5.

Megapode chicks are said to be homeothermic upon hatching (Nice 1962). However, there is no data to confirm this, so an investigation examining the ontogeny of homeothermic capacity in Malleefowl hatchlings is necessary. If hatchlings are good homeotherms, then an investigation into metabolic responses of full term embryos is warranted because in most precocial birds the transition from ectothermy to endothermy takes several days. The response to heating and cooling of near full-term eggs is examined in Chapter 4, while the response to the same environmental conditions in hatchlings is examined in chapter 6. A comparison of thermoregulatory behaviour between Malleefowl and Brush-turkey (Alectura lamthami) hatchlings is also made, to see if the different post-hatch environments of these species have influenced the evolution of their thermoregulatory behaviour.

Desert birds have several adaptations to their arid environment, and these include low standard metabolic rate (Dawson 1976), low evaporative water loss (Weathers 1977) and tolerance of high temperature (Lasiewski and Bartholomew 1966, Lasiewski et. al. 1970). To see if

Malleefowl exhibit any of these properties standard respirometry chamber measurements of metabolism and evaporative water loss are determined in chapter 7.

Malleefowl are ground birds which are relatively immobile and inhabit scrub where surface water is non-existent over the summer months. The major food source during this period is seed which is low in water content. These factors, combined with high diurnal temperatures that provoke evaporative cooling, have the potential to cause problems in water balance. Rates of water turnover of free ranging and captive Malleefowl over different seasons is investigated in Chapter 8.

Frith's (1959a) home range estimates for male and female Malleefowl during the breeding season (0.03 amd 0.20 km^2 respectively) seem very small for birds of their size as food sources occur in low density in mallee scrub. To clarify this point home range, and movements of free ranging adult Malleefowl are investigated with radio tracking equipment. No information is available on chick movement or fate once they escape from the incubation mound, so several chicks were also radio-tagged. The results of these investigations are presented in chapter 9.

Malleefowl do not complete incubation mounds or breed during droughts. A possible reason for mound abandonment is that litter material in the mound does not become moist and consequently does not heat. Hence, the stimulus to complete mounds and begin egg-laying is absent. Another reason could be that food resources are in short supply, and not enough food can be gathered by females for egg

production. A drought over the 1982 - 83 breeding season provided an ideal situation to test the dry litter material hypothesis, the result of which is presented in chapter 10.

An important part of the biology of any species is the reproductive success of the population. How suitable a particular habitat is for a particular population can often be assessed by the breeding success and density of mated pairs. While on various field trips throughout the study, I gathered information on mound density, egg production and incubation success. A comparison with similar data from Frith's (1959a, 1962a) studies from Griffiths NSW is discussed in chapter 11.

CHAPTER 2 GENERAL METHODS

2.1 Study site

The major field study site was located in low rainfall mallee (230 - 260 mm annually) on old Calperum Station 10 km west of Renmark, South Australia. The main physical feature of the area was an east-west sand dune system (Woorinen Formation; Department of Lands 1982) consisting of regularly spaced dunes with relatively wide interdunal swales. Occasional depression areas were located within swales. The dunes were composed of red sand and the interdunal swales of sandy loam (Department of Lands 1982). A detailed description of the vegetation within the study area has been made (Department of Lands 1982) and may be summarized as follows. The area was dominated by a mallee - porcupine grass (Triodia irritans) association. The mallee species included Eucalyptus socialis, E. incrassata, E. foecunda, E. gracilis, E. dumosa, and E. anceps. Various shrubs including Acacia sp., Cassia sp., Dodonaea sp., and Grevillea sp., were scattered throughout the area. This association fits the Class V mallee classification in Frith (1962a).

2.2 Collection and Incubation of Eggs

Malleefowl eggs were collected from natural incubation mounds, chiefly from the Renmark study site. During 1981 five eggs were collected from a mound 20 km north-east of Murray Bridge, South Australia, and eggs collected in 1982 came from mounds located 8 km east of Malinong, South Australia. Eggs were excavated from mounds, a number was written on the blunt pole with a HB grade graphite pencil, and the eggs were placed in insulated foam boxes. The eggs were covered in warm sand taken from the incubation chamber of the mound, and transported by car to the laboratory within 6 h of collection. On arrival in the

laboratory, eggs were weighed to the nearest milligram on an electronic balance (Sartorius 1265 MP) and buried in open plastic containers (32x25x12 cm) with sand taken from natural incubation mounds. Four to six eggs were placed in each container and these incubated in constant temperature cabinets. Four litre plastic containers full of water with large cloth wicks were placed in the temperature cabinets to maintain high humidity within the cabinets.

2.3 Raising and Maintenance of Captive Malleefowl

Malleefowl used in laboratory experiments were raised from hatchlings in an outdoor roofed enclosure (9.1x6.5x2.5 m). The enclosure had a cement floor which was covered with sand and litter material collected from the Renmark study site. Birds had continuous access to a feeder containing commercial pellet food (Pigeon Pex, Milling Industries Limited) and water contained in two drinking troughs. The diet was supplemented approximately twice a week with a mixture of canned dog food (Pal, Uncle Ben's Pet Food Limited) and budgie-seed mix (Blair Athol Grain Store). Occasionally chopped apple was added to the mixture.

2.4 Oxygen Consumption Measurements

Oxygen consumption was monitored with a diaferometer (Kipp and Zonen model MG4-688E) connected to a channel selector (Kipp and Zonen channel selector BA4-695E) and chart recorder (Kipp and Zonen model BD5) early in the study (1981-1982) and a paramagnetic oxygen analyzer (Taylor Servomex model OA184) connected to a chart recorder (Perkin Elmer 56) for the rest of the study. The diaferometer was calibrated by running it in series with the OA184 which was calibrated with nitrogen and dry outside air (20.96% oxygen).

CHAPTER 3 PHYSIOLOGY AND ENERGETICS OF INCUBATION

3.1 INTRODUCTION

Incubation is an important aspect of avian biology and numerous studies address the subject. Incubation in megapodes is particularly interesting because of their underground nesting habit. The underground environment provides conditions of high humidity, elevated carbon dioxide tensions and lowered oxygen tensions (Seymour and Rahn 1978, Seymour and Ackerman 1980). These conditions are not normally encounted in other birds' nests. Malleefowl eggs have several adaptations to underground nesting such as a thin, highly porous eggshell (Seymour and Ackerman 1980) and need not to be turned like other bird eggs. In this chapter several functional aspects of Malleefowl egg incubation are investigated to see what other adaptations the eggs may have.

Although avian incubation temperature is the subject of many studies, there are few studies which measure egg temperature of naturally incubating live eggs (Ackerman and Seagrave 1984). Studies addressing egg temperature usually employ infertile, dead, or model eggs to measure egg temperature (Drent 1970, 1975, White and Kinney 1974, Howell 1979, Vleck 1981a, b, Webb and King 1983, Haftorn 1983, 1984, Williams and Ricklefs 1984). This technique is useful for small eggs in which the metabolic heat production of the embryo has a negligible effect on egg temperature (Webb and King 1983), but is inappropriate for larger eggs in which the developing embryo's metabolism can raise the egg temperature 2 to 3 C above ambient in late incubation (Romanoff 1941, Romijn and Lokhorst 1956, Khaskin 1961,

Drent 1970, 1975, Grant et. al. 1982). In this study eggshell surface temperature is monitored in naturally and artificially incubated Malleefowl eggs.

Bird eggs usually have a relatively narrow range of temperatures at which incubation is successsful (Drent 1970, 1973, 1975, Romanoff and Romanoff 1972, White and Kinney 1974), although short term drops in temperature are tolerated and commonly occur in naturally incubated eggs (McMullan and Eberhardt 1953, Moreng and Bryant 1956, Lundy 1969, Norton 1972, White and Kinney 1974, Vleck 1981b, Haftorn 1983). In some species, notably the procellariiforms, long term drops of incubation temperature to levels well below normal can be tolerated (Boersma and Wheelwright 1979, Vleck and Kenagy 1980, Roby and Ricklefs 1984, Williams and Ricklefs 1984). At such low temperatures embryonic development does not progress (Drent 1975, Vleck and Kenagy 1980, Williams and Ricklefs 1984). Bird eggs incubated for long periods at temperatures just below normal usually fail to hatch because at sub-normal temperatures anomalies such as disproportionate growth of the heart occur (Romanoff 1960, Lundy 1969, Drent 1975). Exposure of eggs to high temperatures for extended lengths of time is fatal (Romijn and Lokhorst 1956, Lundy 1969, Drent 1970, 1975, Vleck 1981b).

Successful incubation periods as short as 44 d and as long as 99 d have been reported for Malleefowl (Bellchambers 1917, Frith 1959a, in Nice 1962), suggesting that this species has an unusually large range of successful incubation temperatures. Because temperature has a direct effect on metabolic processes, it may also affect the amount of energy utilized during embryonic development. Low temperatures lead to longer incubation times and thus more energy may be needed to complete

development. To test the hypothesis that Malleefowl eggs can be incubated successfully over a relatively wide range of temperatures and to investigate the effect of temperature on the energetics of embryonic development, Malleefowl eggs are artifically incubated over a range of temperatures and oxygen consumption measured throughout incubation.

Water economy of incubating eggs forms an important part of incubation physiology. Most birds' eggs lose between 12% and 20% of their initial mass in water over the incubation period (Rahn and Ar 1974, Ar and Rahn 1980). Metabolic water is produced by the developing embryo. The balance between metabolic water production and water loss during incubation appears to be important, as the water content of fresh eggs and neonate birds is the same (Ar and Rahn 1980, Rahn 1984). The high humidity conditions in underground nests retards water loss from incubating eggs (Seymour and Rahn 1978, Seymour and Ackerman 1980, Rahn 1984) and may lead to water balance problems. Water loss throughout incubation in Malleefowl eggs is monitored to see how the potential problem of impeded water loss is overcome.

Because the major substrate metabolised during embryonic development is thought to be lipid (Romanoff 1967), the respiratory quotient (RQ) of developing eggs has been assumed to be close to 0.71. The avian eggshell consists principally of calcium carbonate (Romanoff and Romanoff 1949, Sturkie and Mueller 1976) and during development some of the shell calcium is mobilized to form the developing skeleton consisting chiefly of $Ca_3(PO_4)_2$ (Romanoff 1967, Packard and Packard 1984). This process produces carbon dioxide (CO_2) which is liberated from the egg (Romanoff 1967). In early incubation considerable amounts of CO_2 are released from the albumen (Barott

1937, Romanoff and Romanoff 1949, Romanoff 1967). Because non-respiratory CO_2 may be leaving the egg, measurement of oxygen (O_2) and CO_2 exchange may not give a strictly correct measurement of RQ. I shall use the term respiratory exchange ratio (RE) to describe the exchange of O_2 and CO_2 in eggs. If lipid is the metabolised substrate and CO_2 is also released from the eggshell, RE values greater than 0.71 can be expected. The RE over the last four weeks of incubation is studied in Malleefowl eggs to see if it is similar to other avian eggs.

3.2 MATERIAL AND METHODS

3.2.1 Measurement of natural incubation mound temperatures

Temperature profiles of natural incubation mounds were collected with a temperature probe constructed of a 1.5 m long fibreglass tubular fishing-rod blank with copper-constantan thermocouples inserted at 10 cm intervals along its length. The probe was inserted vertically at the centre of the mound and the temperatures at various depths recorded with an electronic thermometer (Comark, type 1624). The mound was then dug out and the depth at which eggs located recorded.

In a separate experiment, two eggs from a mound located in Pooginook Conservation Park (70 km south-west of the Renmark study site) had thermistors attached to their surface with masking tape and were re-buried in the egg chamber (area where eggs are laid; Frith 1955). Two other thermistors were buried 6 - 8 cm away from the eggs in the surrounding sand. All thermistors were connected to a temperature recorder (Grant Model D) which was buried 50 cm underground and the mound left undisturbed for seventy days. The calibration of the thermistors and recorder was checked on arrival back in the laboratory.

3.2.2 Laboratory incubation procedure

Eggs were collected from natural incubation mounds and incubated in constant temperature cabinets at 30, 32, 34, 36, and 38 C as previously described (section 2.1). When oxygen consumption ($\dot{V}O_2$) of eggs was to be measured, the plastic container holding the eggs was removed from the temperature cabinet and placed on a bench. A previously calibrated 28 gauge copper-constantan thermocouple was slid

down the side of each buried egg in turn so that the tip of the thermocouple was in contact with the eggshell on the side of the egg and the temperature recorded to 0.1 C. Sand temperature in the container was also recorded. An egg was then removed from the sand, weighed to the nearest milligram on an electronic balance (Sartorius 1265 MP) and placed in a respirometry chamber. The plastic container was returned to the temperature cabinet. When $\dot{V}O_2$ measurement had finished the egg was re-weighed, and returned to the appropriate plastic container.

3.2.3 Measurement of oxygen consumption and carbon dioxide production

Respiratory gas exchange was measured in an open flow system. A paramagnetic oxygen analyser (Taylor Servomex OA184) was used for O_2 analysis, and an infrared carbon dioxide analyser (Beckman LB-2 medical gas analyser) used for CO_2 analysis. The CO_2 analyser was only available for a short time in 1984; for measurements at all other times only O_2 analysis was performed. When $\dot{V}O_2$ alone was determined, compressed air was directed through a flow meter (Fischer and Porter FP-1/8-12G5) into a constant temperature cabinet, where it was humidified by passing it through an air-stone immersed in distilled water and directed into a cylindical acrylic respirometry chamber (diameter = 8.5 cm, height = 16 cm). Gas leaving the chamber passed through a water trap consisting of a test-tube in an ice-water bath and was directed through a water absorber (Drierite) and a CO_2 absorber (Ascarite) to the sample channel of the O_2 analyser. Gas leaving the analyser was vented to the atmosphere through a flow meter (Fischer and Porter FP-1/8-12G5). A sample of compressed air was directed through Drierite and Ascarite to the reference channel of the O_2 analyser. Oxygen consumption was calculated from equation (4) of Hill (1972) and

corrected to STP. When CO_2 analysis was also performed, Ascarite was removed from both the sample and reference lines and a sample of gas leaving the flow meter in the sample line on the down stream side of the O_2 analyser directed to the CO_2 analyser. Carbon dioxide production ($\dot{V}CO_2$) and $\dot{V}O_2$ were calculated using equations (2) and (1) respectively of Gleeson (1979). Due to the plumbing arrangment a lag time of approximately 60 s existed between reactions of the O_2 analyser and CO_2 analyser. The O_2 analyser was calibrated as previously described (section 2.3) and the CO_2 analyser calibrated by using CO_2 free air and a calibrated 5% CO_2 in O_2 gas mix. To check the system ethanol was burnt in the respirometry chamber and this gave RE values between 0.65 and 0.68. Burning ethanol should have a RE of 0.67.

3.2.4 Measurement of eggshell thickness

Eggshell fragments from 14 Malleefowl eggs were boiled in 5% NaOH for approximately 20 min to remove organic matter adhering to them (Tyler and Geake 1953). Four of these eggs were infertile, one had died early in incubation, one had died late in incubation, and eight had hatched successfully. After boiling in NaOH fragments were rinsed in water and dried in an oven at 50 C for approximately 40 min. Two fragments from the pole, four from the shoulder, and four from the equator area of each egg had their thickness measured to the nearest 0.0001 mm with a micrometer (Mitutoyo 0-25 mm) which had a ball bearing attached to the fixed end of the measuring gap so that distortion of readings due to the curvature of the shell fragment was avoided.

3.2.5 Measurement of eggshell water vapour conductance

Water vapour conductance (G_{H_2O}) was measured in Malleefowl

shell fragments from the same eggs used in eggshell thinning measurements (see section 3.2.4). Fragments from the pole, shoulder and equator of each egg were treated with 5% NaOH as in section 3.2.4 and sealed to the top of 30 ml glass vials with a hot glue applicator (Bostik). The surface area of shell fragments glued to the vials was 4.5 cm^2. The vials contained 8 ml of water and a wad of cotton wool. The seals were inspected for holes and the vials placed in a desiccator containing silica gel at 34 C overnight. Then they were transferred with gloved hands to an electronic balance (Mettler AE 163), weighed to 0.01 mg, replaced in the desiccator for 3 - 5 d and reweighed.

3.3 RESULTS

3.3.1 Natural incubation temperatures

Temperature within the egg chamber of natural incubation mounds varied considerably both temporally within any given mound, and between mounds at any given time in the breeding season (Figs. 3.1 and 3.2). There did not appear to be any consistent increase or decrease in incubation temperature as the breeding season progressed. At the Renmark study site temperatures as low as 29 C and as high as 38 C were recorded. Mean egg chamber temperature throughout the study was 34.1 $\pm$ 0.4 C ($\pm$ 95% confidence limits). During the course of incubation eggs could be exposed to relatively long term (3 - 4 d) fluctuations in temperature of up to 4 C (Figs. 3.3 and 3.4).

3.3.2 Effect of incubation temperature on egg hatching success

If no increase in $\dot{V}O_2$ of incubating eggs was detected after 25 d of incubation, eggs were opened and examined for signs of embryonic development. In the laboratory, eggs were successfully incubated at temperatures of 32, 34, 36, and 38 C. An incubation temperature of 34 C produced the greatest hatchability (Table 3.1). Despite the fact that no eggs hatched when artifically incubated at 30 C, two eggs in a natural incubation mound hatched after exposure to two periods of 12 d when incubation temperatures were below 30 C (Figs. 3.3 and 3.4).

3.3.3 Effect of incubation temperature on oxygen consumption

Egg $\dot{V}O_2$ data was standardized for weight bias by dividing the raw data by the mass of the egg when the egg was collected. Water loss during the first two weeks of incubation was small, so water lost during this time has a negligible effect on the standarization

Table 3.1. Hatching success of Malleefowl eggs incubated artifically at various temperatures.

Incubation temperature (C)	Number of eggs set	Number of infertile eggs	Number of deaths during incubation	Number of chicks hatched	Percentage hatch[a]
30	6	2	4	0	0
32	11	2	7	2	22
34	12	2	2	8	80
36	10	1	5	4	44
38	10	2	5	3	38

[a] Percentage hatch = $\frac{\text{number of eggs which hatched}}{\text{number of fertile eggs set}} \times 100$

process. Justification for standardization can be seen in egg 42-5. Despite an initial mass of only 92 g compared to a mean of 156 g for the other eggs incubated at 34 C, the mass corrected $\dot{V}O_2$ curve of 42-5 is indistinguishable from the other eggs (Fig. 3.6). Egg 42-5 failed to hatch, but the embryo, when examined, appeared normal and had completely absorbed the yolk sac into the abdominal cavity. Because the exact laying date of eggs was not known, all data are expressed in terms of days before hatch (DBH).

Total oxygen consumed during incubation at each incubation temperature was estimated by integrating the area under the $\dot{V}O_2$ curve throughout incubation (Table 3.2). In eggs where $\dot{V}O_2$ data were missing from early incubation, the VO_2 curve was extrapolated using data from eggs which had been incubated from an early stage and the area under it included in total oxygen consumption calculations. The

amount of oxygen consumed by individual eggs incubated at the same temperature was similar, but differences occurred in amounts of oxygen consumed by eggs incubated at different temperatures (Figs. 3.5 - 3.9, Table 3.2). A Student-Newman-Keuls test for unequal sample sizes (Sokal and Rohlf 1969, p.272) was used to test significance of differences in oxygen consumed at different temperatures. The amounts of oxygen consumed at 36 C and 38 C were not significantly different, but significantly ($P < 0.01$) more oxygen was consumed at 34 C compared to either 38 C or 36 C, and significantly ($P < 0.01$) more oxygen consumed at 32 C compared to 34 C. Analysis of variance showed that there were no significant diffences in egg mass of eggs used at each of the incubation temperatures, so differences in total oxygen consumed by eggs throughout incubation can be attributed to temperature effects rather than egg size effects.

The daily $\dot{V}O_2$ data from eggs incubated at the same temperature were pooled for each day of incubation and a mean $\dot{V}O_2$ curve generated for each temperature (Fig. 3.9). The curve shapes for eggs incubated at 34, 36, and 38 C were similar, $\dot{V}O_2$ increasing until the last week of incubation when it plateaued. Oxygen consumption over this plateau period was highest in eggs incubated at 34 C, lowest in eggs incubated at 38 C, and intermediate in eggs incubated at 36 C. A different $\dot{V}O_2$ pattern occurred in both eggs incubated at 32 C. Oxygen consumption increased during incubation until 7 - 8 DBH, after which $\dot{V}O_2$ declined steadily until hatching (Figs 3.5 and 3.9).

Respiratory exchange ratio over the last four weeks of incubation for six eggs incubated at 34 C, and one egg incubated at 32 C averaged 0.80, but increased significantly from 0.78 to 0.82 during this period (Fig. 3.10).

Table 3.2 Total oxygen consumed by Malleefowl eggs at different incubation temperatures

Egg No.	Initial mass (g)	Incubation temperature (C)	Oxygen consumed ($ml O_2 . g^{-1}$)
35-11	159	32	274
45-2	169	32	270
mean	164 ± 64[a]	32	272 ± 26[a]
12-13	173	34	180
35-13	144	34	199
26-12	153	34	181
17-9	136	34	187
6-1	179	34	209
42-1	150	34	174
42-5	92	34	183
mean	148 ± 26	34	188 ± 9
45-3	166	36	153
35-3	152	36	165
12-3	173	36	151
25-9	164	36	151
mean	164 ± 14	36	155 ± 11
45-4	162	38	166
45-5	167	38	161
40-6	177	38	143
mean	169 ± 19	38	158 ± 31

[a] 95% confidence interval

3.3.4 Mass loss during incubation

Daily mass loss was calculated from the mass difference of eggs between consecutive $\dot{V}O_2$ measurements. Mass loss data were standardized in the same way as $\dot{V}O_2$ data. Data from eggs incubated at the same temperature were pooled and mean curves generated for each temperature (Fig. 3.11). At all temperatures there was a 4 to 6 fold increase in mass loss over the incubation period, almost all of the increase occurring over the last 20 d of incubation. Total mass loss over the incubation period at each incubation temperature was estimated by integrating the area under

the mass loss versus time curve. Incubation periods of 50 d at 38 C, 55 d at 36 C, 60 d at 34 C, and 65 d at 32 C were estimated by relating probable laying date and known hatching dates of eggs incubated at these temperatures. Mean mass loss of infertile eggs at 34 C was 1.31 $mg.g^{-1}.d^{-1}$ and this value used as the daily mass loss at the beginning of the incubation period if actual mass loss data were absent. I assumed that mass loss of infertile eggs was due entirely to water loss. At 34 C mass loss due to high RE was calculated on a daily basis using information on daily VO_2 (Fig. 3.9) and RE (Fig. 3.10) using the equation:

$$\text{Mass loss due to high RE } (mg.g^{-1}.d^{-1}) = \frac{\dot{V}O2 \times RE \times 44}{22.26} - \frac{\dot{V}O2 \times 32}{22.4}$$

where 44 = molecular weight of CO_2

32 = molecular weight of O_2

22.26 = volume (liters) of one mole of CO_2 at STP

22.4 = volume (liters) of one mole of O_2 at STP

Total daily mass loss increased 513%, mass loss due to high RE increased 2,130%, and mass loss due to water loss (calculated by subtracting mass loss due to high RE from total mass loss) increased 416% over the last 31 d of incubation (Fig. 3.12). Hence, at the end of incubation mass loss due to high RE accounts for a significant proportion (24%) of total daily mass loss. At 34 C, total mass loss, mass loss due to high RE, and mass loss due to water loss over the incubation period was estimated by integrating the area under the various mass loss curves over the entire incubation period (Table 3.3). For eggs younger than 32 DBH, mass loss due to high RE was negligible (Fig. 3.12); thus mass loss was attributed entirely to water loss.

Table 3.3 Total mass loss of Malleefowl eggs incubated artificially in sand at various temperatures. Values calculated from figures 3.11 and 3.12 (see text).

Incubation temperature (C)	Mass loss due to high RE ($mg.g^{-1}$)	Mass loss due to water loss ($mg.g^{-1}$)	Total mass loss ($mg.g^{-1}$)
32[a]			141
34	17	110	127
36[a]			105
38[a]			155

[a] Information on RE over the entire incubation period was not obtained so mass loss could not be divided into water loss and high RE components

3.3.5 Egg temperature during incubation

A temperature gradient between the eggshell surface and the surrounding sand developed in artifically incubated eggs at all incubation temperatures, and the gradient reached 1.5 - 2.0 C in full-term eggs (Figs. 3.13 - 3.16). In all cases, the increase in the temperature gradient as incubation proceeded paralleled the increase in $\dot{V}O_2$ over the incubation period. A 3.0 - 4.0 C gradient developed between the eggshell surface and the surrounding sand in two naturally incubated eggs (Figs. 3.3 and 3.4).

Internal egg temperature (T_e) of incubating eggs throughout incubation was estimated using information on eggshell thermal conductance (K), metabolic heat production ($\dot{M}$), evaporative heat loss ($\dot{H}_e$), and external eggshell temperature (T_{es}). Thermal conductance of the eggshell was estimated with the use of a bionic Brush turkey egg (Seymour

Vleck, Vleck, and Booth, manuscript). Briefly, the bionic egg consisted of an infertile Brush turkey egg which had its contents removed and replaced with vermiculite. A precision resistor (333 ohms) was placed within the vermiculite and wires attached to the resistors fed out through a hole in the eggshell. Three thermocouples in parallel were attached to the inside surface of the eggshell, their ends coming out through the hole in the shell. Two pieces of plastic catheter tubing also came through the hole in the eggshell. Resistor wires, thermocouples and tubing were secured in place with epoxy adhesive, making the hole in the eggshell water-tight. Water was syringed into the egg through one of the catheter tubes, the excess air being expelled through the other catheter tube; then both tubes were sealed with plugs. Three more thermocouples in parallel touched the outside surface of the eggshell. A known voltage was applied to the resistor so that heat production was equivalent to an egg just before hatching (330 mW) and the temperature gradient across the eggshell at equlibrium recorded. Heat loss by evaporation was estimated by weighing the bionic egg before and after a measurement period. At equilibrium:

$$\dot{M} - \dot{H}_e = K(T_e - T_{es}) \qquad (3.1)$$

For the bionic Brush turkey egg K was calculated to be 170 $mW.C^{-1}$. Thermal conductance of Malleefowl eggs was assumed to be the same as that of the bionic Brush turkey egg as both species are similar in size and eggshell thickness (Seymour and Ackerman 1980).

By rearranging equation 3.1, T_e can be solved for:

$$T_e = T_{es} + (\dot{M} - \dot{H}_e)/K \qquad (3.2)$$

Equation 3.2 was solved throughout incubation for Malleefowl eggs incubated at 32, 34, 36, and 38 C using values of M from Figure 3.9, $\dot{H}_e$ from Figure 3.11 (32, 36, and 38 C) and Figure 3.17 (34 C), and T_{es} from

Figures 3.13 - 3.16 (Figure 3.17). For incubation temperatures of 32, 36, and 38 C, water loss was assumed to equal mass loss. Although this assumption is probably incorrect, it makes little difference to the overall outcome as M was much greater than $\dot{H}_e$.

Eggs incubated at 32 and 34 C experienced a 4.0 C increase in T_e, eggs incubated at 36 C a 3.3 C increase in T_e, and eggs incubated at 38 C a 3.0 C increase in T_e over the incubation period (Fig. 3.17).

3.3.6 Eggshell thinning

There was a mean thinning of the eggshell during incubation. Eggshell thickness was reduced significantly ($P < 0.001$) by 16.7% from 0.0233 to 0.0194 mm (Table 3.4).

3.3.7 Eggshell water vapour conductance

There was a 3-fold difference between rate of water loss of eggshell fragments before and after incubation (Table 3.5). G_{H_2O} of intact Malleefowl eggs before and after incubation was calculated using the mass loss data in Table 3.5, a water vapour pressure gradient of 39.9 torr (saturated water vapour pressure at 34 C) and assuming a 170 g egg has a surface area of 144.9 cm^2 (Paganelli et. al. 1974). G_{H_2O} was calculated to be 20.2 $mg.d^{-1}.torr^{-1}$ in unincubated eggs and 64.2 $mg.d^{-1}.torr^{-1}$ in incubated eggs.

Table 3.4. Eggshell thickness of Malleefowl eggs before and after incubation. Each value is the mean of ten measurements on the same egg.

Egg No.	Status	Mean thickness (mm)	95% confidence interval
MF6	undeveloped	0.0266	± 0.0006
MF21	early incubation	0.0218	± 0.0008
12-3	undeveloped	0.0229	± 0.0007
16-1	"	0.0239	± 0.0015
45-11	"	0.0215	± 0.0005
Mean	undeveloped	0.0233	± 0.0019
1-5	Hatched	0.0192	± 0.0017
1-8	"	0.0202	± 0.0019
1-9	"	0.0215	± 0.0021
5-1	"	0.0179	± 0.0015
5-2	"	0.0184	± 0.0024
5-3	"	0.0181	± 0.0016
5-4	"	0.0175	± 0.0017
45-14	"	0.0195	± 0.0020
12-5	late incubation	0.0219	± 0.0011
Mean	Hatched	0.0194	± 0.0014

Table 3.5 Water loss through eggshell fragments of Malleefowl eggs, before and after incubation.

	Mass loss of shell fragments ($mg.d^{-1}.4.5\ cm^{-2}$)			
	Whole egg[a]	Pole	Shoulder	Equator
Before				
Mean	25.0	28.0	22.4	22.7
SD	10.5	12.9	10.6	13.4
N[b]	5	5	4	5
After				
Mean	79.6	52.3	90.9	97.7
SD	31.9	28.9	46.2	37.9
N	9	7	10	9
Difference				
Mean	54.6	24.2	68.5	75.0
t	3.66	1.74	2.87	4.19
P	< 0.01	NS	< 0.05	< 0.01

[a] Whole egg is mean of mean of all fragments from each egg

[b] Number of eggshell fragments measured

3.4 DISCUSSION

3.4.1 Natural incubation temperature and effect of temperature on hatchability

Egg chamber temperatures in natural incubation varied considerably in the eleven mounds examined at the Renmark site. In a previous study (Frith 1956, 1957) the egg chamber temperature throughout the incubation season remained remarkably constant (Fig. 1 in Frith 1956). However, the data used to construct this figure were "smoothed by weighting the daily values, the ordinate for each day being the 7-day running mean... as... daily fluctuations in the temperature were rather great and tended to obscure some of the features of the graphs" (Frith 1956). Fluctuations of up to 6.5 C over 48 h were observed at the end of the breeding season (Frith 1956). Because my measurements were instantaneous and not averaged over a period of days considerable variation in egg chamber temperature may be expected.

Almost all egg chamber temperatures recorded from natural mounds at Renmark are in the range 32 - 36 C (Figs. 3.1 and 3.2). These temperatures are at the lower end of the range of egg temperatures reported for other avian species (mean = 35.6 C, Table 1 in Drent 1975). However, as Malleefowl eggs develop, egg temperature rises 3 - 4 C above sand temperature (Fig. 3.17) which brings mean egg temperature throughout incubation at 34 C up to 35.1 C (Fig. 3.17), a level similar to other birds.

Short term, relatively rapid fluctuations in egg chamber temperature due to the mound-opening behaviour of parent birds (Frith 1956) are probably similar in nature to the sorts of temperature

fluctuations experienced by eggs of single parent brooding avian species. Daily fluctuations in egg chamber temperature are only 0.1 - 0.3 C in this study (Figs. 3.3, 3.4), but longer term (4 - 5 d) larger fluctuations in temperature (total range of 3 - 4 C) are characteristic (Figs. 3.3 and 3.4). The slowness of these fluctuations is due to the large thermal inertia of the mound. That Malleefowl eggs developed and hatched successfully under such a thermal regime is remarkable. If Domestic fowl Gallus domesticus eggs are incubated for periods of one or more days at temperatures 3 - 4 C below normal, disproportionate development of organs or limbs occur and the embryos usually die (Romanoff 1960, Lundy 1969, Drent 1975). In general, bird eggs are apparently more tolerant to temperatures well below normal than to temperatures just below normal (Lundy 1969), especially early in incubation (McMullan and Eberhardt 1953, Lundy 1969, Howell 1979). A probable explanation for this phenomenon is that at colder temperatures development ceases but the embryo is still able to maintain itself (Lundy 1969, Drent 1975, Vleck and Kenagy 1980, Williams and Ricklefs 1984), while at the higher sub-normal temperatures development continues but malformations arise (Romanoff 1960, Lundy 1969, Drent 1975).

Malleefowl eggs are successfully incubated artifically at temperatures ranging from 32 to 38 C, although hatchability decreases markedly at the temperature extremes. The incubation temperature of greatest hatchability is 34 C where 80% of eggs hatch, 38% hatch at a temperature 4 C above this, and 22% hatch at a temperature 2 C below this (Table 3.1). The optimal incubation temperature for Domestic fowl is 37 - 38 C (80 - 90% hatch); no eggs survive incubation temperatures 4 C above the optimum, and only 0 - 10% survive incubation temperatures 2 C below the optimum (Romanoff 1936, Barott 1937, Lundy 1969). Virtually no data

are available on the hatchability of eggs incubated continuously at different temperatures for other bird species. Eggs of the Fork-tailed storm petrel Oceanodroma furcata at various stages of incubation failed to hatch when transferred to 26 C, while one near full-term egg hatched after 8 d at 30 C; four other eggs died when incubated at 30 C (Vleck and Kenagy 1980). None of the four fertile Malleefowl eggs set at 30 C developed. However, egg 2-2 from a natural incubation mound in Pooginook Conservation Park experienced an average incubation temperature of only 30.7 C (Fig. 3.4), yet hatched successfully. Egg 2-3 from the same mound also hatched after exposure to similar low temperatures (Fig. 3.3). At one stage egg 2-3 was exposed to 27 C for 3 d (Fig. 3.3) and egg 2-2 to 28 C for 3 d (Fig. 3.4).

Successful hatching of Malleefowl eggs incubated up to 90 d at 27 C has been reported (Frith 1959). The longest successful incubation period recorded for a Malleefowl egg is 99 d (Bellchambers 1917). The shortest natural incubation period recorded is 49 d (Frith 1962b), while an egg hatched after 44 d when incubated artifically at 38 C (Frith in Nice 1962). In this study the exact laying dates of eggs were not known, but because of the frequency at which mounds were visited, eggs were no older than two weeks when collected, and in most cases were less than one week old. Egg 2-2 was about one week old when the temperature monitoring experiment at Pooginook started, making the total incubation period about 70 d at an average temperature of 30.7 C. From my laboratory incubation experiments total incubation times of between 45 - 50 d seem likely for eggs incubated at 38 C. Reported natural incubation periods from 49 d to 99 d would therefore imply continuous incubation at temperatures lower than 30 C for the 99 d period and as high as 38 C for the 49 d period. This wide range of successful incubation temperatures, and hence

incubation periods, is peculiar to Malleefowl, and is probably an adaptation to their unusual form of nidification. Birds that incubate their eggs under the adult are probably capable of regulating egg temperature more precisely than Malleefowl as incubating birds are in intimate contact with the eggs and can detect egg temperature through the brood patch (White and Kinney 1974, Haftorn 1984), whereas Malleefowl can only regulate the temperature of a mound which contains the eggs. Some procellariiform birds have variable incubation periods (Boersma and Wheelwright 1979, Vleck and Kenagy 1980), but the variability is caused by days of neglect when the eggs are not incubated at all (Boersma and Wheelwright 1979, Vleck and Kenagy 1980) and not by eggs being continuously incubated at varying temperatures.

Malleefowl eggs are also tolerant of short term exposure to both high and low temperatures. The full-term eggs used in cooling/heating experiments (chapter 4) hatched after exposure for 1 - 2 h at temperatures of 40 - 42 C, and 25 - 26 C. On one occasion a temperature cabinet was accidently turned off overnight and five eggs at varying stages of incubation were cooled to 25 - 26 C. All these eggs subsequently hatched when normal incubation was continued. Egyptian plover Pluvionus aegyptius (Howell 1979) and Domestic fowl (Lundy 1969) eggs survive short term exposure to temperatures between 40 - 42 C.

3.4.2 Egg temperature throughout incubation

Egg chamber and hence egg temperatures can vary considerably over the natural incubation period in Malleefowl (Figs. 3.1 - 3.4). Even if the egg chamber temperature remains constant throughout incubation the egg temperature changes with time as the increasing metabolism of the growing embryo increases egg temperature above that of the surrounding sand (Fig.

3.17). In fact, naturally incubated Malleefowl embryos can tolerate large changes in temperature. In egg 2-2 from Pooginook, egg temperature (T_e) increased from 28 C early in incubation (Fig. 3.4) to 38 C just before hatching (calculated from the information: T_{es} = 36 C, Fig. 3.4; and VO_2 assumed to equal that of eggs incubated at 32 C which was the temperature of sand in the egg chamber), i.e., the embryo tolerated a 10 C increase in temperature as development proceeded. When eggs of other avian species are artifically incubated, they also experience a rise in temperature as incubation proceeds (Domestic fowl, Romanoff 1941, Romijn and Lokhorst 1956; Domestic duck Anas platyrhnchos, Khaskin 1961; Herring gull Larus argentatus, Drent 1970). Naturally incubating eggs of several avian species are reported to experience a rise in mean egg temperature as incubation proceeds. Herring gull eggs increase from 32 C to 39 C (Drent 1970), Laysan albatross Diomedea immutabilis eggs increase from 34.2 C to 37.3 C (Grant et. al. 1982), Black-footed albatross D. nigripes eggs increase from 33.7 C to 36.3 C (Grant et. al. 1982), and Great tit Parus major eggs increase from 36.1 C to 36.6 C (Haftorn 1983). In eggs which are brooded by parental birds a temperature gradient exists between the top of the egg where the brood patch is in contact with the egg and the bottom of the egg where the egg contacts the nest substrate. This gradient is particularly marked at the beginning of incubation when the embryo is small (Drent 1970). So, despite a 7 C change in mean egg temperature of Herring gulls, the Herring gull embryo experiences a remarkably stable thermal environment between 37.6 C and 39.0 C throughout incubation because at the beginning of incubation the embryo lies at the top of the egg directly under the warm brood patch of the incubating parent (Drent 1970).

Increase in egg temperatures as incubation proceeds may be a

general phenomenon in in naturally incubated avian eggs. The magnitude of this increase appears to be related to egg size. Large eggs produce larger embryos than smaller eggs. Because metabolic heat production depends to a large extent on embryo size, large eggs generate more heat than smaller eggs, and this causes a greater rise in T_e in large eggs. Hence eggs of Domestic fowl (egg mass = 60 g) experience an increase of 3 C in T_e (Romanoff 1941), whereas those of Malleefowl (egg mass = 170 g) experience an increase of 4 C (Fig. 3.17).

3.4.3 Effect of incubation temperature on oxygen consumption

In my study Malleefowl eggs incubated at 34 C consumed an average of 580 $\pm$ 30 kJ of energy (assuming an energy equivalent of 19.64 kJ/litre O_2, Vleck et. al. 1980b). This value is similar to the figures of 613 kJ (calculated from $\dot{V}O_2$ data) and 583 kJ (calculated from energy content of fresh eggs and hatchlings) obtained by Vleck et. al. (1984). Embryonic development is energetically expensive in Malleefowl (Vleck et. al. 1984), taking 188 milliliters of oxygen per gram of fresh egg mass to develop at 34 C compared to the range 65 - 138 $mlO_2.g^{-1}$ found in other avian species (Ar and Rahn 1978, Hoyt and Rahn 1980). The high cost of embryonic development is attributed to maintenance of embryonic tissue over an extended incubation period and the production of an extremely precocious hatchling (Vleck et. al. 1984).

Malleefowl eggs consume more O_2 when incubated at lower temperatures (Table 3.2) indicating a greater energetic cost of development at lower temperature. The increased amount of O_2 consumed is caused by both a greater absolute daily rate of $\dot{V}O_2$ and longer incubation times at lower temperatures (Fig. 3.9). Embryonic development occurs most economically at temperatures between 36 - 38 C, development at

34 C requires 21% more energy and at 32 C 74% more energy. The energetic cost of development in an embryo may be divided into two parts, the cost of tissue synthesis, and the cost of maintenance of embryonic tissue (Vleck et. al. 1980a,b, Vleck et. al. 1984). Energy expenditure due to tissue synthesis is thought to be proportional to embryonic growth rate, and expenditure due to maintenance proportional to embryo mass (Vleck et. al. 1980b). At lower temperatures growth rate is slower (hence incubation period is longer) and the maintenance cost of embryonic tissue is also likely to be lower (because of a Q_{10} effect on tissue metabolism). However, the Q_{10} of growth rate and tissue maintenance may be different so that the proportion of energy spent on either tissue maintenance or growth at any particular stage in development may change with temperature. Because both growth rate and tissue maintenance cost are expected to decrease with decreasing temperature, a decreased peak $\dot{V}O_2$ is also expected at lower temperatures. Exactly the opposite is observed in Malleefowl eggs, i.e., as incubation temperature decreases an increase in peak $\dot{V}O_2$ is observed (Fig. 3.9). Whether this result is due to increased synthetic or maintenance costs is unknown. Studies investigating the effect of temperature on the energetics of tissue synthesis are absent. A large increase in tissue synthesis with decreased temperature must be postulated if it is to account for the increase in energy expenditure observed in Malleefowl eggs. However, because full term Malleefowl embryos incubated at 34 C develop an endothermic response during the last week of incubation (chapter 4), the possibility that embyros increase heat production at low incubation temperatures in a primordial attempt to thermoregulate and thus increase the 'maintenance' energy cost should not be ruled out (see section 4.4.3).

Chicks emerging from eggs incubated between 36 - 38 C presumably

have greater energy reserves and hence should be able to survive longer periods of food deprivation compared to chicks from eggs incubated at temperatures between 32 - 34 C. This potential selective advantage is apparently outweighed by greater hatchability of eggs at 34 C (Table 3.1), as parent birds try to regulate egg chamber temperature around 34 C (Frith 1957, 1959a, Figs. 3.1 and 3.2).

The effect of incubation temperature on embryonic metabolism has been examined in only one other avian species, the Domestic fowl (Barott 1937). Incubation time is found to vary with incubation temperature, eggs incubated at lower temperatures taking longer to hatch. However, the total amount of energy consumed by the chick embryo at various temperatures is similar because the rate of metabolism of embryos at lower temperatures is decreased (Barott 1937). I have re-examined Barott's (1937) data (Fig 3.18) and found that Domestic fowl eggs consume slightly less energy at higher incubation temperatures (Table 3.6). The trend of total energy expenditure to increase with a lowering of incubation temperature in Domestic fowl eggs is the same as for Malleefowl eggs, except that the differences in Domestic fowl eggs are not as great. The energetic cost of embryonic development appears to be temperature dependent in birds.

Interspecific analyses of energetic cost of development and incubation time for birds (Vleck et. al. 1980b, Ackerman 1981) and reptiles (Ackerman 1981) reveal that longer incubation times entail an increased energetic expense. This finding is consistent with the intraspecific results from Malleefowl and Domestic fowl which show that incubation at low temperatures causes longer incubation periods and greater energetic cost.

Table 3.6. Energy consumed by Domestic fowl eggs at various incubation temperatures. Data taken from Barott (1937).

Incubation temperature (C)	Energy consumption (kJ)
35.6	85.0
36.7	78.1
37.2	77.7
37.8	77.4
38.9	75.3
39.7	66.7

Incubating birds' eggs consume 4 - 6 times more O_2 per gram of fresh egg mass than incubating reptile eggs (Ackerman 1981). The increased cost of development in birds' eggs is attributed to the greater incubation temperatures (5 - 8 C) of avian eggs giving rise to higher tissue maintenance costs and this led Ackerman (1981) to state: "There is clearly a real energetic advantage to be gained through development at cooler temperatures." However, results from Malleefowl and Domestic fowl eggs incubated at different temperatures are inconsistent with this statement. A study on the effect of incubation temperature on $\dot{V}O_2$ of eggs in the fresh water chelonian Emydura macquarii showed that the total amount of O_2 consumed to the pre-pipping stage of incubation was the same at temperatures of 25 C and 30 C despite a longer incubation period at 25 C (Thompson 1983). Clearly, more studies examining the effect of incubation temperature on energy consumption during incubation in different species of both birds and reptiles are needed before generalizations about temperature effects on embryo developmental energetics can be made.

The patterns of ontogeny of $\dot{V}O_2$ of Malleefowl eggs at temperatures of 34, 36, and 38 C are similar (Fig. 3.9) and are typical for precocial birds' eggs (Vleck et. al. 1979, Vleck et. al. 1980b). However, eggs incubated at 32 C show a sharp decline in $\dot{V}O_2$ over the last few days of incubation in a manner similar to that of the ratites, Ostrich Struthio camelus (Hoyt et. al. 1978), Emu Dromiceius novaehollandiae, and Rhea Rhea americana (Vleck et. al. 1980a). The decline in $\dot{V}O_2$ at the end of incubation in ratite embryos is attributed to a period when little growth is occurring (Hoyt et. al. 1978, Vleck et. al. 1980a,b). This explanation may apply to Malleefowl eggs incubated at 32 C, but embryonic growth data are not known. In ratites the length of the period of decline in $\dot{V}O_2$ is flexible to some extent and may be a mechanism allowing synchronized hatching within a clutch (Vleck et. al. 1980a). However, Malleefowl eggs do not hatch synchronously, so it is difficult to imagine why a fully developed embryo would stay inside an egg using up energy when it could hatch. Even more puzzling is that this pattern only occurs at 32 C.

3.4.4 Mass loss of eggs during incubation

Incubation temperature does not appear to have a consistent effect on egg mass loss, the highest mass loss rates being recorded at 32 C and 38 C. Water loss is the major component of mass loss (Table 3.3, Fig 3.12). Because incubation sand water content was not controlled (other than humidifiers being placed in temperature cabinets) or monitored in these experiments, variation in sand water content (which affects water loss rate) between different plastic containers and between different temperatures may have masked any effect of temperature if one exists.

A most striking result is that mass loss rate increases 4 - 6-fold during incubation at all the incubation temperatures used (Fig. 3.11). In eggs incubated at 34 C a 6-fold increase in mass loss rate occurs, water loss ($\dot{M}_{H_2O}$) increases 4-fold, the remainder of the increase is due to CO_2 loss (Fig. 3.12). Mass loss is usually assumed to equal water loss of incubating eggs (Ar et. al. 1974). However, if RE is greater than 0.725, mass loss other than that due to water loss occurs because the mass of CO_2 released from the egg is greater than the mass of O_2 consumed. Most of the increase in $\dot{M}_{H_2O}$ occurs over the last 16 d of incubation (Fig. 3.12). Relatively small increases in $\dot{M}_{H_2O}$ with incubation time have been reported for House wrens Troglodytes aedon (Kendeigh 1940), Robins Turdus migratorius (Carey 1979), Redwing blackbirds Agelaius phoeniceus (Carey 1979), Cliff swallows Petrichelidon pyrrhonota (Sotherland et. al. 1980), Bank swallows Riparia riparia (Birchard and Kilgore 1980), Barn swallows Hirundo rustica (Birchard and Kilgore 1980), Laysan albatross Diomedea immutabilis (Grant et. al. 1982), and Black-footed albatross D. nigripes (Grant et. al. 1982).

$\dot{M}_{H_2O}$ can be described by the Fick equation:

$$\dot{M}_{H_2O} = G_{H_2O} \times (P_{H_2O_{in}} - P_{H_2O_{out}}) \quad (3.3) \quad \text{(Ar et. al. 1974)}$$

where G_{H_2O} = water vapour conductance of the egg shell ($mgH_2O.d^{-1}.torr^{-1}$)

$P_{H_2O_{out}}$ = water vapour pressure outside the egg (torr)

$P_{H_2O_{in}}$ = water vapour pressure inside the egg (torr)

$\dot{M}_{H_2O}$ increases if G_{H_2O} increases or the water vapour gradient across the eggshell increases. It is usually assumed that G_{H_2O} remains constant throughout incubation because all gas exchange occurs through pores in the shell, the physical structure of which is set when the shell is formed and this does not alter during incubation (Rahn and Ar 1974, Ar et. al. 1974, Paganelli et. al. 1978). G_{H_2O} depends on the number, length

and effective area of the pores in the shell (Wangensteen et. al. 1970, Ar et. al. 1974). If calcium is mobilized from the eggshell, the eggshell may be thinned and the pore length reduced resulting in an increase in G_{H_2O}. G_{H_2O} of Malleefowl eggs calculated from eggshell fragment data increases 3-fold over incubation from 20.2 $mg.d^{-1}.torr^{-1}$ to 64.2 $mg.d^{-1}.torr^{-1}$. The calculated value for unincubated eggs is close to the value empirically determined for intact unincubated eggs (21.4 $mg.d^{-1}.torr^{-1}$, Seymour and Ackerman 1980) indicating that accurate estimates of whole egg G_{H_2O} can be made from eggshell fragments. Malleefowl eggshell thins from 0.0233 mm to 0.0194 mm during incubation (Table 3.4). If pore length is reduced by an equivalent amount eggshell thinning may account for a 16.7% increase in G_{H_2O} of Malleefowl eggs during the course of incubation. The remainder of the increase in G_{H_2O} must occur by pores in the eggshell becoming larger in diameter and thus increasing the effective pore surface area. The mechanism for this pore enlarging process is unknown, but water condensing in pores may dissolve calcium carbonate ($CaCO_3$) from the inner walls of pores. Once dissolved $CaCO_3$ may diffuse to the outer shell membrane where it is absorbed and transported to the embryo. In Cliff swallows $\dot{M}_{H_2O}$ is 41% greater in eggs with developed chorioallantoises compared to eggs without chorioallantoises; 5.6% of this increase can be accounted for by a thinning of the eggshell which occurs as development proceeds (Sotherland et. al. 1980). Presumably the other part of the increase is caused by an increase in functional pore area.

Egg temperature (T_e) and egg surface temperature (T_{es}) rise during incubation in Malleefowl (Figs. 3.13 - 3.17). An increase in T_{es} causes $\dot{M}_{H_2O}$ to increase because diffusion of water vapour through the eggshell pores is temperature dependent; diffusion being faster at higher

temperatures. An increase in T_e also causes $\dot{M}_{H_2O}$ to increase as $P_{H_2O_{in}}$ increases with increasing temperature. An increase in $P_{H_2O_{in}}$ due to increased T_e accounts for increasing $\dot{M}_{H_2O}$ during natural incubation in Laysan and Black-footed albatross eggs (Grant et. al. 1982). The amount of increase in $\dot{M}_{H_2O}$ due to the rise in T_e and G_{H_2O} of Malleefowl eggs can be estimated. If G_{H_2O} for a 170 g Malleefowl egg is 64.2 $mg.d^{-1}.torr^{-1}$ at the end of incubation and $P_{H_2O_{in}}$ is equal to saturation of water vapour pressure at T_e minus 0.2 torr (the lowering of water vapour pressure due to the osmotic potential of the solutes within the egg fluid), the following calculations can be made. For Malleefowl eggs incubated at 34 C, at the beginning of incubation $\dot{M}_{H_2O}$ is equal to the $\dot{M}_{H_2O}$ of infertile eggs which averages 223 $mg.d^{-1}$ for a 170 g egg. Relative humidity of the incubation sand can be calculated from G_{H_2O} of unincubated eggs (20.2 $mg.d^{-1}$) and $\dot{M}_{H_2O}$ of infertile eggs in this sand, and is 72% (assuming saturated water vapour pressure at 34 C is 39.9 torr). On the day of hatch T_{es} = 35.8 C (Fig. 3.14), and T_e = 37.7 (Fig. 3.17) so $P_{H_2O_{in}}$ = 48.9 - 0.2 torr, and $P_{H_2O_{out}}$ is 31.7 torr (assumming that relative humidity of sand does not change with incubation time). A correction for G_{H_2O} for the increase in T_{es} from 34.0 C to 35.8 C can be made (Paganelli et. al. 1978) and is:

$$(308.8/307.0)^{0.5} = 1.003$$

Therefore, on the day of hatch $\dot{M}_{H_2O}$ = 64.2 x 1.003 x (48.7 - 31.7)

$$= 1092 \text{ mg.d}^{-1}$$

From theoretical calculations $\dot{M}_{H_2O}$ of a 170 g Malleefowl egg increases from 223 $mg.d^{-1}$ to 1092 $mg.d^{-1}$ at the end of incubation. This is close to the observed increase in $\dot{M}_{H_2O}$, as $\dot{M}_{H_2O}$ on the day of hatch is 5.48 $mg.d^{-1}.torr^{-1}$ (Fig. 3.12), which for a 170 g egg is 932 $mg.d^{-1}$.

Further evidence indicating that G_{H_2O} increases in late incubation in Malleefowl eggs comes from three eggs which had well developed but dead embryos in them. These eggs were incubated in the laboratory in the same sand filled containers at the same time as infertile or early incubation eggs from the same mound. In each case the late incubation eggs with dead embryos had $\dot{M}_{H_2O}$ averaging three times that of undeveloped eggs (Table 3.7). Because $P_{H_2O_{in}}$ and $P_{H_2O_{out}}$ would have been similar in these eggs, G_{H_2O} must increase 3 fold in late incubated eggs, an increase similar to that calculated from eggshell fragments.

Table 3.7. Rates of water loss of infertile or dead Malleefowl eggs early and late in incubation at 34 C in sand.

Mound No.	Water loss ($mg.g^{-1}.d^{-1}$)		Ratio
	Early or infertile	Late but dead	Late/early
40	1.047	3.062	2.92
17	1.413	5.787	4.09
6	1.481	2.817	1.90
Mean	1.313	3.889	2.97

3.4.5 Respiratory gas exchange ratio in Malleefowl eggs

The RE of Malleefowl eggs increased from 0.78 to 0.82 over the last

four weeks of incubation. These values are higher than usual, but are within the range previously reported for birds' eggs (Table 3.8). The high RE of Malleefowl eggs does not necessarily imply that substances other than lipid are being metabolized as non-respiratory CO_2 may be being released from the eggshell as a result of calcium mobilization (Romanoff 1967). Malleefowl eggshell thins by 16.7% during incubation (Table 3.4). Eggshell thinning occurs in Domestic Fowl (6.4%, Vanderstoep and Richards 1969), Coturnix Quail (7.3%, Kreitzer 1972), Cliff Swallows (5.6%, Sotherland et. al. 1980), Arctic Terns (8%, Finnlund et. al. 1985), and may be a general phenomenon amongst birds. Presumably thinning occurs as a result of calcium absorption by the embryo. The increase in RE over the last four weeks in Malleefowl eggs may result from an increasing amount of calcium being mobilized and hence an increasing amount of non-respiratory CO_2 being liberated.

Malleefowl eggs incubated at 34 C lose 1.7% of their initial mass as a consequence of high RE. In other avian eggs mass loss through incubation is thought to be due entirely to water vapour loss (Romanoff 1967).

Most of the values reported for avian egg RE are noted incidentally in studies on some other aspect of egg gas exchange. The only extensive information on avian egg RE throughout incubation is for the Domestic fowl (Barott 1937, Romanoff 1967). In Barott's (1937) study RE decreased slowly from 1.0 on the first day of incubation to 0.6 on day 9, and then remained about 0.65 for the remainder of the incubation period. The rather low values of RE after day 8 suggest that the measuring apparatus used in Barott's study was not very accurate. More recent studies have always shown RE in Domestic fowl eggs to be about 0.73 (Table 3.8). The

data in Tables 28 and 30 of Romanoff (1967) allow the calculation of RE for the Domestic fowl egg throughout incubation. Unfortunately these data are gathered from several sources and may not have been taken from the same eggs. However, if the data are combined, RE decreases from 6.75 on day 0 to 0.84 on day 4 and averages 0.83 for the rest of the incubation period. The high RE for the first 3 days is a result of CO_2 dissolved in the albumen being released (Romanoff and Romanoff 1949, Romanoff 1967). High RE in early incubation has also been recorded in Wedge-tailed shearwater Puffinus pacificus chlororhychus eggs (Ackerman et. al. 1980). The RE for Domestic fowl embryo by itself averages 0.80 for the first 13 d and then decreases to 0.71 by the end of incubation (Romanoff 1967). Over the first 13 d the chick embryo metabolizes lipids and proteins and a small amount of carbohydrate, but towards the end of incubation the embryo metabolizes lipid almost exclusively (Romanoff 1967). According to Romanoff (1967) the RE of the whole egg remains high throughout incubation despite a drop in RE of the embryo towards the end of incubation because the amount of CO_2 being released from the eggshell increases as incubation proceeds. On the last day of incubation CO_2 liberated from the shell accounts for 12.1% of all CO_2 released from the whole egg on this day (Romanoff 1967). However, measurements of whole egg RE average around 0.73 (Table 3.8) and not 0.83. Approximately 100 mg of calcium (0.0025 mole) is absorbed by the Domestic fowl embryo from the eggshell throughout incubation (Romanoff 1930, Packard and Packard 1984) for the formation of bones which consist principally of $(Ca)_3(PO_4)_2$. If all of this calcium comes from calcium carbonate, and all of the CO_2 (0.0025 mole) resulting from the break down of calcium carbonate is released a total of 55.7 ml of CO_2 is liberated. This is considerably less than the 358 ml cited in Romanoff (1967), and would have little effect on overall RE.

The average RE for 22 species of bird egg (0.75, Table 3.8) is about what is expected if lipid is the chief substrate being metabolized. However, many of the RE values are about 0.72 or below, so that in these eggs if CO_2 is being released from the eggshell as calcium is being removed, the RE of the embryonic tissue must be below 0.72. To achieve such low RE values the embryo must be transforming fat or protein into sugar (Barott 1937) and this seems unlikely.

Table 3.8 RE data for birds eggs gathered from the literature. Most values are one-off measurements, but for studies where RE was determined over a period of days the mean value is given.

Species	RE	source
Malleefowl *Leipoa ocellata*	0.80	This study
Domestic fowl *Gallus domesticus*	0.70	Barott (1937)
	0.74	Romijn and Lokhorst (1951)
	0.73	Romijn and Lokhorst (1955)
	0.83	Romanoff (1967)
	0.74	Rahn *et. al.* (1974)
Japanese quail *Coturnix cotunix japonica*	0.68	Rahn *et. al.* (1974)
Ring-necked pheasant *Phasianus colchicus*	0.71	Rahn *et. al.* (1974)
Turkey *Meleagris gallopavo*	0.75	Rahn *et. al.* (1974)
Pigeon *Columbia livia*	0.67	Rahn *et. al.* (1974)
African parrot *Agapornis roseollis*	0.72	Bucher and Barnhart (1984)
House wren *Troylodytes aedon*	0.72	Kendeigh (1940)
Fork-tailed storm petrel *Oceanodroma furcata*	0.72	Vleck and Kenagy (1980)
Common tern *Sterna hirundo*	0.69	Rahn *et. al.* (1974)
Herring gull *Larus argentanus*	0.62	Rahn *et. al.* (1974)

Table 3.8 cont.

Bonin petrel *Pterodroma hypoleuca hypoleuca*	0.71	Pettit *et. al.* (1982a)
Laysan albatross *Diomedea immutibilis*	0.76	Pettit *et. al.* (1982b)
Black-footed albatross *Diomedea nigripes*	0.76	Pettit *et. al.* (1982b)
Wedge-tailed shearwater *Puffinus pacificus chlororhychus*	0.82	Ackerman *et. al.* (1980)
Domestic duck *Anas platyrhynchos*	0.69	Khaskin (1961)[a]
Pekin duck *Anas boscas*	0.67	Rahn *et. al.* (1974)
Common shoveler *Anas clypeata*	0.77	Hoyt *et. al.* (1979)[a]
Red-bellied whistling duck *Dendrocygna autumnalis*	0.84	Hoyt *et. al.* (1979)[a]
Tufted duck *Aythya fuligula*	0.84	Hoyt *et. al.* (1979)[a]
Emperor goose *Anser conagicus*	0.89	Hoyt *et. al.* (1979)[a]
Domestic goose *Anser domesticus*	0.81	Rahn *et. al.* (1974)

[a] Calculated from information within the reference

CHAPTER 4 METABOLIC RESPONSES OF MALLEEFOWL EGGS TO COOLING, HEATING, AND HIGH OXYGEN TENSION

4.1 INTRODUCTION

The development of endothermy in hatchling birds has aroused a great deal of interest among comparative physiologists and has been the subject of several studies (see chapter 6). Altricial species are completely ectothermic when they hatch and usually take between one and three weeks to develop into competent endotherms (Kendeigh 1939, Breitenbach and Baskett 1967, Ricklefs and Hainsworth 1968, Hudson et. al. 1974, Clark 1982, Hill and Beaver 1982). Therefore, it is not surprising to find that altricial bird eggs behave in an ectothermic manner throughout incubation. Because eggs of most altricial birds are small, heat generated by the metabolism of the developing embryo, even at full term, has a negligible affect on egg temperature (Webb and King 1983). Most precocial species are also essentially ectothermic at hatching despite increasing oxygen consumption ($\dot{V}O_2$) when exposed to cool temperatures (Romijn 1954, Freeman 1964, Koskimies and Lahti 1964, Dawson et. al. 1972, Spiers et. al. 1974, Aulie and Moen 1975, Dawson et. al. 1976, Dawson and Bennett 1981, Ricklefs and Roby 1983, Hissa et. al. 1983, Eppley 1984). Some precocial species are capable of remaining endothermic on exposure to cold stress when just a few hours old (Koskimies and Lahti 1964, Untergasser and Hayward 1972, Ricklefs and Roby 1983). However, the full term embryos of these species are essentially ectothermic up until hatching (Untergasser and Hayward 1972). The inability of full term embryos in even the most precocial species to increase heat production in response to cold (Domestic chicken Gallus domesticus, Romijn 1954, Freeman 1964, Tazawa 1973, Domestic duck Anas platyrhynchos, Lesser scaup Aythya affinis,

Untergasser and Hayward 1972, Willow ptarmigan Lagopus lagopus, Aulie and Moen 1975) is generally attributed to the inability of immature skeletal muscles to produce thermoregulatory heat and/or limitations in the oxygen conductance (G_{O_2}) of the eggshell and membranes (Untergasser and Hayward 1972).

G_{O_2} is also hypothesized to limit VO_2 of full-term embryonic birds; their metabolic rate being only about half that of adult birds of equivalent body mass (Rahn et. al. 1974, Rahn 1982, Paganelli and Rahn 1984). One way to test this hypothesis is to increase the partial pressure of oxygen (P_{O_2}) of the gas mixture surrounding the full-term egg to see if $\dot{V}O_2$ increases. This experiment is carried out with Malleefowl eggs.

Eggs of several precocial species become progressively warmer and exceed incubator air temperature as they develop in still or forced air incubators (Domestic chickens, Romanoff 1941, Romijn and Lokhorst 1956, Domestic duck, Khaskin 1961, Herring gull Larus argentatus, Drent 1970). The rise in egg temperature is due to the increasing metabolism of the developing embryo. This phenomenon has been described as the first sign of homeothermic development (Romanoff 1941). However, for a demonstration of a true homeothermic response an increase in heat production in response to chilling must be observed.

Hatchling Malleefowl are endothermic just five hours after hatching (chapter 6). Obviously if Malleefowl eggs behave in a similar manner to other precocial species, the switch from ectothermy to endothermy must be extremely rapid. Because the transition from ectothermy to endothermy usually takes several days in other precocial

species (see for example Bernstein 1973, Spiers et. al. 1974, Epply 1984), a more probable alternative is that at least part of the transition in Malleefowl takes place during the last few days of incubation. To test this hypothesis full-term Malleefowl eggs are examined for signs of endothermy by monitoring $\dot{V}O_2$ over a range of ambient temperatures.

Many terrestrial ectothermic verebrates heat faster than they cool when exposed to similar thermal gradients (Grigg et. al. 1979). This phenomenon is caused by changes in peripheral circulation which alters thermal conductance (Grigg et al. 1979). A similar process may occur in full-term birds' eggs. To test this hypothesis rates of cooling and heating of Malleefowl eggs at various stages of incubation are determined.

4.2 MATERIAL AND METHODS

All eggs were incubated at 34 C in mound sand. Methods for measurement of $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) have been previously described (chapter 3). Ambient temperature within the respirometry chamber (T_a), egg surface temperature (T_{es}), and internal egg temperature (T_e) were monitored with 38 gauge copper-constantan thermocouples connected to electronic thermometers (Comark type 1624 or Wescor TH-65 digital TC). Shell surface thermocouples were held in place with a small piece of masking tape and two elastic bands. Internal thermocouples were aseptically inserted ca 15 mm through a 1 mm diameter hole drilled in the shell with a dental drill. The hole was then sealed with cyanoacrylate glue (Cyanobond RS 100, Sumitomo Chemical Co. Ltd., Osaka, Japan). Unfortunately it was not possible to determine the position of the yolk sac within the shell before insertion of the thermocouple. If the thermocouple ruptured the yolk sac the embryo did not survive. After the death of three embryos, further attempts to monitor internal egg temperature were abandoned. In several eggs an attempt was made to quantify movement of the embryo within the shell with 38 gauge wire electrodes connected to an impedance plethysmograph (Model 270 plethysmograph, Parks Electronics Laboratory, Beaverton, Oregon, U.S.A.), the signal of which was amplified and recorded on a polygraph (Grass Model 79D Quincy Mass., U.S.A.). Electodes were inserted on opposite sides of an egg by the method described for internal thermocouples.

Three different protocols were used in experiments. In the first, eggs were taken from the incubation sand and chilled well below normal incubation temperature by placing them in a refrigerator at 5 C

for 15 - 20 min. After this chilling, thermocouples and electrodes were fitted, the egg placed in the respirometry chamber, and the chamber placed in a controlled temperature cabinet set at 25 - 26 C. After 30 - 40 min at this temperature $\dot{V}O_2$ was calculated at 2 - 3 min intervals for 10 - 15 min. The temperature of the cabinet was then increased by 1 - 2 C and measurements taken at the new temperature after 30 - 40 min. Heating continued until the maximum test temperature was reached (usually 38 - 40 C). During serval experiments the fractional concentration of oxygen (F_{O_2}) in gas entering the respirometry chamber was increased from 0.21 to 0.49. The 49% oxygen in nitrogen gas mixture was delivered from a gas mixing apparatus.

In the second protocol, eggs were removed from incubation sand, weighed and immediately placed in the respirometry chamber which was placed in a controlled temperature cabinet set at 34 C. After 10 min the temperature of the cabinet was decreased by 2 C steps every 50 - 60 min until a minimum of 26 C was reached, then the temperature was increased by 2 C steps. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured, and T_a, T_{es} recorded at 5 min intervals throughout the entire experiment.

In the third protocol, eggs were removed from sand, weighed, fitted with a T_{es} thermocouple, and placed in the respirometry chamber which was immersed in a 34 C water bath. After 15 min the chamber was transferred to a 24 C bath for 2 h and then transferred to a 36 C bath for 1 - 1.5 h. $\dot{V}O_2$ was measured, T_a and T_{es} recorded every 5 min throughout the experiment.

4.3 RESULTS

4.3.1 Cooling eggs

Seventeen viable Malleefowl eggs were used in cooling/heating experiments (Table 4.1). Figures describing the chronology of events and the relationship between temperature and $\dot{V}O_2$ for each of these eggs are in Appendix 1 and are prefixed with the letter A.

The metabolic response of Malleefowl eggs to cooling can be assigned to one of three general categories: Ectothermic, Equivocal, or Endothermic.

Ectothermic (Fig. 4.1): $\dot{V}O_2$ decreased as T_a decreased. Typically the Q_{10} for $\dot{V}O_2$ averaged 2.14 (Table 4.1). Respiratory exchange ratio (RE) remained about 7.5 over the entire monitoring period (Fig. A25a). All eggs younger than 7 days before hatch (DBH) behaved in a ectothermic manner, and one egg (42-1) remained ectothermic up to 1 DBH (Fig. 4.1, A30b, Table 4.1).

Equivocal (Fig. 4.2): $\dot{V}O_2$ did not change with a decrease in T_a i.e. $Q_{10} = 1$. $\dot{V}O_2$ became extremely variable, but showed no consistent increase or decrease. Likewise RE was variable but averaged 7.5 throughout the monitoring period (Figs. A18b, A19b). This response was observed in two eggs aged between 3 and 1 DBH (Figs. 4.2, A18b, A19b, A26b).

Endothermic (Fig. 4.3): An increase in $\dot{V}O_2$ was observed when T_a was decreased below a critical temperature. In protocol 1 an endothermic response was observed immediately because the critical temperature had been surpassed when measurements began. When the egg was heated past the critical temperature,

$\dot{V}O_2$ decreased suddenly, and then increased in a ectothermic manner with increasing T_a (Figs. 4.2, A1a - A15b). In protocol 2 (Figs. A16a - A25b), $\dot{V}O_2$ decreased in an ectothermic manner with decreasing T_a until a critical temperature was reached, when a sudden increase in $\dot{V}O_2$ occurred. When the egg was re-heated, $\dot{V}O_2$ typically suddenly decreased again, and then increased in an ectothermic manner with increasing T_a. RE decreased from 8.5 - 8.0 to 7.5 - 7.0 when cooling began, remained at 7.5 - 7.0 while the egg behaved ectothermically during cooling, decreased to 6.5 - 6.0 when $\dot{V}O_2$ suddenly increased, rose to 7.0 - 7.5 while $\dot{V}O_2$ remained elevated, and increased to 8.5 - 9.0 when $\dot{V}O_2$ decreased again at the begining of the re-heating phase (Figs. A20a, A21a, A22a, A23a).

The endothermic response was the most frequently observed metabolic response for eggs aged between 3 and 0 DBH. Weak endothermic responses were observed as early as 5 DBH in eggs 33-18 and 5-5 (Table 4.1, Figs. A2b, A9b).

Evidence for the concept that a critical temperature several degrees below normal incubation temperature must be surpassed before an increase in $\dot{V}O_2$ occurs in eggs exhibiting an endothermic response comes from egg 33-22 1 DBH (Fig. A4). When recording started T_a was 28.5 C and $\dot{V}O_2$ varied between 52 and 59 $ml.h^{-1}$. T_a was then raised to 30.8 C and $\dot{V}O_2$ dropped to 41 - 44 $ml.h^{-1}$. On cooling the egg again to 29 C, $\dot{V}O_2$ remained at a low level (42 - 47 $ml.h^{-1}$), but lowering T_a further to 27.2 C caused an increase in $\dot{V}O_2$ to 52 - 54 $ml.h^{-1}$. When T_a was once again increased to 29.5 C, $\dot{V}O_2$

Table 4.1. Age, metabolic response to cooling during cooling/heating experiments, and Q_{10} values for $\dot{V}O_2$ of Malleefowl eggs over the range of temperatures when eggs exhibited ectothermic behaviour.

Egg number	Age (DBH[a])	Q_{10}	Temperature range for Q_{10}	Figure numbers	Metabolic response to cooling
33-19	2	2.39	30 - 36	A1a, A1b	endothermic
33-18	5	2.37	28 - 36	A2a, A2b	weak endothermic
33-18	3	2.62	29 - 36	A3a, A3b	endothermic
33-22	1			A4	endothermic
33-20	4	2.34	30 - 36	A5a, A5b	endothermic
33-20	1	2.43	28 - 35	A6a, A6b	endothermic
1-15	0			A7a, A7b	endothermic
33-23	3	1.83	27 - 36	A8a, A8b	endothermic
5-5	5	1.90	29 - 33	A9a, A9b	weak endothermic
6-1	3	1.75	30 - 36	A10a, A10b	endothermic
6-2	13	1.94	28 - 37	A11a, A11b	ectothermic
6-2	7	1.89	30 - 36	A12a, A12b	ectothermic
6-2	6			A13	
6-2	3	2.10	32 - 37	A14a, A14b	endothermic
6-2	1	1.93	30 - 36	A15a, A15b	weak endothermic
12-12a	7	2.55	27 - 34	A16a, A16b	ectothermic
12-12a	3	2.02	28 - 35	A17a, A17b	endothermic
35-12	3	0.93	26 - 36	A18a, A18b	neutral
35-12	1	1.00	28 - 36	A19a, A19b	neutral
12-13	3	2.27	29 - 37	A20a, A20b	endothermic
12-13	1	2.26	29 - 37	A21a, A21b	endothermic
26-12	1	1.70	30 - 36	A22a, A22b	endothermic
17-9	3	2.13	30 - 35	A23a, A23b	endothermic
17-9	1	1.87	31 - 36	A24a, A24b	endothermic
35-13	4	2.01	30 - 35	A25a, A25b	ectothermic
35-8	3	1.09	27 - 35	A26a, A26b, A26c	neutral
42-1	42			A27	
42-1	15	2.86	28 - 34	A28a, A28b, A28c	ectothermic
42-1	4	2.07	30 - 35	A29a, A29b, A29c	ectothermic
42-1	1	2.04	29 - 35	A30a, A30b, A30c	ectothermic
Mean		2.14[b] ± 0.13[c]			

[a] Dates when egg were laid were not known so age is expressed in terms of days before hatch.

[b] Does not include eggs classified as Neutral.

[c] 95% confidence limit of the mean

decreased again to 41 - 43 $ml.h^{-1}$. In this case the critical temperature appears to be between 28.5 and 29 C. Further evidence for the critical temperature concept comes from eggs showing endothermic responses in protocol 2 experiments, where a sudden increase in $\dot{V}O_2$ occurred when T_{es} dropped to 28 - 30 C during cooling, and a sudden decrease in $\dot{V}O_2$ occurred when T_{es} reached 29 - 30 C during re-heating (Figs. A21b, A22b, A23b, A24b).

4.3.2 Heating eggs

In seven eggs (33-19, 33-18, 33-20, 33-23, 5-5, 6-1, 6-2) heated to temperatures above 34 C, $\dot{V}O_2$ increased with rising temperature with a Q_{10} of about 2.0 (Table 4.1) until T_a reached 36 - 38 C. At these temperatures the rate of increasing $\dot{V}O_2$ slowed to below the Q_{10} = 2.0 line, while at higher temperatures (38 - 42 C) $\dot{V}O_2$ either plateaued or decreased if F_{O_2} remained at 0.21 (Figs. A1b, A2b, A3b, A5b, A6b, A8b, A9b, A10b, A11b, A12b, A14b).

4.3.3 Effect of increased oxygen tension on oxygen consumption

F_{O_2} of gas entering the respirometry chamber was increased at the end of egg heating experiments in two eggs (6-1, 6-2). On each occasion an increase in F_{O_2} from 0.21 to 0.49 resulted in an increased $\dot{V}O_2$ of between 22 - 29% (Figs, A10a - A15b). In addition, an experiment was performed with egg 6-2 where F_{O_2} was increased from 0.21 to 0.49 for 2 h and when reduced back to 0.21. An increase in $\dot{V}O_2$ was observed with the increase in F_{O_2} to 0.49 and a decrease in $\dot{V}O_2$ was observed when F_{O_2} was reduced back to 0.21 (Fig. A13).

4.3.4 Egg cooling and heating rates

Cooling and heating constants were estimated by calculating the

slope of the regression relating change in T_{es} per 10 min to the average difference between T_{es} and T_a over that 10 min period (Drent 1970). Cooling and heating rates of egg 42-1 were measured under identical conditions on four separate occasions over its incubation period (Figs. A27, A28c, A29c, A30c). Cooling and heating rates of egg 35-8 were measured once when it was three days from hatching (Fig. A26c). The results suggested that cooling and heating constants were not significantly different from each other on the same day, and did not vary significantly as incubation proceeded (Table 4.2).

Table 4.2. Cooling and heating constants of Malleefowl eggs 35-8 and 42-1.

Egg No.	Age (DBH)	Cooling slope	Heating slope	$\frac{\text{Heating slope}}{\text{Cooling slope}}$	Significance[a]
35-8	3	0.269	0.240	0.89	NS
42-1	42	0.186	0.199	1.07	NS
42-1	25	0.217	0.215	0.99	NS
42-1	4	0.198	0.174	0.89	NS
42-1	1	0.181	0.189	1.04	NS
42-1	common[b]	0.192	0.196	1.02	NS

a Slopes compared using analysis of variance (Zar 1974)

b Slopes from all four regressions were not significantly different so a common slope was calculated using data from all four regressions.

4.4 DISCUSSION

4.4.1 Cooling eggs

Malleefowl eggs behave in a completely ectothermic manner when younger than 7 DBH, with their metabolism having a Q_{10} averaging 2.14 between 26 C and 37 C (Table 4.1). This result is consistent with previous studies which have shown birds' eggs to be completely ectothermic up until the day of hatch (House wrens Troglodytes aedon, Kendeigh 1940; Domestic fowl, Romijn 1954, Romijn and Lokhorst 1955, Freeman 1964; Domestic duck, Khaskin 1961, Untergasser and Hayward 1972; Herring gull, Drent 1970; Willow ptarmigan, Aulie and Moen 1975; Leach's storm petrel Oceanodroma leucorhoa, Wilson's storm petrel Oceanites oceanicus, Southern giant fulmar Macronectes giganteus, Southern polar skua Catharacta maccormicki, Williams and Ricklefs 1984). Avian eggs typically have Q_{10} values between 1.5 and 3.0 for temperatures between 25 C and 40 C (Table 4.3).

Two Malleefowl eggs (35-8, 35-12) show an equivocal metabolic response to chilling when close to hatch (Fig. 4.2). Similar behaviour occurs in full-term Domestic fowl eggs (the "neutral" condition) if mild chilling (up to 3 C) takes place, although larger decreases in temperature cause a decrease in metabolism (Pembery et. al. 1895, Freeman 1964). The equivocal metabolic behaviour of Malleefowl eggs occurs over a greater temperature range (7 - 8 C, Figs. 4.2, A18b, A19b, A26b) than in Domestic fowl eggs. The "neutral" condition in Domestic fowl eggs is interpreted as a transitional condition between ectothermic and endothermic behaviour (Freeman 1964).

In most Malleefowl eggs older than 7 DBH, evidence of non-ectothermic behaviour appears and by 3 DBH an increase in $\dot{V}O_2$ occurs if chilling is

Table 4.3. Q_{10} values for oxygen consumption of avian eggs

Species	Age (DBH)	Temperature range (C)	Q_{10}	Source
House wren *Troglodytes aedon*	11	27 - 38	2.54	Kendeigh (1940)
	9	27 - 38	1.97	"
	6	27 - 38	2.90	"
	3	27 - 38	2.42	"
	2	27 - 38	2.68	"
Herring gull *Larus argentatus*	3	30 - 40	1.88	Drent (1970)
	1	30 - 40	1.98	"
	0	30 - 40	1.55	"
Leach's storm petrel *Oceanodroma leucorhoa*	late incubation	25 - 30	2.35	Williams and Ricklefs (1984)
		30 - 35	1.97	
Wilson's storm petrel *Oceanites oceanicus*	"	25 - 30	2.78	"
		30 - 35	2.26	"
Southern giant fulmar *Macronectes giganteus*	"	25 - 30	3.04	"
		30 - 35	2.26	"
South polar skua *Catharacta maccormick*	"	25 - 30	2.22	"
		30 - 35	1.30	"
Malleefowl *Leipoa ocellata*	13 - 0	26 - 37	2.14	Table 4.1

continued for a sufficiently long period (Fig. 4.3, Table 4.1). This increased $\dot{V}O_2$ in response to prolonged chilling is the first non-transient endothermic response (increased $\dot{V}O_2$ could be maintained for at least 2 h, Fig. A2la) recorded for avian eggs. A slight but transient rise in $\dot{V}O_2$ in response to moderate chilling is observable in Domestic fowl eggs 2 and 3 DBH (Freeman 1964), and in Domestic duck eggs 3 and 0 DBH (Khaskin 1961). The difference in the response to chilling of Malleefowl eggs compared to other avian eggs most probably reflects the extremely precocial nature of Malleefowl hatchings.

The increase in $\dot{V}O_2$ with prolonged chilling of full-term Malleefowl eggs does not conform to the 'classic' response of an endotherm, as an increase in $\dot{V}O_2$ does not occur immediately T_a and T_e decrease. In fact, $\dot{V}O_2$ decreases in a ectothermic manner until a critical T_e is reached (28 - 30 C) when a sudden increase in $\dot{V}O_2$ occurs. There is some evidence to suggest that the embryo inside the egg can sense the direction of heat flux, and that the endothermic response is stimulated once T_e approaches the critical temperature and heat is still being lost from the egg to the environment (ie. $T_e > T_a$). The sudden decrease in $\dot{V}O_2$ observed during heating of egg 33-23 (3 DBH) occurred at a time when T_e remained relatively steady, but T_a was increasing (Figs. A8a, A8b). This corresponded to a change in direction of heat flux, from heat leaving the egg to heat entering the egg. A similar situation occurred in egg 6-1 (3 DBH, Figs. A10a, A10b). Further evidence comes from egg 12-13 (3 DBH) where $\dot{V}O_2$ at the same T_{es} was greater during cooling (45 - 60 $ml.h^{-1}$) than during heating (30 - 40 $ml.h^{-1}$, Fig. A20b), and from egg 35-8 (3DBH) where $\dot{V}O_2$ did not decrease with T_a when the egg was being cooled, but dropped suddenly soon after egg re-heating started (Fig. A26a).

The increase in $\dot{V}O_2$ of Malleefowl eggs when the critical temperature is reached during cooling averages 110% of the $\dot{V}O_2$ rate at 34 C (Fig. 4.3), but is not enough to stop T_e from falling further if chilling continues, a situation similar with many hatchling precocial birds.

The increase in $\dot{V}O_2$ observed at low T_a may be due to the embryo becoming restless and attempting to hatch prematurely although no chicks hatched during cooling experiments. Attempts to quantify movement of the embryo during cooling with impedence plethymography failed because the electrodes were fixed to the eggshell and any change in orientation of the

embryo within the egg drastically changed the output of the plethymograph. Premature hatching may be advantagous if the incubation mound is cooling beyond the parent birds' control. Malleefowl incubation mounds have a relatively large thermal inertia, so that once cooled, they take a long time (2 - 3 d) to re-heat (see chapter 3 Figs. 3.3 and 3.4). Over the relatively long cooling periods which may be experienced in natural incubation mounds, increasing $\dot{V}O_2$ at low T_a has little if any adaptive value because it does not prevent T_e from falling. Hence, the increase in $\dot{V}O_2$ is probably an unavoidable consequence of the maturation of thermogenic processes which are extremely well developed in neonate Malleefowl chicks (chapter 6).

The transition from purely ectothermic behaviour to a behaviour where an increase in $\dot{V}O_2$ occurs with prolonged chilling becomes apparent in most Malleefowl eggs 3 DBH (Figs. 4.4, 4.5). If this increase in $\dot{V}O_2$ is indeed a thermoregulatory response, then the transition from an ectothermic to an endothermic state takes 4 - 5 d, a time similar to other precocial birds, the only difference being that in other birds the transition takes place after hatching.

In eggs behaving in a equivocal or ectothermic manner in response to cooling, RE, although quite variable, does not change in any consistent manner, and averages 7.5. In contrast, in eggs having an endothermic response, a consistent decrease in RE is observed when $\dot{V}O_2$ increases in response to cooling, and a consistant increase in RE is observed in the same eggs when $\dot{V}O_2$ decreases in response to heating. These changes are difficult to interpret because an increase in muscular activity (the usual method of increasing heat production in birds) tends to increase carbohydrate metabolism (Freeman 1967) and this causes an increase rather

than a decrease in RE. Increases in RE are observed in Domestic fowl eggs 2, 1, and 0 DBH if they are chilled to temperatures below 35 C (Romijn 1954, Romijn and Lokhorst 1955). This increase in RE is attributed to increased carbohydrate metabolism mediated by increases in muscular activity although an increase in $\dot{V}O_2$ is not observed.

4.4.2 Heating eggs and the effect of increasing the partial pressure of oxygen around full-term eggs

When full-term Malleefowl eggs are heated to temperatures above their normal incubation temperature (34 C), $\dot{V}O_2$ increases in an ectothermic manner, however the Q_{10} of this increase appears to decline at T_a above 36 C. Q_{10} of $\dot{V}O_2$ in bird eggs usually decreases as T_e increases (Williams and Ricklefs 1984). The plateaued $\dot{V}O_2$ observed at high T_a may be due to either a limitation in oxygen diffusing capacity of the eggshell and membranes, a limitation in the oxygen exchanging capacity of the chorioallantois, or an inhibition of metabolic processes due to sub-lethal temperatures.

Newly hatched Malleefowl chicks can tolerate T_b up to 44 C (chapter 6), and in all cases where eggs were heated to high T_a the embryos hatched successfully, so the inhibition of metabolic processess seems an unlikely explanation for plateaued $\dot{V}O_2$ at high T_a.

Ultimately, oxygen conductance (G_{O_2}) must limit $\dot{V}O_2$ if $\dot{V}O_2$ of the embryo continues to increase (Rahn et. al. 1974). The diffusion barrier for oxygen between the outside of the egg and the embyro may be conceptualized by two resistors in series, an outer diffusion barrier consisting of the eggshell and outer shell membrane (G_{O_2}), and an inner barrier consisting of the inner shell membrane, chorioallantoic epithelium,

capillary endothelium, plasma, red cell membrane, and the kinetics of the haemoglobin - oxygen interaction (Paganelli 1980). This inner barrier directly affects the oxygen loading capacity of the chorioallantois. If the limitations of both these resistances are overcome, the intrinsic metabolism of the embryo will limit $\dot{V}O_2$. If the oxygen tension outside the egg is increased by a large enough factor, the G_{O_2} resistance is overcome, and $\dot{V}O_2$ is then limited either by the inner diffusion barrier or the intrinsic metabolism of the embryo.

Evidence suggesting G_{O_2} limitation of metabolism in Malleefowl eggs comes from experiments in which F_{O_2} is increased. When F_{O_2} is increased at high T_a from 0.21 to 0.49, $\dot{V}O_2$ is observed to increase by 22 - 29 % and to fall on or near an extention of the Q_{10} = 2.0 line (Figs. A10b, A11b, A12b, A14b, A15b). Furthermore, in egg 6-2 when F_{O_2} was increased from 0.21 to 0.49 there was a 20% increase in $\dot{V}O_2$, and when F_{O_2} was decreased back to 0.21 again there was a 20% decrease in $\dot{V}O_2$ (Fig. A13). A small but significant increase in $\dot{V}O_2$ (c.a. 8%) has also been reported to occur in Domestic fowl eggs when F_{O_2} is increased to 0.39 (Visschedijk 1980, Visschedijk et. al. 1980). An increase in $\dot{V}O_2$ of up to 22% is observed after the 9th day of incubation in white leghorn eggs and after the 15th day in bantam eggs when eggs are transfered to a 100% oxygen environment (Hoiby et. al. 1983). Oxygen diffusion capacity appears to limit the growth rate of Domestic fowl embryos incubated in room air by limiting the amount of oxygen available for metabolic processes (Metcalfe et. al. 1984, Paganelli and Rahn 1984). Little increase in $\dot{V}O_2$ occurs towards the end of incubation in precocial birds (Vleck et. al. 1979), suggesting that G_{O_2} may limit embryo metabolism in precocial species (Metcalfe et. al. 1984).

Theoretical calculations may indicate what roles G_{O_2}, chorioallantois oxygen loading capacity or some other factor(s) play in controlling $\dot{V}O_2$ in full-term Malleefowl eggs. Inherent in the argument that G_{O_2} may limit $\dot{V}O_2$ is an assumption that there is a critical partial pressure of oxygen (P_{O_2c}) which must be maintained within the gas space inside the shell in order for the embyro to continue normal development. Decreases in P_{O_2} below P_{O_2c} are not tolerated and cause a decrease in metabolic rate. At the end of incubation P_{O_2c} is presumably reached and causes the observed plateau in $\dot{V}O_2$. P_{O_2c} may be estimated from $\dot{V}O_2$ and G_{O_2} at the end of incubation. $\dot{V}O_2$ is related to G_{O_2} by the Fick equation:

$$\dot{V}O_2 = G_{O_2} \times (P_{O_2out} - P_{O_2in}) \qquad (4.1)$$

Where $\dot{V}O_2$ = oxygen consumption ($ml.h^{-1}$)

G_{O_2} = eggshell and membrane oxygen conductance ($ml.h^{-1}.torr^{-1}$)

P_{O_2out} = partial pressure of oxygen outside the egg (torr)

P_{O_2in} = partial pressure of oxygen in the air space inside the egg (torr)

GO_2 can be calculated from the water vapor conductance of the egg (G_{H_2O}) and corrected for changes in T_e (Paganelli et. al. 1978). G_{H_2O} for Malleefowl eggs at the end of incubation at 34 C averages 64.2 $mg.d^{-1}.torr^{-1}$ (chapter 3) and the G_{O_2} calculated from this is 2.77 $ml.h^{-1}.torr^{-1}$ at 36 C (T_{es} of eggs is approximately 2 C warmer than T_a due to metabolism of the embryo, chapter 3). P_{O_2out} can be calculated from information on the ambient pressure, temperature, and F_{O_2} under conditions of measurement. During the last week of incubation the mean $\dot{V}O_2$ of a 170 g Malleefowl egg incubated at 34 C is 66 $ml.h^{-1}$ (Fig. 3.9). Using these values equation 4.1 can be rearranged and P_{O_2in} solved for:

$$P_{O_2in} = (760 - 44.6) \times 0.21 - \frac{66}{2.77} = 126 \text{ torr}$$

This value is significantly ($P < 0.001$) higher than the mean (104 torr) for 23 avian species (Paganelli and Rahn 1984). However, in natural incubation mounds, P_{O_2} in the egg chamber averages 126 torr (Seymour and Ackerman 1980), so for a naturally incubating embryo over the last week of incubation $P_{O_2 in} = 126 - (\frac{66}{2.77}) = 102$ torr. Thus, in naturally incubated Malleefowl eggs the peak metabolism of the embryo causes internal oxygen tensions similar to other birds' eggs, a result consistent with the idea that respiratory gas conductance and pre-pipping metabolism are matched to produce an internal O_2 tension of about 100 torr and CO_2 tension of about 40 torr at the end of incubation (Rahn et. al. 1974, Rahn and Ar 1980, Paganelli and Rahn 1984).

It appears that under the conditions of my experiments (F_{O_2} = 0.21) G_{O_2} is not limiting $\dot{V}O_2$ because $P_{O_2 in}$ at the end of incubation is 126 torr, approximately 20 torr above that in other birds. If $P_{O_2 c}$ in Malleefowl eggs is 126 torr, then naturally incubated eggs would not survive because $P_{O_2 out}$ is 126 torr and consequently there is no movement of O_2 into the egg.

Further evidence suggesting that G_{O_2} is not the ultimate limiting factor of $\dot{V}O_2$ in full-term Malleefowl eggs comes from egg heating experiments in which $\dot{V}O_2$ is observed to increase as T_e increases. For example in egg 6-2, 3 DBH, $\dot{V}O_2$ at 34 C averaged 60 $ml.h^{-1}$, and averaged 80 $ml.h^{-1}$ at 39.5 C (Fig. A14b). Calculations of $P_{O_2 in}$ can be made for both these temperatures:

At T_a = 34 C, $P_{O_2 in} = (760 - 44.6) \times 0.21 - \frac{60}{2.76 \times 1.0033} = 129$ torr

At T_a = 39.5 C, $P_{O_2 in} = (760 - 59.5) \times 0.21 - \frac{80}{2.76 \times 1.0089} = 119$ torr

If the embryo can respire with $P_{O_2 in}$ = 119 torr at T_a = 39.5 C it

should also be able to respire with $P_{O_2 in}$ = 119 torr at T_a = 34 C, and this would allow a greater $\dot{V}O_2$ at 34 C (87 ml.h^{-1}).

Even when F_{O_2} = 0.21, G_{O_2} does not appear to limit $\dot{V}O_2$, so why does $\dot{V}O_2$ increase by 20% when F_{O_2} rises to 0.49? In late incubated Domestic fowl eggs an increase in $\dot{V}O_2$ observed when eggs are placed in pure oxygen is hypothesized to occur as the result of an increase in the saturation level of haemoglobin leaving the chorioallantois from 72% in air incubated eggs to nearly 100% in pure oxygen incubated eggs (Hoiby et. al. 1983). In this case the oxygen exchange of the chorioallantois probably limits $\dot{V}O_2$ because $P_{O_2 in}$ is well above $P_{O_2 c}$ found in Domestic fowl eggs incubated in air. A similar explanation could account for the 20% increase in $\dot{V}O_2$ observed in Malleefowl eggs exposed to high oxygen tension.

4.4.3 Effect of chronic and acute exposure to various incubation temperatures on oxygen consumption

It is of interest to compare the $\dot{V}O_2$ of eggs incubated continuously at various temperatures (chapter 3) to the $\dot{V}O_2$ of eggs normally incubated at 34 C but acutely exposed to changes in T_a (Table 4.4).

If the developing embryo does not acclimate to temperature during incubation I would expect $\dot{V}O_2$ of chronically and acutely treated eggs to be similar at the same temperature. This is the case at 36 C, but differences occur at 32 and 38 C (Table 4.4). Therefore, some thermal acclimation appears to occur at 32 and 38 C temperatures.

$\dot{V}O_2$ of eggs 3 - 0 DBH incubated at 34 C increases by 10% when exposed to prolonged chilling (section 4.4.1). For a 170 g egg this

increases $\dot{V}O_2$ from 66 $ml.h^{-1}$ (Fig. 3.9) to 73 $ml.h^{-1}$, which is remarkably close to the value (72 $ml.h^{-1}$) for a 170 g egg incubated at 32 C. It is tempting to relate the higher $\dot{V}O_2$ in eggs incubated at 32 C to the increase in $\dot{V}O_2$ of chilled eggs incubated at 34 C, and to suggest that both of these phenomena are related to the ontogeny of thermoregulation. However, the threshold temperature for the endothermic response in 34 C incubated eggs is lower than 32 C (28 - 30 C) and only appears over the last few days of incubation; whereas $\dot{V}O_2$ of 32 C incubated eggs is higher than 34 C incubated eggs from the begining of incubation and declines to a level below 34 C eggs during the last week of incubation (Fig. 3.9).

Table 4.4 Oxygen consumption ($ml.h^{-1}$) of chronically and acutely exposed Malleefowl eggs to selected temperature. Oxygen consumption is standardized for a 170 g egg. All eggs are in the last week of incubation. Chronic values calculated from Figure 3.9, acute values from Figures A1b - A15b.

Treatment	Temperature (C)		
	32	36	38
Chronic	72	57	50
Acute	46	60	66
Significance[a]	P < 0.001	NS	P < 0.001

[a] Student's 't' test

In contrast to 32 C, at 38 C chronically exposed eggs have a lower $\dot{V}O_2$ compared to acutely exposed eggs (Table 4.4). T_e of eggs incubated

at 38 C probably approaches sub-lethal levels towards the end of incubation. Prolonged exposure to sub-lethal temperatures may be detrimental to development. If metabolism is reduced, T_e at the end of incubation is also reduced and sub-lethal temperatures may be avoided.

4.4.4 Egg cooling and heating rates

Newton's law of cooling states that the rate of cooling of an isothermal body is proportional to the temperature difference between the body and its environment. This can be stated mathematically as:

$\frac{dH}{dt} = -k(T_b - T_a)$ this equation may be rearranged to give:

$$\ln(T_b - T_a) = -\frac{k}{C}.t + A \qquad \text{(see chapter 6, section 6.2.3)}$$

where A = constant, k is the thermal conductance, C is the heat capacity of the body and $\frac{k}{C}$ is the cooling constant.

The plot of $\ln(T_b - T_a)$ is linear with respect to time and the cooling constant may be determined empirically if T_b and T_a are monitored through time. In my experiments T_{es} rather than T_b was monitored, but 10 min after the chamber containing the egg was transferred to a different water bath and T_a had stabilized, changes in T_{es} should have parallelled those of T_b. Heat lost by evaporation of water from the egg and convection due to air flowing through the chamber is negligible. Unfortunately, eggs that had been incubated for longer than 20 d produced significant amounts of heat via their metabolism during cooling and heating trials which precludes the use of plots of $\ln(T_{es} - T_a)$ against time for determining cooling constants. However, plots of fall in T_{es} over a specified time interval (10 min) against the average temperature difference $(T_{es} - T_a)$ existing during this time interval can be used to estimate cooling constants under these conditions (Drent 1970).

Because thermal conductance is equal to the cooling constant multiplied by the heat capacity of the egg, and the heat capacity of the egg is unlikely to change over the 4 h of a cooling and heating trial, any change in cooling/heating constants indicates a change in thermal conductance. The only way an embryo within an egg can alter its thermal conductance is to alter convective heat flow to the periphery via blood circulation to the chorioallantois. In all cases the cooling and heating constants of eggs are not significantly different (Table 4.2), indicating that convective heat flow from the embryo to the chorioallantois is either unaltered, or if it does alter, it is relatively unimportant compared to heat flow due to conduction. Similar results have been obtained in previous studies on avian eggs (Romijn and Lokhorst 1956, Khaskin 1961, Tazawa and Mochizuki 1978, Tazawa and Nakagawa 1985). The cooling constant of egg 42-1 did not change significantly as incubation proceeded (Table 4.2), indicating that the development of the chorioallontoic circulation has little effect on heat loss from the egg.

The effect of increasing metabolism as development proceeds is not to alter the cooling constant, but to alter the egg temperature at equilibrium when cooling ceases (Fig. 4.6). At equilibrium (extrapolating regression lines in figure 4.6 to when the change in T_{es} per 10 min equals zero), the metabolism of the egg is able to maintain T_{es} above T_a up to 1.5 C with the gradient tending to increase with embryo age (Fig. 4.6). Herring gull eggs give similar results (Drent 1970). That metabolism is responsible for the shift in the intercept and not embryo age per se is demonstrated in egg 42-1 4 and 1 DBH. The metabolism of this egg is greater 4 DBH than 1 DBH (vis Figs. A29b, A30b), and the intercept is greater 4 DBH than 1 DBH (Fig. 4.6).

CHAPTER 5 RESPIRATORY GAS EXCHANGE DURING HATCHING

5.1 INTRODUCTION

The hatching process is similar for most birds and may be typified by the Domestic Fowl Gallus domesticus (Drent 1975). The orientation of the embryo within the egg is important for successful hatching. By the day before hatch in the Domestic fowl, "...the head is turned forward, with the beak emerging behind the posterior margin of the right wing and its point resting near the edge of the air chamber" (Hamilton 1965). The beak then pierces the membranes and enters the air space (internal pipping) and lung ventilation starts although the chorioallantoic membrane continues to be the major gas exchange organ at this stage (Hamilton 1965, Visschedijk 1968, Drent 1975). In a few avian species the eggshell is broken before the beak breaks into the air space (Drent 1975, Ackerman et. al. 1980, Pettit et. al. 1982b).

From the time of internal pipping until the time of hatching (approx 30 h in Domestic fowl) the importance of the chorioallantois for gas exchange diminishes as the lungs become functional (Visschedijk 1968). During this period the metabolic rate of the chick increases greatly (Barott 1937, Freeman 1962, Visschedijk 1968). Concomitant with the change from chorioallantoic to pulmonary respiration and the increase of metabolism are the hatching movements of the chick. The beak pushes against the eggshell and breaks it (external pipping), alternate treading movements of the legs slowly rotate the embryo around in an anti-clockwise direction so that the eggshell becomes broken in a circle around the blunt pole (Drent 1975). Breaking of the shell is facilitated by a knoblike protuberance on the upper

mandible, the egg tooth, which disappears after hatching. The back and forward motion of the head is powered by the hatching (complexus) muscle located on the back of the neck (Clark 1964a, Drent 1975). Eventually the egg is split in two by heaving movements of the shoulders and extention movements of the legs and the chick emerges.

Megapodes hatch in a manner different from other birds. They do not use the an egg tooth or Complexus muscle to hatch, and do not have a fixed air space at the blunt pole (Baltin 1969, Seymour and Ackerman 1980, Vleck et. al. 1984, Seymour 1984). They break the eggshell with their feet and shoulders (Frith 1959, 1962a, Baltin 1969, Vleck et. al. 1984, Seymour 1984). The eggshell is thin, fragile, and breaks relatively easily. Hatching is said to occur in an explosive manner, so that within a short time the entire eggshell and chorioallantois are completely destroyed (Frith 1959, 1962b, Baltin 1969, Vleck et. al. 1984, Seymour 1984). Because there is no internal pipping period in megapodes, when in normal birds a transition from chorioallantoic to pulmonary respiration occurs, the switch over from chorioallantoic to pulmonary respiration must be fast (Vleck et. al. 1984, Seymour 1984).

Some recent studies have examined energy consumption of eggs over the entire incubation period. In most studies oxygen cosumption ($\dot{V}O_2$) has been measured at various times throughout incubation, a temporal $\dot{V}O_2$ curve generated, and the area under the curve intergrated to calculate an energy budget. The following species have been studied in this manner: Domestic Fowl, Gallus domesticus (Barrot 1937, Romijn and Lokhorst 1956); Ostriches, Struthio camelus (Hoyt et. al. 1978); Zebra Finches, Poephila guttata, Village Weaver Birds, Ploceus cullullatus, Pigeons, Columba livia, Japanese Quail, Coturnix coturnix, Domestic Geese, Anser anser (Vleck et. al. 1979);

Emus, Dromiceius novehollandiae, Rheas, Rhea americana (Vleck et. al. 1980a); Wedge-Tailed Shearwaters, Puffinus pacificus chlororhynchus (Ackerman et. al. 1980); Black-Footed Albatross, Diomedea nigripes, Laysan Albatross, Diomedea immutabilis (Pettit et. al. 1982a); Bonin Petrels, Pterodroma hypoleuca hypoleuca (Pettit et. al. 1982b); Brush-turkeys, Alectura lathami (Vleck et. al. 1984); and Malleefowl, Leipoa ocellata (Vleck et. al. 1984). In all these species there is a conspicuous increase in $\dot{V}O_2$ after internal pipping, and in procellariiform species the internal pip-to-hatch period accounts for 40% of total energy used during incubation, depite its relatively short period (Pettit et. al. 1982b).

The energetics of hatching per se has not been well studied (Bartholomew and Goldstein 1984), as only a few studies have monitored gas exchange continuously over the hatching period of birds. Barott (1937) monitored $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) continuously over the incubation period of Domestic fowl eggs, but his apparatus required the measurement of many eggs simultaneously and hatching was not synchronous. More recently Freeman (1962) and Visschedijk (1968) monitored patterns of $\dot{V}O_2$ during hatching of individuals of this species. Oxygen consumption has also been monitored continuously during hatching of Malleefowl and Brush-turkey eggs (Vleck et. al. 1984, Seymour 1984). In the present study a more detailed examination of both $\dot{V}O_2$ and $\dot{V}CO_2$ before and after hatching has been made in hatching Malleefowl in order to increase understanding of the resipatory events of this unusual mode of hatching.

5.2 MATERIAL AND METHODS

Five eggs were incubated in sand at 34 C prior to measurements. Equipment and methods for determining $\dot{V}O_2$ and $\dot{V}CO_2$ were the same as described in section 3.2. The temperature of the respiration chamber was usually held between 34 - 35 C during measurements. Hatching was visible through the clear acrylic respiratory chamber, although occasionally a piece of broken eggshell and/or condensation in the chamber by water vapour released from the broken egg obscured the view. After the first break in the eggshell was detected, the hatching egg was viewed every 5 - 10 min and hatching progress noted (except for events occurring between 23.00 and 8.00 h). A short time after hatching, the hatchling was transferred to a larger plastic chamber (18x18x13 cm) and the humidifier removed, so dry air flowed through the system. This allowed the hatchling's plumage to dry and expand.

5.3 RESULTS

5.3.1 General

The patterns of $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RE) for five hatching Malleefowl from between 23 - 11 h before hatching to 3 - 15 h after hatching were calculated every 5 or 10 min and plotted against time (Figs. 5.1 - 5.5). The fast flow rate and small dead space of the egg chamber system enabled detection of short-term (appoximately 1 min) changes in gas exchange. Sudden changes in gas exchange were characteristic of eggs prior to hatching, so $\dot{V}O_2$, $\dot{V}CO_2$, and RE were calculated at every minute during times when sudden changes occurred. The larger chamber in which the chick was placed in after hatching had a much larger volume and consequently a slower reaction time. It was necessary to interrupt observations for various periods during experiments in order to conduct metabolism measurements on other eggs for the experiments in chapter 3, these periods appear as blanks in figures 5.1 - 5.4.

5.3.2 Patterns of metabolism immediately prior to hatch

Three eggs (12-13, 12-12a, 35-12) of the five studied had similar patterns of metabolism prior to hatch (Figs. 5.1a, 5.2, 5.3a) and shall be dealt with together.

The chorioallantois and shell membranes become separated from the eggshell late in incubation of Malleefowl eggs, so when the eggshell cracks it often falls away from the shell membranes facilitating observations of hatching events (Vleck *et. al.* 1984). In eggs 12-13, 12-12A, and 35-12 the eggshell was cracked and parts of the shell lifted clear of the shell membranes a considerable time (1400 - 950 min) before

hatching. When the eggshell cracked, a small hole was ruptured in the chorioallantois at the base of the egg and a small amount of fluid (approximately 5 ml) was observed to ooze out. The 'spikes' in $\dot{V}O_2$ and $\dot{V}CO_2$ observed when the eggshell was first broken were probably due to the CO_2 rich and O_2 depleted atmosphere inside the eggshell being liberated into the chamber. Despite the small hole, the chorioallantois appeared to be functional as blood vessels could still be seen pulsating, indicating blood was still moving through them. Between 200 - 100 min before hatch regular in-and-out movements of the chorioallantois became visible (Figs. 5.1a, 5.2, 5.3a) and were interpreted as lung ventilation movements. Pulsations of blood vessels in the chorioallantois were not observed over the period when ventilation movements were visible, although the outline of blood vessels could still be seen, so they probably had not completely collapsed. Concomitant with the onset of ventilation movements was a conspicuous drop in RE, followed by its slow recovery until at the time of hatch RE was once again about 0.75. Hatching was achieved by the legs suddenly ripping through the chorioallantois and shattering the remaining eggshell. Little or no blood was lost during this process indicating blood flow through the chorioallantois had already ceased. The large 'spikes' in $\dot{V}O_2$ and $\dot{V}CO_2$ seen at this time were probably caused by the CO_2 rich and O_2 poor air inside the chorioallantois being released into the chamber. During the time between cracking and the time when regular ventilation movements were first visible gas exchange remained relatively constant, but was characterized every few minutes by a sudden decrease followed immediately by a short-lived increase in gas exchange. These changes are clearly seen in both the O_2 and CO_2 tracings. The RE during this time remained relatively stable averaging 0.75 in all three eggs.

Egg 26-12 showed a slightly different hatching pattern. From 1000 min before hatching the characteristic sudden changes in $\dot{V}O_2$ and $\dot{V}CO_2$ were visible, but the eggshell remained intact until just 25 min before hatch when the bottom of the egg was broken and the chorioallantois torn, releasing approximately 10 ml of fluid (Figure 5.4a). Ten minutes before hatching the first ventilation movements became visible. At hatching the eggshell was broken into two and the chorioallantois completely destroyed. No blood was lost. At the beginning of observations (1100 min before hatch), RE was 0.75 and increased to average 0.82 from 950 until 450 min before hatch, then dropped to average 0.78 until hatching.

The hatching pattern of egg 35-13 was different again. The eggshell was broken at its base and the chorioallantois had a small hole in it before measurements started. Regular ventilation movements could be seen at the start of observations 625 min before hatch. About 5 ml of fluid was released at hatching, but no bleeding occurred. Respiratory gas exchange of this egg was more variable than any of the other 4 eggs observed, and the RE appeared to fluctuate quickly, although it averaged 0.75 throughout the observations (Fig. 5.5a).

5.3.3 Metabolic patterns immediately after hatch

Posthatching patterns of metabolism were similar in all five hatchings observed (Figs. 5.1b, 5.2, 5.3b, 5.4b 5.5b). Immediately after the chorioallantois was ripped, 'spikes' of increased $\dot{V}O_2$ and $\dot{V}CO_2$ occurred corresponding to the 'stale' air trapped inside the chorioallantois being released. When the chick was escaping from the eggshell and membranes, large and rapid changes in gas exchange occurred as a result of greatly increased muscular activity. For about 10 min

after hatching RE increased to about 1.0 and then dropped back to 0.75 for the remainder of the observation period. From hatching until 100 - 300 min after hatching average metabolism increased steadily. During this period chicks could be seen shivering, evidently related to evaporative cooling of plumage as the chick dried. Three to four hundred minutes after hatching, chicks were almost dry, metabolism peaked and began to drop again until 600 - 700 min after hatch metabolism stablized at a standard rate.

5.4 DISCUSSION

5.4.1 Gas exchange patterns immediately prior to hatching

Sudden decreases immediately followed by short-lived increases in gas exchange were characteristic of eggs 12-12a, 12-13, 26-12, and 35-12 before ventilation movements became visible (Figs. 5.1a, 5.2, 5.3a, and 5.4A). Such phenomena are not observed in eggs two or three days before hatch, and are probably due to movements of chicks. Movements might temporarily interfere with blood flow to the chorioallantois causing a decrease in gas exchange, while the increase in gas exchange may be brought about by a combination of paying back the oxygen debt caused by the temporary interference in chorioallantoic circulation, and the increased metabolism due to the muscular activity during movements.

Egg 26-12 was the only egg observed to hatch in the rapid manner previously described for megapodes (Frith 1959, 1962b, Baltin 1969, Vleck *et. al.* 1984, Seymour 1984). In the other 4 hatchings the eggshell was cracked, the chorioallantois ruptured 23 - 15 h, and ventilation movements observed 10 - 2 h before hatch. However, pulsations of blood vessels in the chorioallantois were not noted during the period when ventilation movements were visible. Chorioallantoic circulation may have been greatly reduced or ceased at this stage, respiration occurring almost entirely through the lungs, and the chick resting until it finally burst from the shell.

On the other hand, outlines of some blood vessels could still be seen over this period, indicating that blood vessels had not completely collapsed, and that at least some chorioallantoic circulation may be functional. It is possible that the chorioallantoic circulation remains

intact right up until hatching occurs and that all gas exchange takes place through it. If this is the case, the ventilation movements observed could be the chick breathing in fluid in a similar manner to many foetal mammals just prior to birth (Maloney 1984). Evidence for the non-gaseous ventilation of lungs over the period when ventilation movements were visible is indirect and comes from the observation that average gas exchange during this period did not increase. In all other hatching bird species studied, there is a marked increase in gas exchange with the onset of gaseous ventilation of the lungs (Barott 1937, Freeman 1962, Visschedijk 1968, Drent 1970, Hoyt et. al. 1978, Vleck et. al. 1979, 1980, Ackerman et. al. 1980, Pettit et. al. 1982a, 1982b, Bartholomew and Goldstein 1984, Seymour 1984).

A third possibility is that there may be a transition from chorioallantoic to pulmonary respiration in a manner similar to other birds over the pip-to-hatch (paranatal) period in some Malleefowl chicks. In three hatchings (eggs 12-13, 12-12a, 35-12) there was a marked depression of RE corresponding with the onset of lung ventilation movements (Figs. 5.1a, 5.2, 5.3a), RE taking approximately 50 - 100 min to recover to its original value. It is difficult to interpret the events occurring during this period, but it appears that CO_2 is being retained within the egg as it is unlikely that the substrate being metabolized would change. Visschedijk (1968) reported changes in RE associated with hatching in Domestic fowl. In his study, gas exchange occurring over the air space (lung gas exchange) and chorioallantois were monitored separately. At the onset of lung ventilation there was a decrease in RE associated with the air space, and an increase in RE associated with the chorioallantois, but the overall RE remained fairly stable. A similar effect may be postulated for hatching Malleefowl,

except that the increase in RE associated with the chorioallantois would have to be less extreme than in Domestic fowl. Alternately, the chorioallantoic circulation of Malleefowl chicks may rapidly stop at this stage.

The reason for an increase in RE from 0.75 to 0.82 in egg 26-12 950 - 450 min before hatch remains unexplained. This egg hatched just 25 min after the egg was first cracked. A discussion of this very quick hatching process has been published (Vleck et. al. 1984, Seymour 1984). These authors along with Seymour and Ackerman (1980) argue that the fast hatching process is associated with the underground nesting habit of Malleefowl. The high humidity of the atmosphere in the underground nest inhibits evaporation of water from the egg, and consequently the large air space normally found at the blunt pole of birds' eggs does not form. The absence of a large fixed air space prevents internal pipping (Seymour and Ackerman 1980). The combination of no internal pipping and the destruction of the chorioallantois at hatching necessitates 'instantaneous' hatching (Vleck et. al. 1984).

Egg 35-13 was the only egg examined in which characteristic sudden and short-lived changes in gas exchange were not observed. These sudden fluctations were never observed after ventilation movements began in any eggs. Since ventilation movements were visible from the start of observations on egg 35-13, the period when sudden fluctations occur may have past before observations started on this egg.

To summarize, there was considerable variation in the respiratory events leading up to hatching in Malleefowl eggs, ranging from 'viviparous' hatching where the switch over from chorioallantoic to

pulmonary respiration was almost instantaneous, to a situation where there was a lengthy paranatal period in which a transition from chorioallantoic to pulmonary respiration analogous to other hatching birds could be occurring.

5.4.2 Gas exchange patterns after hatch

Compared to prehatching patterns of respiration, posthatching patterns of respiration were remarkably uniform (Figs. 5.1B, 5.2, 5.3B, 5.4B, 5.5B). An increase in RE to approximately 1.0 for a short period (approximately 10 min) immediately after hatching indicates CO_2 is being 'blown off' during this time, and might be attributed at least in part, to the earlier CO_2 'storage' within the chick which is indicated by a drop in RE at the time when pulmonary ventilation starts (see section 5.4.1). Freeman (1971) noted a transient increase in RE in hatching Domestic fowl, and attributed this rise to "...cooling effects of evaporation of surface water stimulating a rise in muscle tone immediately after hatching." This explanation seems unlikely for Malleefowl chicks because RE returns and remains 0.75 for several hours after hatching when the chicks are shivering.

There is an increase in gas exchange for several hours after hatching. Increasing gas exchange immediately after hatch has previously been reported for Malleefowl (Vleck et. al. 1984), Brush-turkeys (Vleck et. al. 1984, Seymour 1984), Domestic Fowl (Freeman 1962), Ostriches (Hoyt et. al. 1978), Zebra Finches, Village Weaver Birds, Pigeons, Japanese Quail, Domestic Geese (Vleck et. al. 1979), Emus, Rheas (Vleck et. al. 1980a), Wedge-Tailed Shearwaters (Ackerman et. al.1980), Laysan and Black-Footed Albatross (Pettit et. al. 1982a), Bonin Petrels (Pettit et. al. 1980b), and probably occurs in birds

generally (Rahn 1982). During the immediate posthatch period the aeration of the lungs, and hence the surface area available for respiratory gas exchange is increasing. Increasing pulmonary diffusing capacity may account for the increase in gas exchange observed over this period (Seymour 1984). The peak metabolic rates observed several hours after hatching (180 - 270 ml $O_2.h^{-1}$) are higher than those previously reported for Malleefowl (120 - 200 ml $O_2.h^{-1}$, Vleck et. al. 1984), but the difference may be due to experimental design. In my experiments chicks were placed in dry air which allowed their plumage to dry. Cooling due to evaporation of water from the plumage caused chicks to shiver elevating metabolic rates. In Vleck et. al.'s (1984) experiments chicks were either kept in humidified air or buried in mound litter. In both cases drying of plumage is impeded and heat lost via evaporation reduced. In my experiments there was a drop in metabolism to 60 - 90 ml $O_2.h^{-1}$ once the plumage had dried and expanded. A drop in metabolism was not observed in Vleck et. al.'s (1984) experiments because the chicks were buried in mound material and were digging from time to time.

5.4.3 Energetic cost of the paranatal period

In most birds the beginning of the hatching process is marked by internal pipping. The total energetic cost during hatching may be calculated by integrating the area under the $\dot{V}O_2$ curve between the time of internal pipping and hatching. For comparative purposes, the energy consumed during the paranatal period is expressed as a percentage of the total energy consumed over the entire incubation period from day 1 until hatch (Pettit et. al. 1982b). Values for the energy consumed over the hatching period expressed in the above manner for 18 species of bird were either calculated or collected from the literature (Table

5.1). The total energy expended during the pip-to-hatch period is about the same in altricial and semi-precocial species (43% of total incubation energy), while it is significantly less ($P < 0.05$) for precocial species (25% of total incubation energy). These figures do not represent the energetic cost of hatching *per se*, because they also include energy used for maintenance and growth of the chick over the hatching period. Bartholomew and Goldstein (1984) extrapolated the energy requirements due to growth and maintenance over the hatching period and subtracted this from the observed energy consumption for this period to determine the energetic cost of hatching in the Brown pelican. They found the extra energy needed for hatching was only 2.5% of the total incubation energy, although during the hatching period 46% (Table 5.1) of total incubation energy was consumed, i.e. maintenance and growth accounted for 94.6% [(46 - 2.5) x 100/46] of energy expenditure over the hatching period. The actual relative energetic cost of hatching may be similar in all birds. Altrical birds continue to grow right up until hatching, whereas precocial birds slow or cease growth towards the end of incubation (Vleck *et. al.* 1979). Growing tissue requires relatively large amounts of energy, whereas matured tissue probably require relatively little energy (Vleck *et. al.* 1979). Hence altrical birds probably require more energy for growth and maintenance during the pip-to-hatch period than precocial birds. The high energy expenditure over the pip-to-hatch period of the semi-precocial species (Table 5.1) can probably be explained by the prolonged nature of this period in these species (Pettit *et. al.* 1982b).

Because the hatching period is so short in Malleefowl, the energy expenditure during hatching is small (3% of total incubation energy, Table 5.1). But after hatching megapodes must struggle up through mound

material to reach the outside world, and this process requires considerable energy (8% and 33% of total incubation energy respectively for Malleefowl and Brush-turkeys (Vleck et. al. 1984).

Table 5.1. Energy consumed during hatching period as percentage of total energy consumed over incubation in 18 species of bird.

Hatchling type	Species	Source of data	% energy consumed
Altricial	Zebra finch	Vleck et. al. (1979)	38[a]
	Village weaver bird	Vleck et. al. (1979)	44[a]
	Pigeon	Vleck et. al. (1979)	42[a]
	Brown pelican	Bartholomew and Goldstein (1984)	46[a]
Semi-Precocial	Herring gull	Drent (1970)	44[a]
	Black-footed albatross	Pettit et. al. (1982b)	42
	Laysan albatross	Pettit et. al. (1982b)	42
	Wedge-tailed shearwater	Pettit et. al. (1982b)	42
	Bonin petrel	Pettit et. al. (1982b)	50
Precocial	Domestic fowl	Barrot (1937)	28[a]
	Ostrich	Hoyt et. al. (1978)	20[a]
	Japanese quail	Vleck et. al. (1979)	31[a]
	Domestic goose	Vleck et. al. (1979)	18[a]
	Emu	Vleck et. al. (1980a)	31[a]
	Rhea	Vleck et. al. (1980a)	32[a]
	Brush-turkey	Vleck et. al. (1984)	33[b]
	Malleefowl	Vleck et. al. (1984)	8[b]
	Malleefowl	Present study	3

a Calculated from area under oxygen consumption curve presented in papers

b Energy includes energy expended while digging out of mound

CHAPTER 6

THERMOREGULATION IN NEONATE MALLEEFOWL AND BRUSH-TURKEYS

6.1 INTRODUCTION

The transition from ectothermy to endothermy in juvenile birds has interested comparative physiologists for many years (for reviews see King and Farner 1961, Hudson et. al. 1974). Birds hatch at varying stages of development ranging from an altricial condition in which chicks hatch with little or no plumage and no endothermic capacity, to a precocial condition where chicks hatch with downy plumage and at least some ability to initiate an endothermic response (increase heat production) when exposed to cold stress (Nice 1962, Dawson and Hudson 1970). Most hatchling birds cope with short term heat stress reasonably well. They become hyperthermic and avoid lethal body temperatures (T_b) by increasing evaporative water loss (EWL) and can probably cope with long periods of heat stress if a supply of water is available (Randall 1943, Ricklefs and Hainsworth 1968, Dawson and Hudson 1970, Dawson et. al. 1972, Hudson et. al. 1974, Dawson et. al. 1976). Initially the only defence against cooling (besides parental brooding) in altricial species is the thermal inertia of their bodies and nests, but they develop homeothermic capacity over the first one or two weeks after hatching due to increases in body size, development of plumage, and maturation of muscles used in shivering thermogenesis (Kendeigh 1939, Breitenbach and Baskett 1967, Ricklefs and Hainsworth 1968, Hudson et. al. 1974, Hill and Beaver 1982, Clark 1982).

Despite a degree of endothermic capacity at hatching, most precocial species need parental brooding to remain homeothermic when

exposed to cold stress (Freeman 1964, 1966, 1967, Koskimies and Lahti 1964, Wekstein and Zolman 1967, 1969, Palokangas and Hissa 1971, Cain 1972, Untergasser and Hayward 1972, Berstein 1973, Spiers et. al. 1974, Aulie and Moen 1975, Aulie 1976a, Misson 1977, Hissa et. al. 1983, Ricklefs and Roby 1983). The only hatchling birds so far studied which possess competent homeothermic capacity are some arctic inhabiting ducks (Koskimies and Lahti 1964, Untergasser and Hayward 1972) and the Antarctic Prion (Ricklefs and Roby 1983). Megapodes produce the most precocious hatchlings known (Nice 1962), and because there is no contact between neonates and parents it has been postulated that megapode neonates should be competent thermoregulators (Nice 1962). This hypothesis is tested by exposing hatchling Malleefowl and Brush-turkeys to cold stress.

Since the classic studies of Scholander, Irving and co-workers (Scholander et. al. 1950a, b, c) many studies on birds have been made in metabolic chambers. These works address the question of whether physical conditions of the environment such as thermal regime and water availability can be correlated with physiological parameters such as thermal conductance (K), standard metabolic rate (SMR), T_b, and EWL. Initially K was thought to be the only factor to vary appreciably with environment, animals from cold climates having smaller K than animals from warm climates (Scholander et. al. 1950c, Drent and Stonehouse 1971). The collection of more data from a larger variety of birds now indicate that changes in other physiological parameters have occurred. Recent investigations have shown that SMR is positively correlated with latitude, presumably because the climate is colder at higher latitudes (Weathers 1979, Hails 1983). Many arid land birds have lower than expected SMR (Dawson and Bennett 1973, Dawson 1976). In mammals SMR can

be correlated with diet (McNab 1980b) but no studies of this type have been carried out in birds. Arid adapted birds may have lower than expected EWL at T_a in and below the thermoneutral zone (TNZ), but possess the ability to rapidly increase EWL at higher T_a (Weathers 1981). Several studies have examined avian T_b, and all have concluded that there is no correlation between T_b and environment (Scholander *et. al.* 1950c, Dawson 1962, McNab 1966, Calder and King 1974).

Malleefowl and Brush-turkey chicks are ideally suited for a comparative physiological study because they are closely related taxonomically (in the same Family), hatch with similar body mass and degree of precociality, but inhabit different post-hatching climates. Malleefowl hatch into an arid environment where diurnal temperatures can be hot (greater than 40 C) and nocturnal temperatures are frequently cool (less than 15 C), while Brush-turkeys hatch into a more mesic jungle or thick scrub environment, where surface water is frequently available, diurnal temperatures moderate (rarely exceed 35 C) and nocturnal temperatures frequently cool. In the first section of this chapter the thermoregulatory ability of neonate Malleefowl and Brush-turkeys are compared to each other and to neonates of other precocial birds.

Thermal conductance may be defined as a measure of the ease of heat transfer from the body by radiation, conduction, convection, and evaporation (Bradley and Deavers 1980), and may be estimated by the equation :

$$K = \frac{\dot{M}}{(T_b - T_a)} \qquad (6.1)$$

where $\dot{M}$ is metabolic rate (King and Farner 1964, Herreid and Kessel

1967, Lasiewski et. al. 1967, Tracey 1972, McNab 1980a, Dawson and Bennett 1981). Although this relationship is an over simplification of the heat transfer processes involved (Lasiewski et. al. 1967, Tracey 1972, Bakken 1976, McArthur 1981) it is useful for making simple comparisons among species (Herreid and Kessel 1967, McNab 1970, Aschoff 1981). Dry conductance (K_n) leaves out the component of heat loss due to evaporation and may be estimated by the equation:

$$K_n = \frac{\dot{M} - \dot{H}e}{(T_b - T_a)} \qquad (6.2)$$

where $\dot{H}_e$ is the rate of heat loss due to evaporation (King and Farner 1964, Dawson and Bennett 1981). K_n and hence K depend on conduction, convection, and radiation components of heat transfer, which in turn depend to some degree on environmental factors such as the conduction medium, wind speed, and external radiation. Therefore K_n and hence K for the same animal may be different for different micro-environments (Tracey 1972, Bakken 1976, McNab 1980a, Chappel 1980, McArthur 1981). In metabolism chamber studies an attempt is made to hold as many factors affecting K constant allowing estimates of K under relatively standard conditions. Such values for K can be reproduced for a given species (McNab 1980a).

From equation 6.1 it can be seen that metabolic rate is equal to K times the difference between T_a and T_b. According to the Scholander - Irving model for a homeotherm, T_b and K remain constant at T_a below thermoneutral, so that $\dot{M}$ increases linearly as T_a decreases. If a bird conforms to the model, K can be calculated as the slope of the regression relating oxygen consumption ($\dot{V}O_2$) to T_a at T_a below thermoneutral (Lasiewski et. al. 1964, Herreid and Kessel 1967, McNab 1980a). If the regression line does not extrapolate to T_b at zero

metabolism (frequently the case because endotherms usually mix behavioural and metabolic thermoregulation below thernoneutrality; Drent and Stonehouse 1971, McNab 1980a, McArthur 1981) a mean conductance may be calculated by applying equation (6.1) to each individual measurement of $\dot{V}O_2$, T_b, and T_a (Calder and Schmidt-Nielsen 1967, McNab 1980a). Another estimate of K can be made using Newton's law of cooling and the cooling curve of a carcass. Newton's law of cooling states that the rate of heat loss from a body is directly proportional to the difference between the body temperature and the environmental temperature. The cooling constant is defined as the constant of proportionality in this relationship. The cooling constant of an intact carcass can be empirically determined and multiplied by the carcass's estimated specific heat to give an estimate of thermal conductance (Morrison and Tietz 1957, Herreid and Kessel 1967, Lasiewski et. al. 1967, Drent and Stonehouse 1971). Thermal conductance calculated by the three different methods are compared for hatchling Malleefowl.

6.2 MATERIAL AND METHODS

6.2.1 Chicks

Eggs of Malleefowl and Brush-turkeys were collected from natural mounds near Remmark, South Australia and Flinders Chase National Park, South Australia respectively and incubated in mound material at 34 C in the laboratory (section 2.1). A total of 21 Malleefowl and seven Brush-turkey chicks were examined. When chicks hatched they were wet and their feathers still ensheathed. A period of at least 5 h after hatching elapsed before any metabolic measurements were made. During this time the chicks' feathers became unsheathed and the plumage dried. Measurements were made on chicks up to 60 h old. After exposure to high T_a chicks had access to water, but they were not fed over the experimental period.

6.2.2 Measurement of oxygen consumption and evaporative water loss

Oxygen consumption and EWL were measured in an open-flow system consisting of an airtight plastic chamber (18 x 18 x 13 cm) located in a temperature control cabinet. Inflowing air was dried by being passed through tubes containing silica gel and then Drierite (anhydrous $CaSO_4$). A sample of this air was directed through a carbon dioxide absorber (Ascarite) to the reference channel of an oxygen analyser (section 2.3), and the rest flowed through the chamber. Gas leaving the chamber was dried in a tube of Drierite and directed through a flowmeter (Gilmont, Fl300 calibrated to within $\pm$ 100 $ml.min^{-1}$) and a sample directed through Ascarite to the sample channel of the oxygen analyser. Flow rates between 1000 and 4000 $ml.min^{-1}$ were utilized.

Chicks in the respirometery chamber sat in the dark on a grid over mineral oil so droppings did not contribute to EWL. Chicks were weighed

and left 10 - 20 min at a selected temperature before recordings began. $\dot{V}O_2$ was recorded at 5 min intervals for 40 - 50 min. At the end of each run, chicks were quickly removed from the chamber, and, within 90s, the cloacal temperature taken by inserting a quick-reading mercury thermometer (WESCO) at least 2 cm into the cloaca. Chicks were then weighed again, placed back in the chamber and the temperature of the cabinet changed.

T_a in the chamber, monitored continuously with a copper-constantan thermocouple connected to a Comark type 1621 electronic thermometer, did not vary by more than 2 C within any run. Flow rates were adjusted so that relative humidity in the chamber was never greater than 21% (calculated from equation 3 of Lasiewski et. al. 1966). EWL was determined by weighing the Drierite tube before and after the 40 - 50 min respirometry period.

$\dot{V}O_2$ was calculated from equation 4 of Hill (1972). An RQ of 0.72 was assumed and the energy equivalent of O_2 taken as 19.79 $J.mlO_2^{-1}$ (Gordon 1982). The latent heat of vaporization of water was assumed to be 2428 $J.g^{-1}$ of water vaporized (Gordon 1982) when calculating heat loss by evaporation. All metabolic and evaporative water loss data were standardized by dividing it by the chick's mean mass during the experimental period.

6.2.3 Cooling curve determination of conductance

Eight Malleefowl chicks died within 4 d of hatching. Carcasses of these chicks were frozen and used later for cooling curve experiments. Carcasses had a thermocouple fastened 5 cm into the cloaca with a small piece of masking-tape, and the legs were tied together with string. The

carcass was heated to approximately 40 C, and then hung by the string in a glass desiccator in a temperature cabinet set at 10 C. No part of the carcass touched the desiccator. Carcass temperature was monitored continuously with a Comark type 1624 electronic thermometer, the output of which was recorded on a Perkin-Elmer 165 recorder. The air temperature within the desiccator was checked every 5 minutes during a cooling experiment with another thermocouple. Each carcass was weighed before and after each experiment. The rate of cooling of each intact carcass was determined twice, then the carcasses were plucked and two more cooling curves determined.

Newton's Law of cooling may be stated mathematically:

$$\frac{dH}{dt} = -K.T$$

where: H = heat content of body (J)
t = time (min)
K = thermal conductance ($mW.C^{-1}$)
T = temperature difference between body and environment (C)

Now $\frac{dH}{dt} = \frac{dH}{dT} . \frac{dT}{dt}$, where $\frac{dT}{dt}$ = rate of temperature change ($C.min^{-1}$)

and $\frac{dH}{dT}$ = heat content change per temperature change (specific heat) ($J.C^{-1}$)

let $\frac{dH}{dT} = A$. Then: $A.\frac{dT}{dt} = -KT$, which can be rearranged to give: $\frac{dt}{dT} = -\frac{A}{K}.\frac{1}{T}$.

Integrating this last expression gives: $\ln(T) = -\frac{K}{A}.t + b$ where b = constant.

This last expression describes a straight line if ln(T) is plotted against t, with $-\frac{K}{A}$ being its slope. Plots of ln(T) against t can be derived from the empirically determined cooling curve and $-\frac{K}{A}$ evaluated.

The specific heat of the carcass (A) can be estimated from carcass mass (specific heat of avian tissue is approximately 3.47 $J.g^{-1}.C^{-1}$, Lasiewski *et. al.* 1967), so K can be evaluated. It was assumed in

calculations that feathers have negligible heat storage capacity (Calder and King 1974) so only defeathered carcass mass was used to calculate carcass specific heat.

6.2.4 Thermal regime of hatching environment

Daily maximum and minimum temperature data for 3 separate hatching periods were obtained from the nearest weather station to the sites where eggs were collected. For Malleefowl the station was 10 km away at Renmark, and for Brush-turkeys it was 25 km away at Cape Borda.

6.3 RESULTS

6.3.1 Malleefowl chicks

Measured physiological parameters from 5 to 60-h-old chicks were compared using analysis of variance and were not significantly different, so data from chicks of all ages were pooled. All data are expressed as means and 95% confidence limits of the means unless otherwise stated. A modified t-test was used to test predicted and determined values for SMR and EWL (Sokal and Rohlf 1969 p.776). The slopes of regressions calculating K and coefficient of heat strain were compared with predicted values, using a regression slope significance test (Zar 1974).

Mean body mass of 21 hatchling Malleefowl was 114.0 $\pm$ 2.5 g.

$\dot{V}O_2$ increased linearly with decreasing T_a below 32 C in chicks maintaining T_b above 39.0 C (Fig 6.1). The TNZ was estimated to be 32 - 39 C, and SMR 0.81 $\pm$ 0.05 $mlO_2.g^{-1}.h^{-1}$. The regression equation for data below the lower critical temperature was:

$$\dot{V}O_2 = 3.66 - 0.087T_a \qquad (r^2 = 0.75,\ P < 0.001) \qquad (6.3)$$

which extrapolates to a temperature of 42.0 C at zero metabolism.

EWL was constant at T_a below 36 C (2.01 $\pm$ 0.18 $mg.g^{-1}.h^{-1}$) but increased steeply at higher T_a (Fig. 6.2). Energetic equivalents of $\dot{V}O_2$ and EWL show that heat production ($\dot{M}$) was greater than evaporation ($\dot{H}_e$) at T_a below 44 C but less than $\dot{H}_e$ at T_a above 44 C (Fig. 6.3).

Mean T_b at T_a below 33 C was 39.5 $\pm$ 0.1 C (Fig. 6.4). At

temperatures above 33 C, T_b increased linearly with T_a according to the relation: $T_b = 25.1 + 0.4T_a$ ($r^2 = 0.86$, $P < 0.01$) (6.4)
During exposure to temperatures above 41 - 42 C, chicks were observed to pant and gular flutter.

Four out of the 21 Malleefowl neonates tested were unable to remain homeothermic at T_a below 25 C. Their T_b and M were below those of of the other Malleefowl chicks, and they subsequently died.

Figure 6.5 indicates the relative frequency of maximum and minimum air temperatures over three hatching periods recorded 10 km from natural incubation mounds.

6.3.2 Brush-turkey chicks

Mean body mass for the seven Brush-turkey hatchlings was 114.5 $\pm$ 10.3 g.

Physiological parameters from 5 h and 60-h-old chicks at T_a above 10 C were compared using analysis of variance. Data were found to be not significantly different, so data at 10 - 40 C for chicks of all ages were pooled.

$\dot{V}O_2$ of homeothermic chicks increased linearly at T_a below 29 C (Fig. 6.6) according to the relationship:

$$\dot{V}O_2 = 3.66 - 0.09T_a \qquad (r^2 = 0.96, P < 0.001) \qquad (6.5)$$

This line extrapolates to 40.5 at zero metabolism.

Body temperature of thermoregulating chicks remained relatively constant at 40.4 $\pm$ 0.2 C for T_a below 36 C (Fig. 6.7). At T_a above

39 C, T_b increased to 44.0 - 44.5 C (Fig. 6.7). Chicks were unable to cope with temperatures greater than 39 C even at low relative humidities (4 - 21%). They became excited and T_b rose quickly. At this stage chicks were removed from the chamber and allowed to recover at cooler temperatures. At T_a below 10 C, 5 to 16-h-old chicks were not considered to be homeothermic as they failed to maintain T_b above 37 C. In one case T_b dropped to 33.4 C during a 50 min exposure to 8 C. $\dot{V}O_2$ of 5 to 16-h-old chicks fell below that of older chicks which remained homeothermic at these temperatures (Fig. 6.6). However the same chicks were able to remain homeothermic under the same conditions the day after they hatched. The TNZ extended from 29 C to about 38 C and SMR was 1.00 $\pm$ 0.04 $mlO_2.g^{-1}.h^{-1}$.

At T_a from 8 C to 38 C EWL appeared to remain constant at 2.57 $\pm$ 0.32 $mg.g^{-1}.h^{-1}$ (Fig 6.8).

Figure 6.9 gives the relative frequency of daily maximum and mimimum temperatures over three hatching periods from a weather station located 25 km from incubation mounds on Kangaroo Island.

6.3.3 Thermal conductance

The regression of $\dot{V}O_2$ on T_a for Brush-turkey chicks (equation 6.5) extrapolates at zero metabolism to within 0.1 C of their measured T_b below TNZ, whereas the same regression for Malleefowl chicks over estimates T_b by 2.5 C (equation 6.3). Thermal conductance calculated from these regressions are 0.478 $mW.g^{-1}.C^{-1}$ for Malleefowl chicks and 0.496 $mW.g^{-1}.C^{-1}$ for Brush-turkey chicks.

Both K and K_n were calculated using equations (6.1) and (6.2)

over the range of T_a utilized for Malleefowl and Brush-turkey chicks (Figs. 6.10, 6.11). As EWL is essentially constant at T_a below 32 C in both species (Figs. 6.2, 6.8), K_n parallels K at temperatures below TNZ (Figs. 6.10, 6.11).

In Malleefowl chicks, the K_n component of conductance caused K to decrease from 0.61 $mW.g^{-1}.C^{-1}$ at 32 C to 0.48 $mW.g^{-1}.C^{-1}$ at 5 C. K averaged 0.53 $mW.g^{-1}.C^{-1}$ over this temperature range. As T_a increased within the TNZ, K_n increased two fold and K three fold (Fig. 6.11). At T_a above 36 C, EWL increased (Fig. 6.2) and was responsible for an increasingly larger proportion of heat loss from the body as T_a approached T_b. At T_a above 43 C, where T_b remained below T_a, K_n decreased (Fig. 6.10).

In Brush-turkey chicks K remained constant at 0.50 $mW.g^{-1}.C^{-1}$ for T_a below TNZ (Fig. 6.11). As T_a increased in the TNZ, K_n tripled (Fig. 6.11). K also tripled in the TNZ (Fig. 6.11). The increase in K was caused entirely by an increase in K_n, because EWL did not increase within the TNZ of Brush-turkey chicks (Fig. 6.8).

Thermal conductance determined by the carcass cooling method was calculated in the following manner. For each intact carcass cooling curve, ln(T) was plotted against t at 4 min intervals and a regression line calculated to determine the slope; for defeathered carcasses a 2 min interval for t was used (Fig. 6.12). Slopes from the two cooling trials on each intact and defeathered carcass were averaged, and multiplied by the defeathered carcass mass and the specific heat of avian tissue to give conductance (Table 6.1). Mass specific conductance was obtained by dividing by the intact carcass mass for intact

carcasses, and the defeathered carcass mass for defeathered carcasses (Table 6.1).

Table 6.1. Carcass mass, feather mass, cooling constants, and calculated conductance of Malleefowl chicks.

Chick No.	Carcass Mass (g)		Feather Mass (g)	Cooling constant		Conductance [mW/(g.C)]	
	Intact	Defeathered		Intact	Defeathered	Intact	Defeathered
11	106.1	98.1	8.0	6.66	18.66	0.356	1.080
15	101.8	94.9	6.9	7.96	18.29	0.430	1.059
21	102.3	93.5	8.8	6.35	18.46	0.336	1.068
3519	106.3	97.0	9.3	6.61	17.93	0.349	1.038
3520	105.6	97.6	8.0	6.87	16.72	0.368	0.968
3521	97.6	91.6	6.0	7.26	17.73	0.394	1.026
3522	90.9	84.9	6.0	5.81	17.92	0.314	1.037
3523	101.5	93.2	8.3	7.13	19.51	0.379	1.129
Mean	101.5 ± 3.7[a]	93.9 ± 3.1	7.7 ± 1.0	6.83 ± 0.52	18.15 ± 0.67	0.366 ± 0.040	1.051 ± 0.055

[a] 95% confidence interval

6.4 DISCUSSION

6.4.1 Standard metabolic rate and body temperature

With the exception of Gulls (Dawson and Bennett 1981), SMR of neonate precocial birds is considerably less than that of adult birds of equivalent body mass (Koskimies and Lahti 1964, Ackerman et. al. 1980, Dawson and Bennett 1981, Hissa et. al. 1983, Table 6.2). Mass - SMR data from Table 6.2 are log - log transformed and plotted in figure 6.13. The allometric equation calculated from these data is:

$$\dot{V}O_2 = 3.585.M^{-0.279} \qquad (r^2 = 0.66; \quad P < 0.001) \qquad (6.4)$$

$\dot{V}O_2$ in $mlO_2.g^{-1}.h^{-1}$, M in g.

A previously published allometric equation (Ackerman et. al. 1980) for SMR in hatchling birds underestimates SMR of 18 out of 21 neonates in Table 6.2. Ackerman et. al.'s (1980) equation was based on 10 species, 6 of which came from the work of Vleck (1978) and Vleck et. al. (1979). I have examined these sources, and could only find data for 'hatching' birds so I have not included it in my analysis. Hatching birds increase their $\dot{V}O_2$ during the hatching process (see chapter 5) and this probably explains Ackerman et. al.'s (1980) results.

Why do neonates have smaller mass specific metabolism than adult birds of equivalent body mass? The answer to this question is probably related to the pre-pipping metabolism of embryos. Pre-pipping metabolism is approximately half that predicted for an adult bird of equivalent body mass and may be limited by the respiratory gas conductance of the eggshell (Paganelli and Rahn 1984). When a bird hatches its metabolism must double to reach adult levels and this process takes several days in most precocious species. Hence,

metabolism is intermediate between pre-pipping and adult levels for several days after hatching. The increase in metabolism is probably related to maturation of skeletal muscle (Untergasser and Hayward 1972) and an increasing functional gas exchange area in the lungs (Seymour 1984).

Table 6.2. Body mass and standard metabolism of some precocious chicks on the day of hatch during the active phase of circadian rhythm.

Species	Mass (g)	SMR ($ml O_2 . g^{-1} . h^{-1}$)	% Predicted Adult[a]	% Predicted Ackerman et. al.[b]	% Predicted Equation (6.4)
Malleefowl (Leipoa ocellata)	114	0.813	56	113	85
Brush-turkey (Alectura lathami)	114	0.995	69	138	104
Black-footed[c] albatross (Diomedea nigripes)	215	0.713	58	118	89
Laysan albatross[c] (Diomedea immutabilis)	208	0.765	62	125	95
Wedge-tailed[b] shearwater (Puffinis puffinis chlororhynchus)	39	0.895	46	93	69
Bonin petrel[d] (Pterodroma hypoleuca hypoleuca)	32	0.992	49	98	86
Mallard[e] (Anas platyrhynchos)	29	1.207	58	116	96
Common teal[e] (Anas crecca)	17	1.738	72	144	118
Common goldeneye[e] (Bucephala clangula)	32	1.603	79	159	118

Table 6.2 Continued

Species	Mass	SMR	%Predicted Adult SMR	%Predicted Ackerman et. al.	%Predicted Equation (6.4)
Common eider[e] (Somateria mollissina)	61	1.264	74	149	111
European widgeon[e] (Anas penelope)	26	1.621	75	152	112
Velvet scooter[e] (Melanitta fusca)	55	1.347	76	154	115
Common merganser[e] (Mergus merganser)	46	1.256	68	137	102
Red-breasted[e] merganser (Mergus serrato)	44	1.247	67	134	100
Tufted duck[e] (Aythya fuligula)	34	1.355	67	136	101
European pochard[e] (Aytha ferina)	40	1.351	70	142	106
Domestic fowl[f] (Gallus domesticus)	35	1.243	62	126	93
Willow ptarmigan[g] (Lagopus lagopus)	14	2.07	81	162	121
Painted quail[h] (Excalfactoria chinensis)	3	2.00	52	105	76
Capercaillie[i] (Tetrao urogallus)	32	1.99	98	196	146
Xantus murrelet[j] (Synthliboramphus hypolecus)	25	1.83	84	168	125
Mean N = 21			68 ± 6[k]	136 ± 11	102 ± 9

[a] equation (4) of Aschoff and Phol (1970) [b] From Ackerman et. al. (1980) [c] Pettit et. al. (1980a) [d] Pettit et. al. (1980b)

[e] Koskimies and Lahti (1964) [f] Misson (1977) [g] Aulie (1976a)

[h] Bernstein (1973) [i] Hissa et. al. (1983) [j] Eppley (1984)

[K] 95% confidence interval

Body temperature is generally lower in hatchling precocial birds than in adults (Hissa et. al. 1983, Table 6.3). Adult T_b appears during the period of post-hatch growth. The time needed to obtain adult T_b varies from species to species, but is generally one to four weeks. Low T_b may be an energy saving mechanism because, for a particular conductance, a greater metabolic rate (and hence greater energy expenditure) is required to maintain a higher T_b compared to a lower T_b. Reduced metabolic rates may be advantagous when food shortages occur or inclement weather prevents foraging (Ricklefs 1974), as hatchling chicks will be able to survive for a longer period of time on the finite amount of stored energy in the internal yolk sac and fat bodies.

Table 6.3 Hatchling and adult T_b of selected precocial birds under thermoneutral conditions during activity phase of circadian rhythm.

Species	Body temperature day of hatch (C)	Adult body temperature (C)	Difference (C)
Malleefowl (Leipoa ocellata)	39.5	39.5	0.0
Brush-turkey (Alectura lathami)	40.4	40.2[a]	-0.2
Domestic fowl (Gallus domesticus)	38-40.0[b,c]	41.5[d]	2.5
Japanese quail (Coturnix coturnix japonica)	40.8[e]	41.6[e]	0.8
Lesser scaup (Aythya affinis)	38.5[f]	41.6[d]	2.8

Table 6.3 continued.

Mallard (Anas platyrhynchos)	38.0[f], 37.2[g]	41.2[d], 41.9[h]	3.0
Pied-billed grebe (Podilymbus podiceps)	38.7[h]	39.3[h]	0.6
Gadwall (Chaulelasmus streperus)	41.5[h]	41.9[h]	0.4
Red-headed duck (Marila americana)	39.3[h]	41.7[h]	2.4
Black-necked stilt (Himantopus mexicanus)	37.0[h]	41.0[h]	4.0
Red-breasted merganser (Mergus serrator)	38.4[g]	41.9[h]	3.5
Common edier (Somateria mollissima)	38.0[g]	42.4[h]	4.4
European widgeon (Anas penelope)	39.9[g]	41.5[h]	2.2
Painted quail (Excalfactoria chinensis)	38.0[j]	40.0[j]	2.0
Capercaillie (Tetro urogallus)	39.4[k]	41.1[k]	1.7
Xantus murrelett (Sythliboramphus hypolecus)	38.8[l]	39.1[l]	0.3
Mean N = 15	39.0 ± 0.7[m]	41.1 ± 0.5	2.1 ± 0.8

[a] Measurement from one individual, [b] Freeman (1970a) [c] Wekstein and Zolman (1967) [d] McNab (1966) [e] Freeman (1970b)

[f] Untergasser and Hayward (1972) [g] Koskimies and Lahti (1964)

[h] Wetmore (1921) [i] King and Farner (1961) [j] Bernstein (1973)

[k] Hissa et. al. (1983). [l] Eppley (1984) [m] 95% confidence interval

Despite lower than expected (adult) SMR, hatchling Malleefowl and Brush-turkeys regulate T_b at adult levels (Table 6.3), reflecting the

extremely precocial state of these chicks. The relatively small conductances of these two species (section 6.4.2) probably makes adult T_b levels possible even with a reduced metabolic rate. In contrast, most hatchling birds' plumage is not as well developed as an adult bird's plumage of the same size, so hatchlings lose more heat to the environment than adults.

6.4.2 Comparison of methods used to calculate thermal conductance

Because Brush-turkey chicks conform almost perfectly with the Scholander-Irving model for a homeotherm, estimation of K from the regression and averaged methods are almost identical (Table 6.4). In contrast, because the regression of $\dot{V}O_2$ on T_a does not extrapolate to T_b at zero metabolism in Malleefowl chicks, the regression method underestimates K compared to the averaged method (Table 6.4). In this case the averaged method should give a better estimate of K (McNab 1980a).

Estimates of thermal conductance from metabolic data and cooling curve data from dead chicks can be quite different. The metabolic data method probably gives a better estimate because it takes into account heat lost via EWL, respiratory ventilation, and convective heat transfer from the core to exposed peripheral areas of the body such as the head and feet, whereas the cooling curve method does not.

Malleefowl chick conductance estimated by the cooling curve method is considerably smaller than estimated by the other two methods, even when it is compared to K_n (Table 6.4). Cooling curve conductance probably should be compared to K_n, as there is little or no water loss component to heat loss in this method. In live Malleefowl chicks heat loss through respiratory ventilation accounts for some of their greater conductance

Table 6.4 Comparison of thermal conductance estimated by different methods.

Technique	Conductance below TNZ ($mW.g^{-1}.C^{-1}$)	
	Malleefowl	Brush-turkey
Regression (K)	0.478	0.497
Averaged[a] (wet) (K)	0.526	0.500
Averaged[b] (dry) (K_n)	0.470	0.405
Cooling curve[c] (intact)	0.366	
Cooling curve[c] (defeathered)	1.051	

[a] Mean of values obtained using equation (6.1) at T_a below TNZ.

[b] Mean of values obtained using equation (6.2) at T_a below TNZ.

[c] Mean obtained from Table 6.1.

compared to dead birds, but most of the difference probably arises by covective heat flow from the body core to peripheral areas such as the exposed legs and head via the circulatory system. Thus heat transfer from the core to the peripherary where it is lost to the environment is facilitated in live birds, but this form of heat transfer does not occur in dead birds. Herreid and Kessel (1967) expected 'higher conductance from dead birds since feather fluffing and postural changes were absent', but they did not find this. Conductance calculated from metabolic data and

cooling curve data from live hummingbirds going into torpor were almost identical (Lasiewski et. al. 1967). Live 3-day-old Mourning doves cooled faster than dead ones when exposed to 2 C (Breitenbach and Baskett 1967). Suggested reasons for this were increased heat loss due to respiratory ventilation and extensive heat loss from well vascularized but poorly insulated areas of skin (Breitenbach and Baskett 1967).

Defeathered Malleefowl chick carcasses have conductances almost three times greater than intact carcasses (Table 6.1), a result consistent with that of other bird carcasses (Herreid and Kessel 1967). This highlights the importance of the well developed plumage of these chicks. If the plumage was not developed, and T_b was still to be maintained, metabolic rate would have to increase up to three times, a considerable energetic cost.

6.4.3 Cold-hardiness in Malleefowl and Brush-turkeys hatchlings

Compared to other galliform species, both Malleefowl and Brush-turkey hatchlings are extremely cold-hardy. All other galliform hatchlings so far studied can not remain homeothermic even under mild cold stress (temperatures between 20 C and 25 C) on their first day [Black grouse (Lyrurus colchicus), Koskimies 1962; California quail (Lophortyx californicus), Koskimies 1962; Capercaillie (Tetrao urogallus), Koskimies 1962, Hissa et. al. 1983; Domestic fowl (Gallus domesticus), Koskimies 1962, Freeman 1964, 1966, 1967, Misson 1977; European quail (Coturnix coturnix), Koskimies 1962; Japanese quail (Coturnix coturnix Japonica), Spiers et. al. 1974; Painted quail (Excalfactoria chinensis), Bernstein 1973; Ring-necked pheasant (Phasianus colchicus), Koskimies 1962; Willow ptarmigan (Lagopus lagopus), Aulie and Moen 1975, Aulie 1976a)].

There are three major factors which affect the ability of hatchling birds to remain homeothermic under cold stress: (1) body mass, (2) insulation, and (3) the ability to increase heat production when exposed to cold (Farner and King 1961). Body mass affects the surface to volume ratio. Heavier bodies have relatively greater amounts of thermogenic tissue per unit of heat losing surface area. Thus heavier bodies are more resistant to chilling than lighter ones. Malleefowl and Brush-turkeys hatch at a much larger mass (114 g) than other galliform species (2 - 40 g), and this contributes to their greater cold hardiness.

Insulation is obviously important to cold-hardiness as it effects the thermal resistance between the body and the environment. Most gallinaceous birds hatch with downy plumage which has good heat conserving properties. There has been some doubt as to whether megapodes chicks hatch with down or with contour feathers (Nice 1962, Clark 1960, 1964a). In fact, they hatch with a plumage homologous with other galliform species, but the feathers are primarly pennaceous in contrast to the 'downy plumage' of more typical galliform chicks (Clark 1960, 1964a). This type of plumage provides the birds with excellent insulation (section 6.4.2).

The most important factor contributing to cold-hardiness in neonate birds is the ability to increase heat production in response to cold (Koskimies and Lahti 1964, Wekstein and Zolman 1967, Dawson et. al. 1972, Untergasser and Hayward 1972, Spiers et. al. 1974, Aulie and Moen 1975, Dawson et. al. 1976, Misson 1977, Dawson and Bennett 1981, Hissa et. al. 1983, Ricklefs and Roby 1983). Most neonate galliform birds are incapable of doubling their $\dot{V}O_2$ when exposed to cold temperatures (Koskimies 1962); whereas Malleefowl can increase $\dot{V}O_2$ at least 3 times a few hours after hatching (Fig. 6.1), and Brush-turkeys can do the same the day after

hatching (Fig. 6.6). The other precocial hatchlings which remain homeothermic at low T_a are also capable of increasing their metabolism at least 2 - 3 times upon cold exposure within hours of hatching (Koskimies and Lahti 1964, Untergasser and Hayward 1972, Ricklefs and Roby 1983).

Mechanisms of increased heat production in birds are still not fully understood. In adult birds shivering thermogenesis appears to be the main, if not the only, mechanism of increasing regulatory heat production, there being little evidence of non-shivering thermogenesis (West 1965, Calder and King 1974). Brown adipose tissue which is the site of non-shivering thermogenesis in many mammals appears to be absent in most birds (Freeman 1967, Johnston 1971). Evidence of brown adipose tissue has been found in Ruffed grouse (Bonasa umbellus) and Black-capped chickadees (Parus atricapillus), but its thermogenic properties, if any, have not been demonstrated (Oliphant 1983). The suggestion that Domestic fowl chicks utilize non-shivering thermogenesis before shivering thermogenesis develops in a manner similar to some neonate mammals is based on the observation that 'little or no shivering' is observed in cold exposed chicks, but an increase in $\dot{V}O_2$ occurs (Freeman 1966, 1967, Wekstein and Zolman 1967, 1969). However, the absence of visible shivering should not be considered evidence of non-shivering thermogenesis, as increased muscle tone and mild shivering can often be detected electromyographically, but not visually (Calder and King 1974). Malleefowl and Brush-turkey chicks visibly shiver when exposed to T_a below 20 C, confirming shivering thermogenesis as the heat production mechanism in these species. It now seems likely that shivering thermogenesis is the major avenue of increasing heat production in neonate birds (Odum 1942, Aulie and Moen 1975, Aulie 1976b, Hissa et. al. 1983).

At T_a below 10 C homeothermy failed in 5 to 10-h-old Brush-turkeys,

but these birds remained homeothermic on their second day. In contrast, Malleefowl hatchlings are homeothermic at T_a down to 3 C only 5 h after hatching. The greater tolerance to cold exposure of Malleefowl, compared to Brush-turkeys may be related to differences in the incubation biology of the two species. Malleefowl eggs have a longer incubation time, and longer period of peak $\dot{V}O_2$ immediately prior to hatching compared to Brush-turkey eggs (Vleck et. al. 1984). Brush-turkey chicks spend up to three days in the incubation mound after hatching, while Malleefowl chicks only spend up to one day (Vleck et. al. 1984). During the extended period of peak $\dot{V}O_2$ within the egg, Malleefowl may be developing cold defence mechanisms (see chapter 4), while this development occurs in Brush-turkey chicks during the post hatch period in the warm environment of the mound.

Maturation of thermogenic mechanisms can be rapid in hatchling birds. Malleefowl are competent homeotherms 5 h after hatching, Brush-turkeys after 24 h. Mallards (Anas Platyrhynchos), Lesser scaups (Aytha affinis), and Common eiders (Somateria mollissima) show no sign of thermoregulation before hatching, but are competent homeotherms by the end of the day of hatch (Untergasser and Hayward 1972). Newly hatched (wet) Willow ptarmigans (Lagopus lagopus) are unable to increase $\dot{V}O_2$ in response to cold, but are able to do so when half a day old (Aulie and Moen 1975). Rapid maturation of muscles, oxygen transport systems, or a combination of both must be occuring in these hatchlings. Embryonic muscles may be capable of increasing heat production, but this function could be inhibited by oxygen transport barriers such as the chorioallantois and eggshell (Untergasser and Hayward 1972). Full term Malleefowl eggs exhibit an endothermic response after prolonged chilling (chapter 4), but it is not as marked as in neonate chicks. Even after hatching the bird lung-air sac system is still developing and may limit the degree to which respiratory gas

exchange can be increased (Seymour 1984).

6.4.4 Heat stress in Malleefowl and Brush-turkey hatchlings

At T_a > 36 C, neonate Brush-turkeys become hyperthermic (Fig. 6.7) and are unable to tolerate T_a above 39 C for more than a few minutes. Hatchling Brush-turkeys probably never experience heat stress because temperatures inside Brush-turkey mounds are regulated around 34 C and rarely exceed 37 C (Seymour pers. comm.), and post-hatching thermal maxima are moderate (Fig. 6.9).

In contrast to neonate Brush-turkeys, neonate Malleefowl can cope with high T_a. In hatchling Malleefowl EWL increases in an exponential fashion at temperatures above 36 C (Fig. 6.2), and is responsible for an increasingly larger proportion of heat loss from the body as T_a approaches T_b. At T_a above 43 C, heat lost via evaporation exceeds heat produced via metabolism (Fig. 6.3), consequently T_b remains lower than T_a (Fig. 6.4). Increased EWL is achieved by panting and gular fluttering. The onset of gular fluttering occurs at T_b about 42 C, a temperature similar to the onset of panting and gular flutter in other avian species (Dawson and Bartholomew 1968). Gular fluttering increases evaporative heat loss with minimal muscular effort and consequently little increase in metabolism (King and Farner 1961, Dawson and Hudson 1970, Calder and King 1974). Malleefowl neonates are very tolerant of short-term exposure to high T_a, as shown by their high upper critical temperature (39 C) and small increase in metabolism at temperatures above TNZ (Fig. 6.1).

Weathers (1981) introduced a concept for assessing the cost of thermoregulation during heat stress, the coefficient of heat strain (h_s) which is the slope of metabolism on T_a above the upper critical

temperature. His allometric equation predicts that a 114 g bird should have an h_s of 0.57 $mW.g^{-1}.C^{-1}$. The calculated value for Malleefowl neonates of 0.26 $mW.g^{-1}.C^{-1}$ is significantly smaller ($P < 0.001$) and indicates Malleefowl chicks are exceptionally adapted to cope with heat stress.

Within the TNZ, T_b of Malleefowl chicks increases from 39.5 C to 41.4 C (Fig. 6.4). $\dot{V}O_2$ remains constant throughout the TNZ, ie. Q_{10} = 1, a phenomenon discussed at length in Weathers (1981). He suggests this may be a water saving strategy.

6.4.5 Thermal conductance of Malleefowl and Brush-turkey hatchlings

Thermal conductance of Malleefowl and Brush-turkey neonates calculated by both the regression and averaged methods (Table 6.4) were not significantly different from each other [analysis of variance used for comparison of regression method; Student's t test for unequal sample sizes (Simpson et. al. 1960 p.176) used for comparison of averaged method]. These values are similar to the value of an adult bird in the activity phase of the circadian rhythm (0.525 $mW.g^{-1}.C^{-1}$; Aschoff 1981), indicating the excellent insulating properties of these chicks' plumage.

At T_a below TNZ neonate Brush-turkeys behave in a manner similar to the Scholander - Irving model of a homeotherm, ie. K is minimal and remains constant. However, in Malleefowl chicks, changes in the K_n component of conductance caused K to decrease at temperatures below TNZ. Decreasing K_n indicates insulation increases, probably by postural and ptiloerectional changes. Ptiloerection increases in Black-capped chickadees as T_a decreases, even though $\dot{V}O_2$ data indicates that the birds are out of their TNZ (Hill et. al. 1980). Similar observations have been made in other avian

species (McFarland and Baher 1968).

Although at temperatures below TNZ Malleefowl and Brush-turkey chick K is similar, K_n (Table 6.4) is significantly ($P < 0.001$) smaller in Brush-turkey chicks because EWL, and hence, heat loss via evaporation are significantly higher (Table 6.5) than in Malleefowl chicks at these temperatures.

In both Malleefowl and Brush-turkey neonates K increases 3-fold within the TNZ. The major changes in K are caused by changes in K_n brought about by a flattening of plumage and vasodilation of exposed skin areas such as the feet and the underside of wings. These changes facilitate heat loss from the body to the environment. In Malleefowl K_n increases 2-fold and a further increase in K is caused by an increase in EWL as T_a increases at the upper end of the TNZ (Fig. 6.2). In contrast, increases in K_n are entirely responsible for the 3-fold increase in K observed in the TNZ of Brush-turkey chicks as no increase in EWL occurs (Fig. 6.8). Despite an increase in EWL of Malleefowl chicks at the top end of their TNZ and no increase in EWL of Brush-turkeys, EWL of both species is similar at 38 C as Brush-turkeys have an intrinsically higher rate of EWL than Malleefowl (Table 6.5).

Under conditions of extreme heat stress ($T_a > T_b$) K_n of Malleefowl chicks decreases (Fig. 6.10). This strategy is adaptive as it decreases the amount of heat entering the body from the environment by conduction, convection, and radiation and thus conserves water that would otherwise be lost by thermoregulatory evaporation. This strategy may be particularly important in Malleefowl chicks as T_a greater than T_b are encountered on 5% of days during the hatching period and there is no

drinking water available to replace water lost by evaporation.

6.4.6 Metabolic water production in hatchling Malleefowl

Because drinking water is usually unavailable to hatchling Malleefowl, oxidative or metabolic water production (MWP) may play an important role in overall water economy. MWP may be calculated from metabolic rate if the substrate being metabolized is known (Bartholomew and Dawson 1953, Bartholomew and Cade 1963). Malleefowl chicks feed principally on insects and seeds (Frith 1962b) so MWP is likely to be intermediate between the rates for lipid and carbohydrate metabolism. MWP increases as T_a decreases below TNZ (Fig. 6.14) because metabolic rate increases. Even at thermoneutral temperatures MWP accounts for 23% of total water influx of free-ranging chicks (52 $ml.kg^{-1}.d^{-1}$ = 2.2 $mg.g^{-1}.h^{-1}$; chapter 8). At 20 C MWP can account for over 50% of this water influx, and because night temperatures frequently fall below 20 C over the hatching season (Fig. 6.5) MWP must account for an appreciable amount of water influx.

6.4.7 Differences in thermoregulatory parameters of Malleefowl and Brush-turkey hatchlings

Hatching body mass and K are similar in Malleefowl and Brush-turkey neonates, but SMR, T_b, and EWL at T_a below 36 C are significantly less in Malleefowl chicks (Table 6.5). These data may reflect differences in climatic conditions of the post-hatch environment of the two species. Brush-turkeys hatch during the period September - February into a mesic environment, where daily thermal maxima are rarely extreme (1% of days reach temperatures greater than 35 C, Fig. 6.9), and surface water is usually available. Malleefowl hatch from November through to March into an arid environment where surface water is rarely available, and high temperatures regularly encountered (22% of days reach temperatures greater than 35 C,

Table 6.5. Thermoregulatory parameters of Malleefowl and Brush-turkey neonates.

Parameter	Malleefowl	Brush-turkey	Significance[a] of comparison
Body mass (g)	114.0	114.5	NS
SMR ($ml O_2 . g^{-1} . h^{-1}$)	0.813	0.995	$P < 0.001$
EWL at T_a below 36 C ($mg . g^{-1} . h^{-1}$)	2.006	2.547	$P < 0.001$
K at T_a below 30 C ($mW . g^{-1} . C^{-1}$)	0.478	0.496	NS
T_b at $10 < T_a < 36$ (C)	39.5	40.4	$P < 0.001$
TNZ (C)	32 - 39	29 - 38	

[a] Body mass, SMR, T_b, and EWL values compared using a modified 't' test (Simpson et. al. 1960 p. 176). K compared using a regression slope comparison test (Zar 1974)

Fig. 6.5). Low metabolic rate favours water economy, especially at high temperatures, and has been found in several arid zone birds (Dawson

1976, Weathers 1981,Marder and Bernstein 1983), and is generally considered to be an adaptation to hot and/or low productivity environments (Dawson 1976).

If avian T_b is adaptive to thermal environment, birds which regularly encounter high temperatures should have a higher T_b than birds which experience cooler temperatures (Scholander et. al. 1950c,. Calder and King 1974). At low T_a, a low T_b decreases the thermal gradient between the bird and the environment and consequently saves energy. A high T_b at high T_a saves water because a higher T_a is reached before EWL must be increased in order to maintain T_b. Because Brush-turkeys have a higher T_b than Malleefowl, but encounter a cooler thermal regime than Malleefowl, it can be concluded that T_b is non-adaptive to thermal environment, a finding consistent with previous studies (for review see Calder and King 1974). Avian T_b is determined by an interaction between the rate of heat produced and the rate of heat loss from the body. Heat production is determined by metabolic rate. Heat loss is determined by thermal conductance and the difference between T_b and T_a. Low T_b could therefore be the result of low metabolic rate, high conductance, or both. Because neonate Malleefowl and Brush-turkeys have similar thermal conductance, but Malleefowl have a lower SMR, it is not surprising to find that Malleefowl also have a lower T_b.

In nature, cold temperatures may be more stressful to neonate Malleefowl and Brush-turkeys than heat as 53% and 88% respectively, of days over the hatching period have thermal minima below 15 C (Figs. 6.5, 6.9). The ability to maintain elevated metabolism continuously during this time depends on energy stores in the body and food availability.

Malleefowl and Brush-turkey neonates have extensive sub-cutaneous fat bodies and a substantial amount of yolk within the abdominal cavity (Clark 1964a, Vleck et. al. 1984, personal observation) and are capable of foraging within hours of emerging from the incubation mound. Their energy stores and ability to obtain food soon after hatching should enable them to maintain elevated metabolism for long periods of time and be adequate protection against death through hypothermia.

At T_a below 36 C Malleefowl chicks lose water at the rate of 2.0 $mg.g^{-1}.h^{-1}$, which is significantly lower ($P < 0.001$) than the expected value (2.3 $mg.g^{-1}.h^{-1}$, Crawford and Lasiewski 1968), while the observed maximum EWL (11.8 $mg.g^{-1}.h^{-1}$) is only 49% of the predicted maximum (24.0 $mg.g^{-1}.h^{-1}$, Calder and King 1974). Low EWL is an obvious advantage in an arid environment, and arid adapted birds have lower EWL at T_a below 40 C than birds inhabiting more mesic environments (Weathers 1981). Ideally, arid adapted species should have a low EWL at temperatures in and below TNZ, and the ability to increase EWL extensively when T_a approaches and exceeds T_b (Weathers 1981). Neonate Malleefowl behave in this manner (Fig. 6.2). Presumably, because Brush-turkey chicks are unlikely to experience water shortages they can afford higher EWL.

The similarity of K in Malleefowl and Brush-turkey neonates may be somewhat surprising, as early work indicated that it is the K component of an animal's thermoregulatory physiology which is usually modified to suit thermal conditions of an environment (Scholander et. al. 1950c). More recent work indicates K is largely dependent on body size (Herreid and Kessel 1967, Lasiewski et. al. 1967, Drent and Stonehouse 1971, Aschoff 1981), and this probably explains why K is similar in Malleefowl

and Brush-turkey neonates.

In concluding this section, I suggest the differences in thermoregulatory behaviour of Malleefowl and Brush-turkey chicks reflect differences in their post-hatching environments. The difference in cold-hardiness on the first day may be related to differences in the post-hatch residence time in the incubation mound. The lower SMR, EWL, and greater heat tolerance of Malleefowl compared to Brush-turkey chicks are probably adaptations to the drier, hotter environment experienced by Malleefowl. The higher T_b of Brush-turkeys is probably a consequence of their greater SMR.

CHAPTER 7 THERMOREGULATION IN TWO-YEAR-OLD MALLEEFOWL

7.1 INTRODUCTION

Malleefowl inhabit semi-arid mallee country where diurnal summer temperatures are frequently high, and surface water is usually unavailable (Frith 1962b). Most of the breeding season occurs during summer and adult birds expend a great deal of energy tending their mounds and producing eggs. Because birds inhabiting low latitudes or hot environments generally have lower weight specific standard metabolic rate (SMR) (Hudson and Kimzey 1966, Trost 1972, Dawson and Bennett 1973, Dawson 1976, Weathers 1977, 1979, Ellis 1980, Hayworth and Weathers 1984) and higher thermal conductance (K) (Scholander et. al. 1950c, Calder 1964, Aulie 1976b) than birds inhabiting high latitudes or cold environments, and certain arid zone species also have evolved lower evaporative water loss (EWL) compared to birds inhabiting more mesic environments (Dawson and Fisher 1969, Bartholomew 1972, Weathers 1981, Hayworth and Weathers 1984), one might expect Malleefowl to possess similar physiological adaptations. Here I report measurement of these parameters on two-year-old Malleefowl in order to test these predictions.

7.2 MATERIAL AND METHODS

Open flow respirometry was used to measure oxygen consumption ($\dot{V}O_2$) and EWL. Compressed air was past through water absorbers (silica gel and Drierite), a sample directed through a carbon dioxide absorber (Ascarite) to the reference channel of a Taylor Servomix OA18 oxygen analyser, while the remainder flowed through a flow meter (Fischer and Porter model 10A3555A, accurate to $\pm$ 300 ml.min^{-1}) into a respirometry chamber constructed from a 60 l black plastic barrel in a temperature cabinet (Fig. 7.1). A sample of gas leaving the chamber was first drawn through a tube of Drierite and then a tube of Ascarite by a pump (Cole-Parmer model 7015), and directed to the sample channel of the oxygen analyser where it was vented to the room through a flow meter (Fischer and Porter model C-1812-5A-L).

$\dot{V}O_2$ was calculated according to equation (4) of Hill (1972). EWL was estimated by weighing the Drierite in the sample line before and after a 50 - 60 min measurement period. EWL was calculated with the formula:

$$EWL\ (mg.min^{-1}) = \frac{W \times F1}{t \times F2}$$

where W = change in mass of Drierite tube (mg)

t = time of measurement period (min)

F1 = flow rate of gas entering chamber (ml.min^{-1}, STP)

F2 = flow rate of gas through sample line (ml.min^{-1}, STP)

Ambient temperature (T_a) in the chamber was monitored continuously with a thermocouple suspended within the chamber connected to a electronic thermometer (Comark type 1621). Body temperature (T_b)

of birds was measured to $\pm$ 0.2 C within 90 s of the end of a measurement period, by inserting a quick reading mercury thermometer (WESCO) or a thermocouple connected to a electronic thermometer (Comark type 1624) approximately 5 cm into the cloaca.

Respiratory frequency (R_f) was monitored on a polygraph (Grass model 79D) via a volumetric pressure transducer (Grass model PT5 A) connected to the respirometry chamber with a 10 cm length of plastic tubing (internal diameter 7 mm).

Birds were starved for 24 h prior to making measurements, but had access to water over this period. All measurements were made between 8.00 and 18.00 h. Birds were weighed on an electronic balance (Mettler PC 4000) and placed in the respiratory chamber at least 30 min before measurements started. They sat in the dark on a grid over mineral oil to prevent water from droppings contributing to the water vapour in the chamber. Flow rates of gas through the chamber were regulated (11 - 15 $l.min^{-1}$) so that relative humidity, calculated by equation (3) of Lasiewski *et. al.* (1966), never exceeded 20%. $\dot{V}O_2$ was calculated at 5 min intervals throughout a 50 - 60 min measurement period. It was normally possible to make measurements at five or six different temperatures on a bird within one day. At the end of the day, the bird was weighed again.

7.3 RESULTS

7.3.1 Body mass

All results are presented as means and 95% confidence limits of the mean unless otherwise stated. Four sub-adult Malleefowl (2.5 years old) were used in these experiments, their mean body mass was 1390 g (Table 7.1). $\dot{V}O_2$ and EWL data were standardized by dividing them by the mean mass of the bird over the experimental period.

Table 7.1 Body mass of sub-adult Malleefowl over the three week period when measurements were made.

Bird No.	Minimum mass (g)	Maximum mass(g)	Mean mass (g)
3514	1310	1446	1384 ± 43[a]
3518	1294	1429	1350 ± 29
3524	1650	1769	1724 ± 52
3525	1045	1153	1100 ± 20
Pooled	1325	1449	1390 ± 408

[a] 95% confidence interval

7.3.2 Oxygen Consumption and thermal conductance

$\dot{V}O_2$ increased linearly with decreasing T_a at T_a below 22 C according to the relationship:

$\dot{V}O_2 = 1.14 - 0.03T_a$ $\quad (r^2 = 0.87, P < 0.001)$

$\dot{V}O_2$ in $mlO_2.g^{-1}.h^{-1}$, T_a in C, which extrapolates to 42.4 C at zero metabolism (Fig 7.2). The thermoneutral zone (TNZ) appeared to extend from 22 C to 37 C with mean $\dot{V}O_2$ being 0.52 ± 0.01

$ml.g^{-1}.h^{-1}$ within this zone. At T_a between 37 and 40 C $\dot{V}O_2$ increased slightly (Fig. 7.2). At T_a above 40 C $\dot{V}O_2$ increased greatly because birds became restless and continually moved around inside the chamber kicking at the walls (Fig. 7.2).

Thermal conductance was calculated from the slope of the regression line relating $\dot{V}O_2$ to T_a at T_a below 22 C and using a conversion factor of 5.5 mW per $mlO_2.h^{-1}$. Thermal conductance was 0.148 $mW.C^{-1}.g^{-1}$.

7.3.3 Evaporative water loss

At T_a below 36 C EWL remained relatively constant at 1.03 $\pm$ 0.08 $mg.g^{-1}.h^{-1}$. At higher T_a EWL increased 7-fold (Fig. 7.3).

7.3.4 Body temperature

At T_a below 32 C, T_b increased linearly with decreasing T_a according to the relationship:

$$T_b = 40.3 - 0.04T_a \qquad (r^2 = 0.40, \quad P < 0.001)$$

T_a and T_b in C, and averaged 39.7 $\pm$ 0.2 C over this temperature range (Fig. 7.4). At T_a between 32 and 40 C T_b increased linearly according to the relationship:

$$T_b = 32.7 + 0.22T_a \qquad (r^2 = 0.76, \quad P < 0.001)$$

T_a and T_b in C, while at T_a greater than 40 C, when birds were restless, T_b rose to 44.6 C (Fig. 7.4).

7.3.5 Respiratory frequency

At T_a between 22 C and 32 C, R_f remained relatively constant averaging 14.2 $\pm$ 0.7 $breaths.min^{-1}$ (Fig. 7.5). Below TNZ R_f increased according to the relationship: $R_f = 19.2 - 0.23T_a$

($r^2 = 0.54$, $P < 0.001$) and reached 18.7 breaths.min^{-1} at 2 C. At T_a between 34 and 37 C R_f increased moderately to about 30 breaths.min^{-1}, and at T_a above 37, R_f increased to 200 - 400 breaths. min^{-1} (Fig. 7.5). Birds were observed to pant and gular flutter when exposed to T_a above 37 C.

7.4 DISCUSSION

7.4.1 Oxygen consumption and thermal conductance

Studies of $\dot{V}O_2$, EWL, T_b, and K in birds in respirometry chambers have generated a large data base from which interspecific comparisons can be made. Despite the problems of differing experimental procedures influencing the results of these studies (Lasiewski 1969, Lasiewski and Seymour 1972), and the fact that the conditions of measurement are artifical (Porter 1969, Lasiewski 1969, Bakken 1976, Robinson et. al. 1976), data generated from respirometry studies have enabled the formulation of generalizations which have predictive power.

Malleefowl SMR is significantly lower than predicted for a bird of equivalent body mass (Table 7.2), a finding consistant with other arid adapted birds (Dawson 1976, see chapter 6). The upper critical temperature (37 C) is relatively high, indicating that mild heat stress is well tolerated in this species. Unfortunately, at temperatures greater than 40 C birds did not rest in the metabolism chamber, so a comparative assessment of thermoregulation at high T_a is not possible. However, wild birds in their natural environment cope with heat stress extremely well. On hot days Malleefowl normally sit quietly in a dust bath in the shade. If the mound needs to be dug out, the bulk of the work is done in the relative cool of early morning, and the remainder done gradually over the rest of the day. If the mound is disturbed during the day while the male bird is in the vicinity, he will leave the shade and immediately repair the mound. On 28 December 1982, a hot sunny day on which the air temperature reached 34 C, I opened a mound and left it in strong sunshine. On my leaving the male bird immediately moved in and began to refill the mound. Within 3 min the

bird started to gular flutter and pant, and the wings were held out from the body exposing the relatively unfeathered skin under the wings. After 22 min of continuous digging saliva dripped from the bird's mouth, but digging continued for another 8 min. After this initial 30 min bout of digging, the bird sat for 12 min in a partially shaded dust bath. He returned to work the mound for another 3 min before returning again to the shaded dust bath. The bird did not stop gular fluttering and panting during the entire 65 min observation period. The high air temperature, exposure to full sunshine, and the 30 min of continuous digging must have forced this bird to incur a considerable heat load.

Table 7.2 Metabolic parameters of two-year-old Malleefowl

Parameter	Observed	Predicted	% Predicted	Significance[a]
SMR ($ml.g^{-1}.h^{-1}$)	0.52 ± 0.01	0.74[b]	71	$P < 0.05$
EWL ($mg.g^{-1}.h^{-1}$)	1.03 ± 0.08	0.89[c]	114	NS
Thermal conductance ($mW.C^{-1}.g^{-1}$)	0.15 ± 0.01	0.16[d]	95	NS
R_f (min^{-1})	14.2 ± 0.7	15.5[e]	92	NS

a Compared to 95% confidence interval of predicted variable (Simpson et. al. 1960, p.238)

b Aschoff and Pohl (1970)

c Crawford and Lasiewski (1968)

d Aschoff (1981)

e Calder (1968)

Thermal conductance of Malleefowl is similar to the predicted value

(Table 7.2). Small arid-adapted birds normally have relatively high thermal conductances and high lower critical temperatures, a condition which allows their metabolic water production to approach EWL at low T_a (Bartholomew 1972). However, the relationship between EWL and metabolic water production is not as critical to overall water economy in large birds as it is in smaller ones because large birds lose relatively less water and have lower lower critical temperatures (Bartholomew and Cade 1963, Bartholomew 1972). Because Malleefowl are relatively large birds, increased metabolic water production at temperatures below TNZ are probably unimportant.

7.4.2 Evaporative water loss

EWL of Malleefowl is similar to the predicted value for a non-passerine bird of equivalent mass (Table 7.2). This result is in contrast to results for Malleefowl chicks in which EWL is significantly lower than predicted (chapter 6). Reduced EWL may be expected for an arid adapted species (Weathers 1981). Because Malleefowl are relatively immobile, they do not have access to free water and must obtain all their water from food items. A large proportion of their diet consists of seeds (Frith 1959, Booth 1985) which are low in preformed water (Bartholomew 1972). Under such circumstances water may be potentially limiting, making any water conserving mechanism advantagous. Low EWL is an obvious way to restrict water loss. Under the conditions of measurement experimental birds were fully hydrated. If the experimental birds had been water deprived before measurements were made, EWL may have been lower as EWL can be reduced under conditions of water deprivation in some avian species (Calder 1964, Willoughby 1969, Lee and Schmidt-Nielsen 1971, Bartholomew 1972). Later in the study, under conditions of water deprivation, the same four experimental birds were able to reduce their

total water efflux measured by the tritiated water method to an average of 21.4 $ml.kg^{-1}.d^{-1}$ (chapter 8). If during this trial the birds had an EWL of 1.03 $ml.g^{-1}.h^{-1}$, they would be losing water at the rate of at least 24.7 $ml.kg^{-1}.d^{-1}$. Clearly, Malleefowl are capable of restricting EWL under conditions of water stress.

7.4.3 Body temperature

The T_b of Malleefowl falls within the range of birds, but is lower than the mean T_b for 22 galliform species (41.8, Neumann *et. al.* 1968). The biological significance of a lower T_b is dubious as avian T_b does not appear to be related to climate in an adaptive manner (chapter 6).

An increase in T_b as T_a is reduced below TNZ is an unusual phenomenon and is probably caused by an overshoot of the increased metabolism stimulated by cold exposure. Similar observations have been recorded for the Common raven, *Corvus corvax* (Schwan and Williams 1978), Mute swan, *Cygnus olor* (Bech 1980), Black grouse *Lyrurus tetrix* (Rintamaki *et. al.* 1983), and Japanese quail *Coturnix coturnix japonica* (Nomoto *et. al.* 1983).

Within the TNZ T_b rises by 1.0 C, yet $\dot{V}O_2$ remains constant, i.e., $Q_{10} = 1$, a phenomenon which is common to many avian species (Weathers 1981). The absence of increased metabolism with an increase in T_b helps reduces water loss during moderate heat stress (Weathers 1981).

7.4.4 Respiratory frequency

Malleefowl have a respiratory frequency slightly lower than predicted but the difference is not significant (Table 7.2). More recent

values of R_f when measured in the TNZ of completely unrestrained birds are lower than values predicted from Calder's (1968) allometric equation (see Bucher 1985 for discussion). The moderate increase in R_f observed as T_a approaches the upper critical temperature (34 - 37 C) is probably induced by the need to increase heat loss by evaporation through the respiratory system because $\dot{V}O_2$ remains constant (i.e. the increase in R_f can not be attributed a need to increase the rate of respiratory gas exchange), and EWL doubles at these temperatures (Fig. 7.3). Increases of R_f wihin the TNZ in birds are caused by thermoregulatory needs (Bucher 1985). Between 37 C and 40 C, R_f increases to panting levels (Fig. 7.5) and EWL increases greatly (Fig. 7.3), while $\dot{V}O_2$ increases by 8% (Fig. 7.2). Presumably the increase in $\dot{V}O_2$ represents the metabolic cost of panting and gular fluttering.

Because metabolic rate in Malleefowl increases by 108% as T_a decreases from 22 C to 2 C, and R_f only increases by 30%, tidal volume and/or oxygen extraction efficiency must also increase. Tidal volume and R_f increase, whereas oxygen extraction efficiency does not appear to change in the Fish crow Corvus ossifragus (Bernstein and Schmidt-Nielsen 1974) and the parrots Bolborhynchus lineala (Bucher 1981) and Ammazona viridigenalis (Bucher 1985) at T_a below TNZ, so it is probable that in Malleefowl tidal volume also increases at low T_a.

In summary, Malleefowl have lower than predicted SMR, a phenomenon consistent with other arid-adapted birds, and an EWL slightly greater than predicted, a result inconsistent with results from other arid-adapted birds. However, Malleefowl can reduce their EWL under conditions of water deprivation.

CHAPTER 8 WATER TURNOVER

8.1 INTRODUCTION

Water and energy requirements are two of the post important aspects of any animal's biology. Energy is needed to drive the chemical processes within the body, and water is the medium within which these processes take place (Bartholomew 1972). Because water is a by-product of catabolism, an increase in metabolism increases water as well as energy turnover. For a given food item there is usually a relatively constant content of energy and preformed water, so a need to consume a fixed quantity of food to meet energy requirements also entails the consumption of a fixed quantity of preformed water. Because metabolic and preformed water are associated with energy consumption, water turnover is usually positively correlated with energy turnover (Streit 1982). In some species, all body water needs may be obtained through preformed and metabolic water. In these cases energy and water enconomies are tightly linked. In other animals water and energy economies are uncoupled to varying extents. When the water content of food stuffs is not high enough to meet overall body water needs, extraneous water must be obtained through drinking. The need to increase evaporative water loss (EWL) to regulate body temperature at high ambient temperatures also uncouples water and energy economies.

There are many laboratory studies on the water economies of birds (see Bartholomew 1972 for review), but relatively few on wild birds (Dawson et. al. 1983) because of the inherent difficulties of working with free-ranging animals. Although laboratory studies are useful for comparisons among species, there are difficulties in extrapolating

laboratory results to the field. For example, the Zebra finch (Poephila guttata) can be maintained on air dried seed without drinking water in the laboratory (Calder 1964, Cade et. al. 1965), but in the wild it is one of the most water dependent bird species inhabiting Australian arid areas (Fisher et. al. 1972, Davies 1982).

The availability of isotopically labeled water has made the study of water turnover in free-ranging animals possible. Mammals are better studied than birds in this respect. In many mammals there are seasonal variations in water turnover which are related to seasonal changes in diet or climatic factors (Holleman and Dieterich 1973, Nicol 1978, Grubbs 1980, Green and Eberhard 1983). In general, arid inhabiting mammals have lower water turnover rates than more mesic species (MacFarlane and Howard 1972, Nicol 1978, Streit 1982). Unfortunately, similar studies on wild birds are scarce so comparisons and generalizations among free living birds are tentative. There have been few studies on wild birds over different seasons, and none on water turnover of individuals throughout a year.

A study of water turnover in Malleefowl is interesting because this species inhabits arid regions where surface water is usually unavailable. It is a relatively immobile, largely granivorous, bird that breeds during the hottest, driest time of year (Frith 1962b), so one might expect this species to have low water turnover rates. In this study both laboratory and field monitoring of water turnover in Malleefowl is carried out for periods up to one year.

8.2 MATERIAL AND METHODS

8.2.1 Water flux and body water of captive chicks

Captive chicks were housed in sand-lined outdoor enclosures (90X90X200 cm). Water and a mixture of canned dog food and budgie seed mix was supplied ad libitum. For estimation of total body water (TBW) chicks were weighed to 0.1 g (Mettler PC 4000 electronic balance) and 0.5 ml of 100 $\mu Ci.ml^{-1}$ tritiated water (HTO) in 0.9% saline injected intramuscularly into the right thigh muscle (peronaeus longus) with a 22 gauge needle connected to a previously calibrated 1.0 ml tuberculin syringe (Yale B-D 2027). In all cases where injections or blood samples were taken the skin around the injection or sampling point was swabbed with 70% ethanol solution. An hour after injection a blood sample was taken from either the left or right brachial vein by inserting a 24 gauge needle and collecting approximately 0.5 ml of blood in a heparinized 5 ml syringe. The blood was then transferred to 70 µl heparinized microhaematocrit tubes which were sealed with Critocaps® (Sherwood Medical Industries Inc.) and stored. If chicks had previously been injected with HTO an initial blood sample was taken before re-injection with HTO. Further blood samples were taken several days after injection with HTO. Chicks were weighed and blood samples taken as previously described. Water turnover was estimated by HTO washout between subsequent sampling times (Nagy and Costa 1980).

8.2.2 Water flux in free-ranging chicks

Water flux was estimated in two free-ranging chicks at the Renmark study site in February 1984. Chicks with radio transmitters (Wildlife Materials Inc. USA., PB-1220-LD) strapped to their backs were transported to the field and released 18 h after injection with HTO.

Chicks did not drink or feed during transport. Three days after release, the chicks were located, caught with an insect sweep net, weighed to 1 g with a spring balance (Salter Super Samson 200 g) and bled.

8.2.3 Water flux and body water of captive adults

Four adult Malleefowl were maintained in an outdoor enclosure as previously described (chapter 2). For estimation of TBW birds were placed in a weighed calico bag and weighed to the nearest 1 g (McDel 7 rotating weight balance), and a 0.5 ml blood sample taken either from a clipped toe or a brachial vein with a 22 gauge needle and 5 ml heparinized syringe. A 0.5 ml aliquot of 1 $mCi.ml^{-1}$ HTO in 0.9% saline was injected into the right thigh muscle with a previously calibrated 1.0 ml tuberculin syringe (Yale B-D 2027). An hour after injection a further 0.5 ml blood sample was taken. This proceedure was repeated at one to two month intervals between August 1983 and November 1984.

8.2.4 Water flux and body water of free-ranging adults

Wild adult Malleefowl were first trapped on their breeding mounds (chapter 9). Upon capture a radio transmitter (Wildlife Materials Inc. USA., HLPB-2120-LD) was strapped to the bird's back, enabling them to be tracked to their roosts on subsequent field trips. They were caught in a 70 cm diameter net mounted on an extendible aluminium pole and treated as previously described (section 8.2.3) except that weighing was done to the nearest 10 g on a 5 Kg spring balance (Salter Super Samson) and 1.0 ml 1 $mCi.ml^{-1}$ HTO in 0.9% saline was injected.

8.2.5 Analysis of tritiated water in blood samples

Water was distilled from blood samples as soon as they arrived in the laboratory with the method of Nagy (1983). Distillate was flame sealed in glass tubes and stored at room temperature until analysed. Tritium activity was quantified by pipetting 10 µl (Drummond microcaps) of a sample into 3 ml of xylene scintillation fluid (Packard Scintillator 299TM) delivered from an auto-pipetter (Oxford model SA 400 Auto-pipetter) and counted for 10 min in a scintillation counter (Packard Model 3330 Tri-carb Scintillation Spectrometer) at 50% gain and a window length of 50-1000. Where possible 5 replicates of each sample were counted. Five blanks consisting of 10 µl of distilled water in scintillant and five standards consisting of 10 µl of a standard tritiated water solution (1.0 ml 100 $\mu Ci.ml^{-1}$ in 1000 ml of distilled water for chicks, 0.5 ml 1 $mCi.ml^{-1}$ in 1000 ml distilled water for adults) were run at the same time blood water samples were analysed. All standard and sample counts used in calculations were corrected for background activity indicated by the blanks.

8.2.6 Calculation of total body water

Total body water was estimated by HTO dilution. If HTO had not previously been injected, TBW of chicks was calculated according to the formula:

$$TBW\ (ml) = \frac{StH^*}{T_{60}H^*} \times 1000 \times \frac{0.4918}{0.9935} \qquad (8.1)$$

TBW of captive adult birds:

$$TBW\ (ml) = \frac{StH^*}{T_{60}H^*} \times 1000 \times \frac{0.4918}{0.4963} \qquad (8.2)$$

TBW of free-ranging adult birds:

$$TBW\ (ml) = \frac{StH^*}{T_{60}H^*} \times 1000 \times \frac{0.9908}{0.4963} \qquad (8.3)$$

where StH* = activity of HTO in standard solution

$T_{60}H^*$ = activity of HTO in blood water after 60 min equilibration period

and the fraction at the end of the equations represent the ratio of the amount of HTO solution injected into birds to the amount of HTO solution injected into 1000 ml standard.

When HTO had previously been injected, TBW was calculated using equations (8.1), (8.2), or (8.3) except that the first term on the right hand side of the equations was replaced with $\frac{StH^*}{(T_{60}H^* - T_0H^*)}$

where T_0H^* = activity of HTO in blood water prior to further injection with HTO

I assumed 1 ml of water has a mass of 1 g, so percentage body water was calculated using the formula:

$$\text{Body water (\%)} = \frac{\text{TBW (ml)}}{\text{Mass (g)}} \times 100 \qquad (8.4)$$

8.2.7 Water flux calculations

Any mass changes in birds occurring over the period between subsequent blood sampling times were treated as if they occurred linearly, so average water efflux during the experimental period was calculated with equation (4) of Nagy and Costa (1980):

$$\text{efflux } (ml H_2O.kg^{-1}.d^{-1}) = \frac{2000(W2 - W1).\ln(H1^*.W1/H2^*.W2)}{(M1 + M2).\ln(W2/W1).t} \qquad (8.5)$$

where W1 = TBW at time 1 (ml)

W2 = TBW at time 2 (ml)

H1* = activity of HTO in blood water at time 1

H2* = activity of HTO in blood water at time 2

M1 = mass of bird at time 1 (g)

M2 = mass of bird at time 2 (g)

t = time elapsed between time 1 and time 2 (d)

Total body waters used in equation (5) were calculated by HTO dilution using the appropriate equation. In cases where W2 was not determined by HTO dilution, an estimate was made by multipling M2 by the percent body water calculated at time 1. Average influx of water during the experimental period was calculated using equation (6) of Nagy and Costa (1980):

$$\text{influx } (\text{ml}H_2O.\text{kg}^{-1}.\text{d}^{-1}) = \text{efflux} + \frac{2000(W2 - W1)}{t(M1 + M2)} \qquad (8.6)$$

symbols are the same as in equation (8.5).

8.3 RESULTS

8.3.1 HTO equilibration time trial

Before water turnover experiments were begun, a trial on two captive adult Malleefowl was carried out to determine the equilibration time for the injected dose of HTO. The result indicated that equilibration occurs within 60 min (Table 8.1). A 60 min equilibration time was therefore used in all subsequent trials.

Table 8.1 Results of tritiated water equilibration trial. Numbers in table are number of counts per 10 min from a 10 µl aliquot of water distilled from blood samples.

Bird No.	3524	3525
Mass (g)	1808	1257
Time after injection (min)		
20	12069	15465
40	12017	15032
60	11892	16539
120	11956	16461

8.3.2 Water flux and body water of chicks

Total body water of Malleefowl chicks of varying ages averaged 72.9% of body mass (Table 8.2). Three chicks (4543, 4546, 4547) had TBW determined on two separate occasions. In each case percentage body water decreased slightly as the chicks became older (Table 8.2). Despite this trend in individuals, pooled data from chicks of all ages

did not indicate a consistant relationship between age and percentage water content (Fig. 8.1).

Table 8.2 Body water content of Mallee Fowl chicks of different ages.

Chick No.	Age (d)	Mass (g)	Body water (ml)	% Body mass
4531	4	107	77.3	72.2
4532	22	143	106.7	74.6
4533	13	120	88.7	73.9
4534	11	122	95.7	78.5
4543	14	119	89.3	75.0
4543	44	187	136.2	72.7
4544	1	124	91.1	73.2
4545	0	111	77.6	69.6
4546	1	112	79.7	71.1
4546	19	126	88.3	70.1
4547	1	101	73.1	72.7
4547	15	115	81.4	70.9
4548	11	101	75.4	74.6
4549	2	89	61.4	68.7
4550	0	100	76.0	76.0
Mean				72.9 $\pm$ 1.5[a]

[a] 95% confidence interval

Water efflux and influx in chicks were closely coupled for any measurement period with the exception of chick 4547 under field conditions (Table 8.3). In all but one of the measurements in enclosures (4550), chicks gained mass and consequently water influx was always greater than water efflux. For the two chicks measured in the field (4547, 4550), the converse was true; both lost mass and water efflux was greater than water influx. In enclosure trials, water flux from different individuals varied by 200%, and sequential measurements on the same individuals varied up to 160%. Water influx of chicks in the field was approximately half that measured in enclosures. Water efflux was also greatly reduced in the field compared to enclosure values.

Table 8.3 Water efflux and influx of Malleefowl chicks during January and February 1984.

Chick No.	Duration (Days)	Mass (g) Initial	Mass (g) Final	Age (days) Initial	Age (days) Final	Efflux ($ml.kg^{-1}.d^{-1}$)	Influx ($ml.kg^{-1}.d^{-1}$)
4543	11	119	142	14	26	114.5	124.7
4543	18	142	187	26	44	103.0	113.6
4544	11	124	134	1	13	61.7	66.4
4544	18	134	178	13	31	94.1	105.7
4545	15	111	116	0	15	103.7	105.9
4545	11	116	131	15	26	137.7	145.5
4546	7	112	117	1	8	59.2	64.6
4546	11	117	126	8	19	62.1	65.3
4547	14	101	115	1	15	101.4	106.9
4547*	4	115	107	15	19	80.5	56.3
4548	9	88	101	2	11	112.0	126.7
4550	5	100	96	0	5	100	94.0
4550*	4	96	93	5	9	54.5	48.2

* Indicates chicks measured in the field

8.3.3 Water flux and body water of captive adult Malleefowl

Sexes of captive birds were unknown, but the greater weight of 3524 suggest that this bird was a male and the others females.

Body mass of birds 3514, 3518, and 3524 remained relatively constant throughout the study (Figs. 8.2 - 8.5). A sudden, unexpected large increase followed soon after by a decrease in body mass occurred in bird 3525 in late Feburary 1984 (Fig. 8.5). There was also a corresonding large decrease in percentage water content of this individual at this time.

Water efflux and influx values were almost identical throughout the monitored period (Figs 8.2, 8.3, 8.4, 8.5) indicating that birds were in water balance. Over the last 16 days of monitoring, drinking water was removed which resulted in some mass loss and reduced water flux in all four birds (Figs. 8.2 - 8.5). Drinking water accidently ran out in late January 1984 resulting in reduced water flux (Figs. 8.2, 8.3, 8.5). Bird 3524 had a consistently greater water flux compared to other birds, but this was reduced to a level similar to others when drinking water was deprived (Table 8.4).

Table 8.4. Mean water efflux ($ml.kg^{-1}.d^{-1}$) of captive adult Malleefowl.

Bird No.	Efflux water *ad libitum*	95% confidence interval	Efflux water deprived
3514	28.7	± 2.9	19.5
3518	25.4	± 2.0	19.1
3524	49.7	± 6.0	23.7
3525	24.8	± 2.6	19.2
Mean except 3524	26.3	± 1.5	19.3

Percentage water content of captive birds did not vary in any regular temporal manner (Figs. 8.2, 8.3, 8.4, 8.5). Mean percentage water content was not significantly different among birds and the overall mean during the study was 65.4% (Table 8.5).

8.3.4 Water flux and body water of free-ranging adult Malleefowl

Water efflux and influx were similar for free-ranging adult birds, so only values of water efflux are presented (Figs. 8.6, 8.7). Three females (4530, 4537, 4540) and one male (3529) were monitored for varying lengths of time. Monitoring ceased when birds were killed by

Table 8.5. Mean percentage water content of captive adult Malleefowl.

Bird No.	Number of times estimated	Mean water content (%)	95% confidence interval
3514	10	64.6	± 1.8
3518	10	66.1	± 1.6
3524	10	65.3	± 1.2
3525	8	65.4	± 2.1
Mean		65.4	± 1.0

foxes, but in two cases (3529, 4530, Fig. 8.6) birds were followed for 12 months before they were eaten. In the case of 4530 the carcass was found within an hour of death on the last field trip, enabling water distilled from muscle tissue to be used for estimating water efflux.

At the time of first capture all birds were in breeding condition. The male bird (3529) maintained body weight throughout the 12 month monitoring period (Fig. 8.6), but the three female birds lost mass by the end of the breeding season (Feburary, Figs. 8.6, 8.7). Bird 4530, the only female followed for 12 months, began gaining mass again in May and reached peak mass in September which was the beginning of the next breeding season, although this bird did not appear to breed.

In all wild birds percentage body water content did not vary in any temporally regular manner (Figs. 8.6, 8.7). Percentage body water contents of wild birds were not significantly different from each other, but were significantly greater than captive birds ($P < 0.001$, analysis of variance), averaging 67.4% (Table 8.6).

Water efflux was consistently low from November 1983 to August

1984 in birds 3529 and 4530, but increased greatly in September 1984 before decreasing again in October and November 1984 (Fig. 8.6). Water efflux was consistent and low in bird 4537 from January 1984 to June 1984, while it was quite variable and relatively high in bird 4540 (Fig. 8.7). Mean water efflux of free-ranging adult birds (49.0 $ml.kg^{-1}.d^{-1}$, Table 8.7) was significantly higher ($P < 0.001$, t-test for unequal sample sizes, Simpson et. al. 1960 p. 176) than the mean for non-drinker laboratory birds (26.3 $ml.kg^{-1}.d^{-1}$, Table 8.4).

Table 8.6. Mean percentage water content of free-ranging adult Malleefowl.

Bird No.	Sex	Number of estimates	Mean water content (%)	95% confidence interval
3528	F	1	67.4	
4530	F	9	66.7	± 2.8
4537	F	5	66.6	± 2.0
4539	M	1	67.7	
4540	F	4	68.0	± 1.6
4542	F	1	66.9	
3529	M	10	69.0	± 1.4
Mean			67.6	± 0.9

Table 8.7 Mean water efflux of free-ranging adult Malleefowl

Bird No.	Sex	Number of estimates	Mean water efflux ($ml.kg^{-1}.d^{-1}$)	95% confidence interval
4530	F	9	54.4	± 17.6
4537	F	4	32.8	± 1.9
4540	F	3	41.4	± 22.6
3529	M	9	53.3	± 17.2
Mean		25	49.0	± 8.5

8.3.5 Relationship between body water content and body mass

As TBW was determined up to ten times in each individual bird, and the birds' body mass varied throughout the study, regression analysis of body mass on % TBW was performed to see if the two variables were related (Figs 8.8, 8.9, 8.10). In all laboratory birds the regressions were significant (Figs. 8.8, 8.9), % TBW being negatively correlated with body mass, but the regressions for the two field birds for which enough data were available were not significant (Fig. 8.10).

8.4 DISCUSSION

8.4.1 Justification of HTO technique

Ideally the use of tritium labeled water to estimate water content and flux should be experimentally verified by sacrificing birds and dehydrating carcasses in the case of water content, and simultaneous measurement of water and food consumption in the case of water flux. This is not possible for Malleefowl as they are difficult to obtain, and do not feed properly when confined to small cages needed for direct water budget measurements. However, the system has been verified several times for mammals (Nagy and Costa 1980), and birds (Degen et. al. 1981, Williams and Nagy 1984, Williams 1985).

In mammals HTO estimates of water volume usually overestimate water volume measured by dehydration by 1 to 6%, probably due to incorporation of tritium into tissues and the turnover of tritium between the times of injection and initial sampling (Nagy and Costa 1980). HTO dilution space averages 72% of body mass in six 0 - 2 day-old Malleefowl chicks which is 4% greater than the water space determined by dehydration (68%, N = 3, Vleck et. al. 1984), but the difference is not significant ($0.05 < P < 0.1$, t-test for unequal sample sizes, Simpson et. al. 1960, p. 176). Body water calculated by tritiated water dilution overestimates body water by 2.5% in pigeons Columba livia (Siri and Evers 1962), 15% in Domestic fowl Gallus domesticus (Farrell and Balnave 1977); the differences ranges between -2.1 and 3.2% in Chukar partridges Alectoris chukar (Degen et. al. 1981) and between - 0.7 and 5.4% in Sparrows (Williams 1985).

When estimating water turnover by monitoring the rate of tritium

activity loss it is assumed that body water is a single exchangable pool, and that exchange with extraneous water occurs at a constant rate. Neither of these assumptions is strictly true. Several water pools which differ in exchange rates exist within the body (Streit 1982), and exchange with extraneous water is not constant (Nagy and Costa 1980). However the errors involved in ignoring these problems are relatively small. For example, in mammals errors are usually less than 4% (Nagy and Costa 1980). In Chukar partridges and Sand Partridges Ammoperdix heyi, water flux estimates of HTO washout are between 90.7% and 113.3% of measured water flux (Degen et. al. 1981).

8.4.2 Water flux and body water of chicks

Water flux of chicks maintained in enclosures varies considerably (Table 8.3). Two of the chicks (4543, 4546) maintained relative constant water flux rates over two separate measurement periods while in enclosures, whereas two others (4544, 4545) increased their water flux considerably over a second measurement period. Apparently these two chicks increased their drinking rate during the second period. Minimum water influx for growing Malleefowl is probably about 65 $ml.kg^{-1}.d^{-1}$ as chicks can gain mass with influxes of this magnitude (4544, 4546), while chicks in the field lose mass at influxes around 50 - 55 $ml.kg^{-1}.d^{-1}$, although differences in food quality and/or quantity may also contribute to mass loss of field chicks.

Chick 4550 was the only chick which did not gain mass while being kept in an enclosure. This probably results from its young age because Malleefowl chicks gain little or no mass in the week following hatching (Booth, unpublished data). In all chicks gaining mass, water influx exceeds water efflux because total body water increases as new tissue is

synthesized.

The two chicks in the wild (4547, 4550) had greatly reduced water influxes compared to when they were in enclosures, presumably because they did not have access to drinking water. An estimate of water influx due to oxidative water production in wild chicks can be calculated assuming: (1) chicks do not feed, (2) that their average metabolic rate is three times standard (2.44 $ml.g^{-1}.h^{-1}$, this is probably an overestimate as each time chicks were tracked down they were found sitting quitely in the shade of low bushes), and (3) that lipid is the substate being catabolized. Oxidation of 1 g of lipid yields 1.07 g of water and requires 1,945 ml of oxygen (Peters and Van Slyke 1946). For a 100 g Malleefowl chick the amount of oxidative water produced per day is:

$$\frac{2.44 \times 100 \times 24 \times 1.07}{1945} = 3.22 \text{ ml}$$

Hence, water influx due to catabolism is 32.2 $ml.kg^{-1}.d^{-1}$. As humidity was relatively low during the field trial it is unlikely that a significant quantity of extraneous water entered the body via pulmocutaneous avenues. Because the measured water influx of free-ranging chicks (67.5 $ml.kg^{-1}.d^{-1}$) is higher than that calculated for a theoretical non-feeding chick, free-ranging chicks must have consumed some food during the field trial.

Predicted water turnover for a 100 g bird is 190 $ml.kg^{-1}.d^{-1}$ (Dawson et. al. 1983) which is considerably greater than any of the measurements obtained for Malleefowl chicks. Malleefowl chicks are exceptional because they are able to gain mass with water turnovers about one third of the predicted value. Such low water turnover rates indicate that selection for physiological adaptations restricting water

turnover has taken place, presumably because Malleefowl inhabit arid environments.

Body water of chicks averaged 72.9% of body mass (Table 8.2) which is significantly higher ($P < 0.001$, analysis of variance) than adult birds (captive 65.4%, free-ranging 67.6%, Tables 8.4, 8.5), a finding consistent with other hatchling birds (Medway and Kare 1957, Ricklefs 1967, Ricklefs and White 1981). The higher water content of hatchling birds compared to adult birds is attributed mainly to the higher water content of hatchling bird integument as the quills of developing feathers consist primarily of water, but as feathers grow and mature their water content decreases (Ricklefs 1974).

8.4.3 Water flux and body water of adult birds

Water fluxes of three captive birds (3514, 3518, 3525) are similar throughout the study period, but that of 3524 is considerably higher (Table 8.5). However, when drinking water is removed water flux of 3524 is similar to the other birds, suggesting that this bird may be called a 'drinker'. In studies on captive birds certain individuals occasionally drink far more water than others (Bartholomew and Cade 1963). When water deprived, water flux of 'non-drinker' Malleefowl decreases by only 7 $ml.kg^{-1}.d^{-1}$ (Table 8.5) indicating that these birds do not drink much water even when it is available. Predicted water turnover for a 1600 g bird is 91 $ml.kg^{-1}.d^{-1}$ (Dawson *et. al.* 1983). Captive 'non-drinker' Malleefowl water turnover averages 26.3 $ml.kg^{-1}.d^{-1}$ (Table 8.5) which is significantly less than the predicted value ($P < 0.05$, confidence interval test, Simpson *et. al.* 1960, p. 238) indicating these birds are well adapted to an arid environment. Relatively low water turnover is indicated in arid inhabiting birds (Degen *et. al.*

1981) and mammals (MacFarlane and Howard 1972, Nicol 1978). However, allometric prediction of water turnover in birds is still tentative because of the small number of species for which data are available (Dawson et. al. 1983), and the fact that the experimental conditions under which measurements are made strongly influence the outcome (Table 8.8).

Table 8.8. Body water content and water turnover of birds determined with HTO.

Species	Mass (g)	Conditions of measurement	Water content (%)	Turnover ($ml.kg^{-1}.d^{-1}$)	Source
Domestic fowl Gallus domesticus	1694	F* caged	62.0	125.2	1
	2600	M* caged	64.1	61.0	1
	3490	F caged	54.1	64	2
	5090	M caged	64.3	73	2
	3440	F caged, laying	61.6	121.6	2
	4900	M caged	70.9	72.2	2
	3530	F caged, laying	63.8	125.3	2
	4640	M caged	66.6	60.1	2
Road runner Geococcyx californianus	277	F caged	56.0 - 65.0	165.3	3
	308	M caged	72.0 - 55.0	129.4	3
	271	F caged, no water, mice	60.5	79.3	3
	299	m caged, no water, mice	66.0	90.6	3
Japanese quail Coturnix coturnix japonica	117	F caged	61.8	201	4
	105	M caged	66.5	220	4
Burrowing owl Speotyto cunicularis hypogaea	140	caged	41.9	73	4
Northern petz conure Aratinga canicularis	300	caged	64.2	56	4
	325	caged	59.4	58	4
Vulturine fish eagle Gypohierax angolensis	1590	caged	70.7	84	4
Snowy plover Charadrius alexandrinus	33.7	caged, 25 C	69.6	433	5
	32.2	caged, 40 C	72.8	485	5

Table 8.8 continued

Semipalmated	24.3	caged, 25 C	58.5	545	5
sandpiper	22.5	caged, 40 C	55.9	566	5
Calidris pusillus					
Killdeer	71.1	caged	67.2	463	5
Charadrius vociferus					
Glaucous-winged	763	caged, fresh water	81.3	64	6
gulls	817	caged, sea water	76.9	64	6
Larus glaucescens					
Purple martin	47.7	F Wild, nesting	66.5	249.4	7
Progre subis	50.3	M wild, nesting	64.5	212.2	7
Domestic duck	3130	caged, catherterized	61.6	233.5	8
Anas platyrhnchos					
Zebra finch	13.4	caged, hydrated	63.0	346.3	9
Poephila guttata					
Phainopepla	22.7	wild		945	10
Phainopeplas nitens					
Chukar	378	caged	64.4 -74.9		11
partridges	403.5	caged		57.5	11
Alectoris	388.6	caged, dry food, water	69.2	70.7	12
chukar	353.8	caged, dry food, greens	75.5	90.5	12
	420	caged, dry food, water	67.6	72.4	13
	436	caged, dry food, greens	65.6	50.2	13
	405	caged, dry food, greens water	64.4	59.7	13
	318	greens only	68.1	103.1	13
	490	F wild	67.8	144.1	14
	412.7	M wild	69.0	182.0	14
	444.5	wild, summer	67.4	100.6	15
Sand partridge	159	caged		88.8	11
Ammoperdix heyi	173.1	wild, summer	69.8	122.5	15
Silver eye	10.1	caged		2000	16
Zosterops	9.5	wild, spring	78.3	1459	16
lateralis	10.2	wild, summer	77.6	2188	16
	9.2	wild, summer, vineyard	69.4	718	16
White-browed	11.4	wild		820	16
scrubwren *Sericornis frontalis*					
White-fronted	12.4	wild		620	16
chat *Epthianura albifrons*					

Table 8.8 cont.

Emu	32700	caged, hydrated		60	16
Dromaius	30400	caged, dehydrated		20	16
novaehollandiae	38800	enclosure, hydrated	61.0	44.8	17
	34200	enclosure, dehydrated	61.0	14.8	17
	30200	semi-natural, summer	66.5	54.2	18
	34500	semi-natural, winter	66.3	53.4	18
	40200	pre-incubating adult	61.0	68.4	18
	2500	3-4 week old chicks	71.3	285	18
	6600	4-5 month old chicks	68.8	193	18
Ostrich	95400	enclosure, water	68.0	86.3	19
Struthio	80700	enclosure, no water		6.8	19
camelus					
Malleefowl	123	chicks, caged	72.9	101.8	20
Leipoa ocellata	103	chicks, wild		52.3	20
	1634	caged, water	65.4	32.2	20
	1650	caged, no water		20.4	20
	1818	wild	67.6	49.0	20

* F = female, M = male

References for Table 8.7.

1 = Chapman and Black 1967
2 = Chapman and Mihai 1972
3 = Ohmart et. al. 1970
4 = Chapman and McFarland 1971
5 = Purdue and Haines 1977
6 = Walter and Hughes 1978
7 = Utter and LeFebvre 1973
8 = Thomas and Phillips 1975
9 = Skadhauge and Bradshaw 1974
10 = Weathers and Nagy 1980
11 = Degen et. al. 1981
12 = Degen et. al. 1982
13 = Degen et. al. 1984
14 = Alkon et. al. 1982
15 = Degen et. al. 1983
16 = Rooke et. al. 1983
17 = Dawson et. al. 1983
18 = Dawson et. al. 1984
19 = Withers 1983
20 = This study

Water turnover values of adult Malleefowl in the field are consistently higher than those in the enclosure (Figs. 8.2 - 8.7, Tables 8.4, 8.7), a finding consistent with other studies (Alkon et. al. 1982, Fig. 8.11). The allometric relationship for laboratory measurements from 18 avian species ranging in body mass from 10 g to 95400 g (Table 8.8) is:

$$\text{Log}_{10}(\text{turnover ml.d}^{-1}) = \text{Log}_{10}(-0.066) + 0.666\text{Log}_{10}(\text{body mass g}) \quad (8.7)$$

$$r^2 = 0.83, \quad P < 0.001$$

and for field measurements from 9 species ranging in body mass from 10 g to 34500 g (Table 8.8) is:

$$\text{Log}_{10}(\text{turnover ml.d}^{-1}) = \text{Log}_{10}(0.125) + 0.681\text{Log}_{10}(\text{body mass g}) \quad (8.8)$$

$$r^2 = 0.88, \quad P < 0.001$$

The regression coefficients (slopes) of equations (8.7) and (8.8) are statistically indistinguishable and are similar to previously published values for birds (Ohmart et. al. 1970, Walter and Hughes 1978, Degen et. al. 1982, Dawson et. al. 1983), but the y-intercepts are significantly different ($P < 0.001$). Field measured water turnovers are probably greater than laboratory ones because the greater activity of free-ranging birds increases their metabolic rate and, hence, their food and water consumption.

Other factors that influence water turnover include rainfall, temperature, food type and availability, behaviour, and reproductive condition (Degen 1977, Grubbs 1980, Withers et. al. 1980, Alkon et. al. 1982). Rain may increase water turnover by wetting food items and therefore increase incidental water intake. Temperature may increase water turnover in two separate ways. At low temperatures birds may have to increase their metabolic rate to maintain body temperature. This increases metabolic water production, and necessitates an increase in food consumption which further increases water intake. On the other hand, an increased water turnover may be caused by the need to increase evaporative water loss in order to maintain body temperature at high ambient temperatures.

Mean air temperature and daily rainfall rate were compared to daily water turnover rate in field birds by multiple regression analysis but no statistically significant relationship was found. Despite seasonal changes in air temperature, water turnover of birds in enclosures remains relatively constant when water is available ad

libitum. These findings suggest that other factors such as diet, activity, and behaviour have a more important influence on water turnover in Malleefowl than air temperature or rainfall. Water turnover does not increase in Desert chukars Alectoris chuckar sinaica during rainy periods, but increases shortly thereafter when green vegetation becomes available (Alkon et. al. 1982).

A conspicuous increase in water turnover occurs in both the female and male free-ranging Malleefowl during August - September 1984 (Fig. 8.6) which coincides with the beginning of the 1984-85 breeding season, suggesting either a switch to a diet of higher water content or an increase in activity due to the onset of breeding at this time. The diet of Malleefowl is not well known, but they eat seeds, small herbs, shoots, and any invertebrates found while scratching in ground litter material (Frith 1962a). An increase in the proportion of herbs and shoots would elevate water turnover. A switch in diet from primarily seeds to green vegetation increases water turnover in Desert chukars because nutrients are less concentrated and the water content higher in green vegetation than in seeds (Alkon et. al. 1982). Egg laying doubles water turnover in Domestic fowl Gallus domesticus (Chapman and Mihai 1972) and an increase in water turnover may be expected in breeding Malleefowl. However, because water turnover of the male Malleefowl also increases, the increase in water turnover is not substained for more than a month, and neither of the monitored birds appeared to breed in the 1984-85 breeding season, it seems unlikely that the observed increase in water turnover was due to breeding activity per se. Never the less an increase in water turnover also occurs over August - September in laboratory birds 3514, 3518, and 3525 (Figs. 8.2, 8.3, 8.5), so the possibility that a photoperiod induced hormonal release from the

pituitary which stimulates metabolism and activity in preparation for breeding (Ricklefts 1974) at this time of year should not be ruled out.

TBW responds to environmental stress and may be a useful indicator of body condition, because elevated TBW reflects a loss of body solids (pricipally fats) indicating poor body condition (Alkon et. al. 1982). TBW is significantly higher in free-ranging Malleefowl than in laboratory maintained birds (Tables 8.5, 8.6), but both are well within the range normally found in birds (Table 8.7). Presumably laboratory birds have a slightly higher fat content than free-ranging birds because they always have access to food and are not as active as free-ranging birds.

Relative TBW of captive adult birds is negatively correlated with body mass (Figs. 8.8, 8.9), indicating that changes in body mass are primarily due to changes in fat content as is expected in non-growing adult birds (Alkon et. al. 1982). This relationship is highlighted in bird 3525 during Janurary - Feburary 1984 where a sudden large increase in body mass is also accompanied by a sudden decrease in relative water content (Fig. 8.4). However, the regressions relating TBW to body mass for two field birds are not significant (Fig. 8.10). The procedure for measuring TBW may obscure a relationship. The tritium dilution technique estimates all interchangable water within the body, including gut contents. A correlation between % TBW and body mass may not be obtained because the water content of food material within the gut may have varied throughout the study.

All field birds were in breeding condition when first caught. All females when next caught towards the end of the 1983-84 breeding

season had ceased egg-laying and lost considerable body mass (Figs. 8.5, 8.6). Mass loss over the breeding season is a common phenomenon among birds (Ricklefs 1974). In the female that was followed until the beginning of the next breeding season, body mass remained low through to May when it began increasing until it reached a peak value in September, the beginning of the 1984-85 breeding season. This pattern suggests that female birds lose condition over the breeding season due to the energetically demanding egg-laying process. Malleefowl lay up to 3 times their own body weight in eggs in a single breeding season (Frith 1959). It would appear that female Malleefowl spend the non-breeding season building up body condition in preparation for the next breeding season.

8.4.4 Conclusion

Malleefowl are the only megapode species to inhabit an arid environment. The other megapodes inhabit jungle or thick scrub environments (Frith 1959b) where water availability is presumably not a problem.

As predicted, Malleefowl have evolved exceptionally low water turnover and this has enable them to exploit arid environments. The physiological and/or anatomical mechanism/s enabling such low water turnover are unknown. In desert rodents which also have low water turnover, behavioural traits such as nocturnal activity and spending the day in underground burrows, and physiological traits such as nasal counter current water conservation and high renal concentrating capacity are all responsible for increased water economy (Grubbs 1980). Most birds are diurnal and have only a moderate renal concentrating capacity, even the most developed avian kidney has poor concentrating capacity compared to arid inhabiting rodents (Skadhauge 1981). However, many

birds posses extra-renal water saving mechanisms in the form of nasal salt glands and/or urine modification in the hindgut (Skadhauge 1981). Malleefowl chicks and adults do not posses nasal salt glands (personal observation), but both have well developed rectal caeca (personal observation). Rectal caeca have been implicated in post-renal modification of urine in other avian species (Skadhauge 1972, 1981, Thomas 1982, Thomas et. al. 1984) and are therefore worthy of further investigation in Malleefowl.

CHAPTER 9 HOME RANGE OF MALLEEFOWL

9.1 INTRODUCTION

Home range is the area over which an animal normally travels in search of food (Burt 1943). Hence in birds, the size of the home range is positively correlated with food density (Schoener 1968). For any species, home range may vary in size in different habitats, at different times of the year, and may overlap to some degree with the home range of adjacent conspecifics. Knowledge of the home range size and the degree of overlap is important for wildlife management because these factors enable an estimate of the potential carrying capacity of an area for a particular species. Once the carrying capacity of habitat type is known appropriate management practices can be implemented. Radio telemetry is particularly useful for the estimation of home range, as once an animal is radio-tagged it may be followed without interference from the observer.

Malleefowl are becoming increasingly rarer, yet their spatial requirements and seasonal movements have been little studied (Brickhill 1983). An estimate of Malleefowl home range in high rainfall mallee near Griffiths, New South Wales can be made from Frith's (1959a) study. He reported that during the breeding season males were never seen more than 100 yds from their breeding mounds, and that females could be found up to 250 yds away. As for movement during the non-breeding season Frith (1959a) stated that the birds "may wander from the area and move relatively long distances, one pair having been seen nearly 400 yds from their mound." Casual observations during field trips early in this study suggested that Malleefowl in low

rainfall Murray mallee 10 km west of Renmark, South Australia, move far greater distances than have previously been indicated, so a study on Malleefowl movement using radio telemetry was commenced.

Two important components of a species' population dynamics are juvenile recruitment and juvenile dispersal (Morris 1984). Nothing is known about the fate of Malleefowl chicks once they leave the incubation mound (Frith 1962a). During this study an attempt was made to follow radio-tagged chicks to gather information on their dispersal distance and survival rates.

9.2 MATERIAL AND METHODS

9.2.1 Radio tracking adult birds

Adult birds were trapped at their incubation mounds in enclosures with a trap door fitted in one side. A 2-stage transmitter weighing 30 g (HLPB-1220-LD, Wildlife Systems Inc. U.S.A.) was attached to the backs of birds with garter-elastic passed around the base of each wing (Fig. 9.1). The transmitter settled underneath the feathers on the bird's back. The flexible whip antenna was parallel to the back and pointed posteriorly. The mounted transmitter appeared not to impair running or flight, nor did it interfere with the mating of birds, as one female laid a fertile egg several days after a transmitter was attached.

Birds were located by triangulation from two radio tracking stations located 1.4 km apart on top of sand dunes. At each station extendable aluminium poles (maximum extension 6.5 m) were used to mount directional antennae. A vertical metal shaft was driven into the ground, and a circular aluminium disc which was marked in 2 degree intervals was clamped to it so that 0 degrees pointed towards magnetic north. The aluminium pole was mounted on top of the disc. A pointer at the base of the pole lined up with the directional antenna on top of the pole enabling bearings to be read from the disc. The whole pole-antenna system could be rotated about the metal mounting shaft. A 3-bar Yagi directional antenna (F151-3FB, Wildlife Materials Inc. U.S.A.) and a 24-channel (150-152 MHZ) tracking receiver (Merlin 12) were used to pick up transmitter signals at station 2 and a 4-bar Yagi directional antenna (AVM Instrument Company, U.S.A.) with a 12-channel (150-151 MHZ) tracking receiver (LA12 AVM Instrument Company, U.S.A.)

used at station 1.

Radio-tagged birds were located by two methods. The first method involved taking simultaneous or near simultaneous bearings on birds from the two tracking stations and calculating the birds' location by triangulation. When two people were available simultaneous bearings were taken. If only one person was available bearings were taken at one station, then at the other as soon as possible after the first (time elapsed between taking bearings rarely exceeded 20 minutes). Direction of a radio-tagged bird from a tracking station was determined by the location of nulls in the signal and averaging the bearing between nulls. Birds could be detected up to 9 km from the tracking station using this system. The second method of location used a portable directional antenna to follow the signal direction until the bird was sighted.

9.2.2 Radio tracking chicks

Malleefowl eggs were collected from mounds and incubated in the laboratory at 34 C in sand taken from mounds (chapter 2). After hatching, chicks were wing-tagged and kept in an outdoor enclosure (chapter 8). They were released at the study site one to sixteen days after hatching. Five radio tagged chicks were released. One-stage transmitters (MPB-1220-LD, Wildlife Material Inc. U.S.A.) weighing 5 g were attached to chicks by two methods. The first method used the garter elastic method described for adults, but this proved to be unsatisfactory (see discussion). The second method involved gluing the transmitter with Cyanobond RS100 (Sumitomo Chemical Co. Ltd., Osaka, Japan) to the skin and feathers between the shoulders on the back of chicks. The low power of chick transmitters did not permit location by

triangulation using tracking stations (maximum distance at which signal could be detected was approximately 500 m), so chicks were located using the portable antenna to follow the signal direction until they were sighted.

9.3 RESULTS

9.3.1 Adult Malleefowl location

A total of eight adult Malleefowl were caught between 25.X.1983 and 11.I.1984 (Table 9.1.). Radio transmitters were attached to six of these birds. Transmitters were not attached to the other two birds for the following reasons: male 4538 from mound 40 escaped before a transmitter could be attached, and male 4539 from mound 26 died during handling. Only three of the six radio-tagged adult birds remained in the study area for more than 6 months. Female 3528 from mound 45 laid one more egg after being captured. She was located on 21.XI.83 9 km from her mound, but was not detected again. Presumably this bird continued to move away from the study site and moved out of the range of the tracking equipment. The transmitter from female 4542 was found on 24.III.84 approximately 1 km from where she was last caught. This bird was probably eaten by a fox as the transmitter casing had fox teeth marks in it and the tip of the whip antenna had been chewed. However, there were no signs of Malleefowl feathers or remains on the ground where the transmitter was found. The transmitter and remains (head, part of sternum, part of pelvic girdle, part of a femur, and many feathers) of female 4540 were found on the ground on 5.VI.1984 approximately 1.5 km from where she was originally caught. She was probably eaten by a fox or feral cat. The signal from female bird 4537 could not be detected after 9.XII.84, and it is not known if the transmitter failed or the bird moved out of range. The carcass of bird 3529 was found on 17.XI.84; the transmitter had fox teeth marks in it. Bird 4530 was killed by a fox during the process of recapture on 17.XI.84.

Table 9.1. Details of adult Malleefowl caught.

Bird No.	Sex	Mound where Caught	Date of Initial Capture	Fate
3528	F	45	25.X.83	Moved out of study area
3529	M	45	25.X.83	Eaten by fox 17.XI.84
4530	F	16	26.X.83	Eaten by fox 17.XI.84
4537	F	40	14.XII.83	Disappeared after 9.VIII.84
4538	M	40	14.XII.83	Escaped before radio tagged
4539	M	26	14.XII.83	Died during handling
4540	F	26	17.XII.83	Eaten by fox or cat 5.VI.84
4542	F	12	11.I.83	Eaten by fox 24.III.84

Area of home range was estimated by drawing the smallest possible convex polygon containing all the locality data (Jennrich and Turner 1969). A compensating planimeter was used to calculate its area (Table 9.2).

Birds moved over areas ranging from 3.9 to 4.6 km^2 during the study (Figs. 9.2 - 9.7; Table 9.2). Only two birds (male 3529 and female 4530) continued to attend their mounds after they had been caught. In these cases the locality data was broken up into two categories, (1) data taken while still working mound (breeding season), and (2) data taken after mounds had been abandoned (non-breeding season). The breeding and non-breeding home ranges of these birds covered different territory (Figs. 9.3 and 9.4, Table 9.2).

Table 3. Home range size of adult Malleefowl in Murray mallee 10 km from Renmark, South Australia.

Bird no.	Sex	season[a]	Home range (km^2)
3529	M	B	2.6
3529	M	NB	2.4
3529	M	B&NB	4.0
4530	F	B	3.7
4530	F	NB	1.7
4530	F	B&NB	3.9
4537	F	NB	4.6
4540[b]	F	NB	1.4

a B = Breeding season, NB = Non-breeding season
b Home range is probably under estimated as the bird was eaten by a predator before sufficient locality data were accumulated.

9.3.2 Chick location and movement

Five radio-tagged chicks were released and attempts made to follow their movements. The first chick was released on the morning of 17.XI.1983 at mound 45. Three days later an intensive search around the point of release failed to detect the chick. The search proceeded by walking in two concentric circles around the point of release, the first with a radius of approximately 500 m and the second with a radius of approximately 1000 m. Every 300 - 400 m an attempt was made to detect the radio signal without success. Therefore, it was assumed the chick was not within 1500 m of the point of release. On the morning of 22.II.1984 four radio-tagged chicks were released at station 1. An

adult transmitter was attached to the largest chick to enable detection over a much greater range (5 - 7 km). The movements of these chicks over the next 4 days were monitored and they did not move more than 500 m from the point of release (Fig. 9.7). On the next visit to the area (12 - 15.III.1984) none of the chicks could be detected.

9.4 DISCUSSION

9.4.1 Home range of adult Malleefowl

The home range of the only male observed is similar in size during the breeding and non-breeding seasons, but these two ranges do not overlap entirely (Fig. 9.3). The shift in home range is probably due to the bird changing mounds during the observation period. In the 1983-84 breeding season mound 45 was worked by this bird, but mound 45 remained unworked during the 1984-85 breeding season. The home range of male 3529 during the breeding season (2.6 km^2) is 80 times larger than previously indicated for male Malleefowl. When discussing the activities of male Malleefowl during the breeding season, Frith (1959a) stated: "...(The male) is seldom absent from the mound for more than 2 hours at a time. The remainder of the time is spent in a camp....always within sight of the mound and usually within a few yards of it. No male has ever been seen more than 100 yds from the mound during the breeding season." Several times in the present study male 3529 was observed to visit the mound in the early morning only, and then wandered off into the scrub. On one occasion he was observed at the mound in the morning and again in the afternoon 1.2 km from the mound.

The two female Malleefowl have larger home ranges than the male (Table 9.2), although the male's total home range (area covered in both breeding and non-breeding seasons) is similar in size to that of females. The breeding season home range of female 4530 is twice the area of her non-breeding home range (Table 9.2). Female Malleefowl possibly cover more territory during the breeding season to gather enough food for the energetically demanding process of egg laying (Frith 1959a). During the non-breeding season a female's territory need not be

as large because her food requirements during this time should be no greater than a male's. A need to meet energy requirements is a reasonable explanation for the change in home range size of females from the breeding season to the non-breeding season, although the female in the present study ceased egg-laying at the time she was radio-tagged. Female 4537 had the largest home range (Table 9.2) and this was recorded during the non-breeding season. In Frith's (1959a) study female Malleefowl were sighted up to 250 yds from their mounds during the breeding season. If 250 m is taken as the maximum distance from a mound that a female Malleefowl moves during the breeding season, the home range is 0.2 km^2 or only 1/20th the size observed in the present study (Table 9.2). During the non-breeding season the birds in Frith's (1959a) study moved further than in the breeding season, being sited up to 400 yds from their mound. All birds observed in the present study moved distances of 1 km or more during and after the breeding season. One bird (Bird 3528) moved a distance of 9 km in 2 weeks before disappearing out of the study area.

At least part of the large discrepancy in home range size between the Malleefowl of Frith's (1959a) study and the present study can be attributed to differences in the quality of the habitat. In Frith's study area located in high rainfall mallee there was a total of 5.5 mounds/km^2 of which 2.8 mounds/km^2 were active during the 1953-54 breeding season. A maximum density of 5.5 active mounds/km^2 was observed in 1958-59, while during a drought the density fell to 2.5 active mounds/km^2. In contrast, the total mound density in my study area was 4.4 mounds/km^2, while the density of active mounds over two breeding seasons was only 1.1 mounds/km^2 (chapter 11) which is less than half the density of active mounds in Frith's study area during a

drought. Over the 1982-83 drought period no Malleefowl managed to breed in my study area (chapter 10). The vegetation description given by Frith for his study area is quite different from my study area, there being more understory shrubs in Frith's area. Because these shrubs are major food sources for Malleefowl (Frith 1959a, 1962a), birds may not have needed to move as far in Frith's study area to meet their food requirements. The average clutch size for birds over six breeding seasons in Frith's study was 18 (range 5 - 33; Frith 1959a), while in the three successful breeding seasons of my study it was only 14 (Chapter 11). Mean egg mass of 844 eggs in Frith's (1959a) study was 187 g while in my study it was 168 g (chapter 11). These factors suggest that food is more difficult to obtain in my study area, necessitating larger home ranges.

The home ranges of birds 3529, 4530 and 4537 which all worked different mounds overlapped each other; in fact the ranges of birds 3529 and 4537 almost completely overlapped (Fig. 9.9). Although males vigorously defend the area immediately around their mound during the breeding season (Frith 1959a) they do not defend their whole home range against conspecifics, a phenomenon common with many other birds (Forsman *et. al.* 1984).

If food availability is the major factor determining home range size, two strategies may be envisaged. The first is that a relatively small area is vigorously defended and the intrusion of other Malleefowl prevented. The second is that a larger area is utilised, but other Malleefowl are tolerated over much of the territory. The strategy chosen probably depends on the amount of energy and time available to defend a territory as presumbably larger territories require greater

time and energy to maintain. Because male Malleefowl spend large amounts of time maintaining their breeding mounds, and females spend most of their time during the breeding season feeding (Frith 1959a), it is probably not feasible for them to defend an area against conspecifics, so a larger, undefended home range is utilized.

9.4.2 Malleefowl chick movement

Because of the relatively short range of the transmitters used on chicks, little useful information was gained from this part of the study. After the first trial in which the chick could not be located just 3 days after release, I decided it would be best to change the method of transmitter attachment, because if the chick was still alive the elastic attachment of the transmitter would interfere with the chick's growth. Gluing the transmitters directly to the chick's back avoided this problem, but the transmitters were easily dislodged. One transmitter fell off after 2 days during trial 2. When chicks were tracked down during trial 2, they were usually found sitting quietly under a low shrub and were difficult to detect visually. When disturbed they would fly 20 - 50 m approximately 2 m off the ground and then run 50 - 100 m before crouching on the ground under a bush or at the base of a mallee tree. During the first 4 days of the second trial none of the chicks moved very far (Fig. 9.8), yet none of them could be detected on the next trip 15 days later. One of these chicks was wearing an adult transmitter which had a much greater range (5 - 7 km). Either the transmitter failed, the chick moved a long distance, or the transmitter was damaged when the chick was eaten by a predator. The most probable alternative was fox predation because transmitters were extremely reliable, and chicks did not move great distances during the first four days of the trial. The loss of signal from the other smaller

transmitters may also have been caused by predator damage.

9.4.3 Fox predation of adult Malleefowl

An unexpected result was the high degree of fox predation on adult birds. At least four out of the six radio-tagged adults were taken by foxes (Table 9.1). Previously it was thought that fox predation of Malleefowl eggs and probably of chicks was high, but predation of adults low (Frith 1959a). Handling of birds when taking blood samples may have caused the death of birds and foxes picked up and consumed the carcasses. This scenario seems unlikely because four captive birds treated in the same way once a month for over 12 months showed no sign of ill effect, and two of the wild birds (3529, 4530) were caught 9 times over 12 months before they were taken by foxes. Bird 4530 was taken by a fox while it was being pursued along the ground by me after I had scared it out of a roosting tree, so its death was certainly due to observer interference.

9.4.4 Conclusion

The home range of Malleefowl in this study was much larger than in Frith's (1959a) study with a breeding pair needing about 4 km^2 of scrub, although home ranges of birds from different mounds overlap to a considerable degree. The difference in home range size between the two studies is probably due to differences in vegetation, and hence food availability.

Fox predation of adult birds was much greater than expected. During the study at least 67% of radio-tagged adult birds were taken by foxes.

CHAPTER 10. EFFECT OF ADDING WATER TO MALLEEFOWL MOUNDS DURING A DROUGHT

10.1 INTRODUCTION

Malleefowl do not breed in drought years (Bennett in Campbell 1901, Mattingley 1908, Lewis 1939, McGilp 1948, Frith 1959a). However, in drought years mound renovation is started but is abandoned at a stage when litter has been piled into the mound crater.

Frith (1959a) suggested that an increase in mound temperature stimulates birds to complete mounds and begin egg-laying. According to this hypothesis, the failure of microbial heat production in drought years prevents the birds from breeding. A second possibility could be that rain influences the abundance of available food and contributes to the success of breeding adults and their hatchlings. It is possible to test the former hypothesis by adding water to renovated mounds during a drought year. The exceptional drought conditions that occurred over much of south-eastern Australia during 1982 provided an opportunity to carry out this test.

10.2 MATERIAL AND METHODS

During 1981, eight active mounds were located at the Renmark study site. In 1982, a drought year, only four of these mounds were worked by the birds. Two of these four mounds had water artifically added to them, the other two mounds were left untouched as controls. Mounds were visited several times over the period of mound preparation in both years. On these visits temperature at various depths within the mound were recorded as previously described (chapter 3), samples of litter material were taken for water analysis, and observations on the type and amount of work done by birds noted. Mean mound temperature was calculated by averaging temperatures from all levels within the mound. Litter samples were sealed in plastic bags and returned to the laboratory where they were placed in pre-weighed crucibles, weighed to the nearest milligram with an electronic balance (Sartorius 1265 MP), dried for 24 h at 70 C in an oven and reweighed. Percentage water content was calculated on a wet weight basis [100(wet wt - dry wt)/wet wt].

On 14 October 1982 15 l of water from a jerry can was added to each of the artifically watered mounds, equivalent to about 2 mm of rain over the entire mound. On 22 October a further 400 l of water (ca 57 mm of rain) was added to each of the watered mounds from a water tanker by means of a small fire-fighting unit. Water was sprayed into the air so that it fell onto the mounds like rain.

Rainfall and air temperature data were obtained from a Commonwealth Burea of Meterology Station located 10 km away at Renmark.

10.3 RESULTS

10.3.1 Rainfall patterns in 1981 and 1982

The pattern and amount of rain was similar in 1981 to those of years (Fig. 10.1). However, in 1982 total rainfall was only 34% of normal amount received, and only 22 mm fell over the critical months of May, June, and July (Fig. 10.1).

10.3.2 Mound renovation in 1981

Mound renovation followed the typical pattern in 1981. Good rainfalls over the months of May, June, and July throughly wetted the litter material in the mound crater and induced microbial heat production. Mound temperatures increased from July through to October, but there was no obvious relationship between litter water content and mound temperature (Fig. 10.2). All mounds showed a peak water content at the end of July, after which water content gradually dropped until a stable level of about 9% was reached in mid-September (Fig. 10.2). By mid-September an 8 - 10 C gradient existed between mean air and mound temperatures, and mounds reached suitable incubation temperatures (Fig. 10.2). The first eggs appeared in the mounds in mid-September and on 28 October all mounds were covered in sand and contained eggs.

10.3.3 Mound renovation in 1982

In 1982 very little rain fell during the period of mound renovation (Fig. 10.1) and consequently the water content of litter was low (Fig. 10.3). Mounds were first visited in late August when litter was still being piled into the crater. The addition of water on 14 October had little effect on mound temperature (Fig. 10.3). However, the addition of a larger volume of water on 22 October caused mound

temperature to increase dramatically (Fig. 10.3). The increase in water content to levels similar to those in the previous year (cf. Figs. 10.2 and 10.3), immediately triggered microbial respiration and heat production. When the litter dried out in late November and December, microbial heat production ceased and mound temperature once again approached air temperature (Fig. 10.3). The temperature of the two unwatered mounds rose with increasing air temperature, but never greatly exceeded it, and failed to reach apropriate incubation temperatures (Fig. 10.3).

Fresh foot-prints, scratchings, and faeces indicated that the birds continued to visit the two unwatered mounds until about 12 November, but then abandoned the mounds. The two watered mounds were visited and worked by birds until about 6 December, but were abandoned also. Neither watered nor unwatered mounds were covered by sand and no eggs were laid.

10.4 DISCUSSION

Birds responded to mound watering by thoroughly mixing the litter material and constructing egg chambers. The failure of birds to cover the watered mounds with sand and to begin egg-laying despite mounds reaching suitable incubation temperatures for a period of more than one month (a period long enough to stimulate commencement of egg laying, Frith 1959a), indicates that heating of mound material alone does not trigger Malleefowl to complete mounds and start egg-laying.

It is significant that Malleefowl are able to obtain and maintain suitable incubation temperatures in mounds that do not contain any litter at all (Frith 1955, 1956, 1959a). This is possible during summer and autumn when the birds manipulate the sand over mounds to heat it with solar radiation. Solar heat is usually utilized by birds only towards the end of the breeding season, in mid-January, February, and March (Frith 1956, 1957, 1959a). The fine hot weather usually associated with a drought should make operation of an entirely solar heated mound possible. The fact that Malleefowl do not make use of solar heated mounds during droughts suggests that factors other than mound temperature influence egg-laying.

Breeding in a drought year is disadvantageous compared to normal years because of poor primary production and a consequent reduction in food resources (Mattingley 1908, Frith 1959a, Earle 1982). Females may have difficulties in finding enough food to form the very large energy rich eggs they lay, and chicks hatching at the end of summer may starve (Frith 1959a). Breeding in birds requires a large energy investment (Ricklefs 1974, Earle 1982), especially in a species like Malleefowl

which produce an abnormally large mass of eggs each season (Lack 1968) and work on a mound for several months (Frith 1959a). Female Malleefowl lose considerable mass over the breeding season (chapter 8) indicating that egg production is a considerable energetic burden. It is of selective advantage if birds can anticipate the environment at the time when chicks will hatch and avoid egg-laying if conditions are likely to be unfavourable. This may be difficult in Malleefowl because of the two month incubation period. Therefore the cue for egg-laying is more likely to be certain aspects of the environment that are related to seasonal climatic patterns rather than to mound temperature alone.

CHAPTER 11 MOUND DENSITY AND HATCHING SUCCESS OF MALLEEFOWL OVER THE STUDY PERIOD

11.1 INTRODUCTION

A study on several aspects of Malleefowl biology near Griffith in New South Wales provided information on fecundity and breeding success for this species in high rainfall (ca 350 - 400 mm annually) mallee (Frith 1959a, 1962a). Unfortunately, high rainfall mallee is also highly suitable for wheat and sheep farming and consequently little of this country has been left uncleared. Today the majority of remaining Malleefowl populations inhabit low rainfall (ca 220 - 300 mm annually) mallee, land less suited for agricultural purposes. The present study area is located in low rainfall (ca 260 mm annually) mallee near Renmark, South Australia. Information on fecundity and hatching success is presented to provide some data from low rainfall mallee and to compare this with the more extensive data of Frith's (1959a, 1962a) study.

11.2 MATERIAL AND METHODS

11.2.1 Mound survey

In early 1981 most of the study area was systematically surveyed for Malleefowl mounds by walking along compass bearing transects spaced 30 m to 100 m apart depending upon vegetation density and effective visual distance. During 1983 a further section of the study site was surveyed resulting in the location of six more mounds. When a mound was located, its position was mapped. During August, September and October of each year the previously located mounds were visited and checked for signs of mound renovation and breeding activity.

11.2.2 Fecundity and hatching success

During the breeding seasons of 1981-82, 1983-84 and 1984-85 active mounds were visited once every two to four weeks. Mounds were dug out and eggs removed for weighing with a spring balance (Salter Super Sampson 200 g). Any new eggs were marked with a number using a HB graphite pencil. After weighing and marking, eggs were returned to the egg chamber and the mound re-built. Interfering with mounds did not cause birds to abandon their breeding efforts. On several occasions parent birds were in the vicinity when mounds were checked for eggs, and on most occasions as soon as I moved away from the mound the parent bird moved in and re-built the mound.

Successful hatching of eggs was evident from eggshell fragments and pieces of shell membrane where the eggs had been incubating. Eggs broken during the course of incubation were easily detected by smell, and chicks which broke the eggshell but failed to escape at hatching were also obvious. Eggs which remained in the mounds for more than

three months were opened and examined for signs of embryonic development. If no sign of development was detected the eggs were classified as infertile. Several eggs were accidently broken during the study while i dug out mounds. During the study approximately sixty eggs were collected for laboratory experiments. Ninety percent of the fertile eggs collected for the laboratory were classified as successful hatchings because about 10% of naturally incubated fertile eggs fail to hatch (see results).

11.2.3 Rainfall patterns

Monthly rainfall data for the duration of the study were obtained from a Commonwealth Bureau of Meterology Station located 10 km from the study site at Renmark, South Australia.

11.3 RESULTS

11.3.1 Mound survey

A total of 47 Malleefowl mounds were located within the 10.7 km^2 study area (Fig. 11.1), but only a few of these were active in any breeding season (Table 11.1). One new mound was subsequently found after the area had been surveyed, so a few mounds within the study area may not have been located. Sixteen mounds were used over the three successful breeding seasons; five mounds were used in more than one breeding season (Table 11.1). Mounds did not appear to be associated with soil type, vegetation type or aspect. Active mounds were located in light and heavy sands, in several vegetation associations, and on top of and between sand dunes.

11.3.2 Fecundity and hatching success

Twenty-two clutches containing a total of 289 eggs were followed and the fate of the eggs recorded (Table 11.2). Clutch sizes varied from two to thirty-four over the entire study period (Table 11.3). The clutches laid in the 1984-85 breeding season contain significantly ($P < 0.005$, analysis of variance) more eggs than either the 1981-82 or 1983-84 breeding seasons. Over the three seasons egg mass varied between 92 g and 202 g, the mean mass being 168 g (Table 11.3, Fig. 11.2). Mean egg mass varied between clutches in any one breeding season, and was different for each season (Table 11.3, Fig. 11.2).

Table 11.1 Active Malleefowl breeding mounds in the study area over the years 1981 - 1984.

Breeding Season			
1981-82	1982-83[a]	1983-84	1984-85
Mound No.	Mound No.	Mound No.	Mound No.
1	12	12	4[c]
5	16	16	35
12	38	17	42
16	45	25	44
24		26	46
33		35	
38		40[b]	
45		44[b]	
		45	
		46[b]	

[a] Mounds were prepared by birds, but no breeding occurred.
[b] Mounds were not found until the 1983-84 breeding season.
[c] Mound not monitored throughout breeding season.

Table 11.2 Fate of Malleefowl eggs over 3 successful breeding seasons

Breeding season	Number of clutches	Number of eggs	Fate (%) Hatched	Infertile	Died in shell	Broken by birds	Broken by me
1981-82	8	94	77.7	7.5	7.5	2.1	5.3
1983-84[a]	10	85	70.6	8.2	15.3	2.4	3.5
1984-5	4	110	87.3	2.7	8.2	0.9	0.9
Total	22	289	79.2	5.9	10.0	1.7	3.1

[a] Data from mound 44 excluded as this mound was probably robbed by humans.

Table 11.3 Clutch size and egg mass from three breeding seasons

Mound No.	1981-82 Clutch size	1981-82 Mean egg mass (g)	1981-82 Range	1983-84 Clutch size	1983-84 Mean egg mass (g)	1983-84 Range	1984-85 Clutch size	1984-85 Mean egg mass (g)	1984-85 Range
1	17	174 ± 3[a]	164-186						
5	16	172 ± 5	155-182						
12	11	182 ± 7	166-194	14	186 ± 5	172-202			
16	8	178 ± 9	162-189	6	177 ± 7	171-185			
17				8	164 ± 13	135-178			
24	3	157	153-161						
25				15	158 ± 7	119-167			
26				11	166 ± 9	134-188			
33	23	178 ± 4	162-195						
35				13	159 ± 4	143-166	29	167 ± 3	151-181
38	3	186	174-193						
40				8	170 ± 7	156-181			
42							34	153 ± 5	92-173
44				2[b]	162	158-166	18	176 ± 3	161-185
45	13	160 ± 3	152-173	8	167 ± 2	162-172			
46				2	165	163-167	29	169 ± 3	146-183
Mean	12 ± 6	174 ± 2	152-195	9 ± 3	167 ± 3	119-202	28±11	164 ± 3	92-185
Mean for 3 seasons				13.8 ± 4.1	168 ± 2	92-202			

[a] 95% confidence interval of the mean.

[b] This mound was ignored in clutch size analysis as eggs had probably been taken by humans.

11.3.3 Rainfall patterns

Long term and yearly rainfall patterns over the four years of the study are presented in figure 11.3.

11.4 DISCUSSION

11.4.1 Mound density

Malleefowl mound density in the study area was 4.4 mounds.km^{-2}, but only 1.1 mounds.km^{-2} were active in 1981-82 and 1983-84. In the 1984-85 breeding season active mound density was even lower being 0.6 mounds.km^{-2}. No breeding occurred during the drought over the 1982-83 breeding season. In contrast mound density at a site near Griffith, New South Wales had a mound density of 5.5 mounds.km^{-2}. Maximum active mound density at this site was 5.5 mounds.km^{-2}, and the minimum which occurred during a drought year was 2.5 mounds.km^{-2} (Frith 1959a). Clearly the breeding pair density (as indicated by active mound density) is different between the two study areas. The difference can probably be explained by differences in rainfall and vegetation type. Malleefowl density depends on the availability of food which in turn is dependent on the type and density of vegetation (Frith 1959a, 1962a). Malleefowl feed extensively on the seeds of shrubs found in their habitat; the legumes Acacia sp., and Cassia sp., are the most important food species (Frith 1959a, 1962a). In mallee country the density of the shrub understory depends primarily on rainfall (Frith 1962a), and because my study site has an average rainfall of only 264 mm (Fig. 11.3) compared to 386 mm of the Griffith study site (Frith 1959a) it is not surprising to find correlations in vegetation and Malleefowl mound density. Frith (1962a) classified mallee vegetation into 5 classes, each of which has a characteristic vegetation association and Malleefowl breeding density. The Griffith study site (Class II mallee) has the highest active mound density (5.3 mounds.km^{-2}), whereas my study site (Class V mallee) has the lowest mound density (1.5 mounds.km^{-2}; Frith 1962a). To highlight the differences in the two study sites even further, the rainfall which

was classified as a drought in the 1957-58 Griffith breeding season (234 mm; Frith 1959a, 1962a) is only 34 mm below the average for my study area.

11.4.2 Egg mass, clutch size and hatching success

Clutch size varied considerably over the study period (Table 11.3), a finding consistent with Frith's (1959a) study. Clutch size in Malleefowl is determined chiefly by the egg laying rate of the female, which in turn depends upon the amount of available food (Frith 1959a, 1962a). Small clutches indicate a shortage of food, and large clutches an abundance. Mean clutch size was similar in 1981-82 and 1983-84, but was significantly greater ($P < 0.005$, analysis of variance) in 1984-85 (Table 11.3), indicating greater food availability in that season. An indication of the amount of energy available to females for breeding in any particular season is the total mass of eggs laid. The average mass of eggs in a clutch in 1981-82 was 2042 g, 1585 g in 1983-84 and 4529 g in 1984-85. Hence food was probably much more abundant over the 1984-85 breeding season compared to the other two seasons. Mean egg mass was significantly smaller ($P < 0.005$, analysis of variance) in 1984-85 compared to the other two breeding seasons (Table 11.3), but this may be a consequence of the smaller number of clutches examined in 1984-85.

Obviously the timing of rainfall plays an important part in determining the seed set in Malleefowl food plants as the total rainfall in 1984 was less than either 1981 or 1982 (Fig. 11.3), yet food availability as indicated by egg production was greatest in 1984-85. Good rains over the summer of 1983-84 and follow up rains in July, August and September of 1984 (Fig. 11.3) probably created excellent conditions for seed set and plant growth in 1984.

Egg size is genetically determined and relatively constant among avian species (Lack 1968). If food supplies to the laying female are scarce during the breeding season, she normally reduces the clutch size or does not breed at all; rarely if at all is egg size reduced (Lack 1968).

Average clutch size in the present study was smaller than the average clutch size in Frith's (1959a) study, but the large variation in both studies meant that the difference was not significant (Table 11.4). However the mean egg mass was significantly smaller in the present study (Table 11.4). The smaller egg mass can not be explained by differences in female mass as the weight of female birds in both studies were similar (Table 11.4). Food availability may explain the difference in egg mass despite Lack's (1968) assertion that egg mass is unaffected by food supply. Evidence from active mound densities in the present and Frith's (1959a) study, and from the vegetation descriptions of the two study areas suggests that Mallee food plants, and hence Malleefowl food was more abundant in Frith's study area. It should be noted, however, that the smallest mean egg mass in the present study occurred in the 1984-85 breeding season when clutch size was greatest. In Frith's study egg mass was smallest in years when clutch size was smallest suggesting that in these years food was more difficult to obtain.

The most unexpected finding of this study was no predation of eggs by foxes (Table 11.2) despite an abundance of foxes within the study area. In only one mound did eggs disappear (mound 44 in 1983-84) and these were probably taken by humans as there was no sign of broken eggshell in the mound. When foxes take eggs they bite the top of the egg and lick out the egg contents leaving the eggshell *in situ* (Frith

1959a). If foxes do raid a mound they eat every egg within it. Several infertile eggs remained in mounds of the present study for the entire breeding season, confirming that fox predation of eggs was absent. In contrast, 37% of all eggs laid were taken by foxes at Griffith (Frith 1959a, 1962a). The only large difference between egg fate of my study and Frith's (1959b) study occur between the number of eggs which hatched and the number of eggs taken by foxes (Table 11.5). The greater hatching success in the present study can be accounted for entirely by the lack of egg predation.

Foxes were present in my study area. They were frequently seen at night, and their footprints and faeces were often present at Malleefowl mounds. The difference in the predation rate of eggs may be explained by the way mounds were maintained in the present study. Even at the very beginning and ends of the breeding season mounds were built up high, there usually being at least 40 cm of sand over the top of eggs. The loose dry nature of the sand makes it difficult for foxes to dig down to the eggs. The only way foxes could obtain eggs at my study site would be to surprise adult birds excavating the mound but this obviously did not happen. In Frith's study, mounds were not built up early and very late in the breeding season and this is the time when almost all predation of eggs occurred. However, when mounds were built up in mid-summer, virtually no egg predation occurred (Frith 1962a).

Malleefowl have a high fecundity (Lack 1968), and their nesting success is also relatively high compared to other avian species. This means that most mortality occurs at the post hatching stage. Most mortality probably occurs within the first few weeks after hatching (Mattingley 1908, Frith 1962b).

Table 11.4. Comparisons of egg mass, clutch size and female body mass between the present and Frith's (1959a) study.

	Present study			Frith's study			Comparison[a]
	Sample size	Range	Mean	Sample size	Range	Mean	
Clutch size	21	2 - 34	13.8	54	5 - 33	17.3	NS
Egg mass (g)	281	92 - 202	168	844	117 - 275[b]	187	$P < 0.001$
Female mass[c]	5	1520-2050	1768	4	1788-1901	1830	NS

[a] Comparisons made using a modified 't' test (Simpson et. al. 1960).

[b] Calculated from data on egg volume (in Frith 1959a) and initial egg density (in Vleck et. al. 1984).

[c] In my study mass of birds at the beginning of the breeding season is used. Females lose mass over the breeding season (chapter 8). The time when females were weighed was not specified in Frith (1959a).

Table 11.5. Comparison of egg fate between the present and Frith's (1959a) study.

	Sample size	Fate (%) Broken	Eaten by foxes	Fail to hatch	Hatched
Present study	289	4.8 (1.7)[a]	0	15.9[b]	79.2
Frith's (1959a) study	1094	1.4	37.2	11.9	49.5

[a] Eggs broken by birds.

[b] Includes infertile eggs and chicks which died while hatching.

LITERATURE CITED

Ackerman, R.A. (1981). Oxygen comsumption by sea turtle (Chelonia caretta) eggs during development. Physiological Zoology 54:316-324.

Ackerman, R.A., and R.C. Seagrave (1984). Parent-egg interactions: Egg temperature and water loss. In G. C. Whittow and H. Rahn (eds). Sea Bird Energetics, Plenum N.Y. (In press).

Ackerman, R.A., G. C. Whittow, C.V. Paganelli, and T.N. Pettit (1980). Oxygen consumption, gas exchange, and growth of embryonic Wedge-tailed shearwaters (Puffinus pacificus chlororhynchus). Physiological Zoology 53:210-221.

Alkon, P.U., B. Pinshow, and A.A. Degen (1982). Seasonal water turnover rates and body water volumes in desert Chukars. CONDOR 84:332-337.

Ar, A., and H. Rahn (1978). Interdependence of gas conductance, incubation length, and weight of the avian egg. Pages 227-236 in J. Piiper (ed). Respiratory functions in birds, adult and embrryonic. Springer-Verlag, Heidelberg.

Ar, A., and H. Rahn (1980). Water in the avian egg: Overall budget of incubation. American Zooligist 20:373-384.

Ar, A., C.V. Paganelli, R.B. Reeves, D.G. Greene, and H. Rahn (1974). The avian egg: Water vapor conductance, shell thickness, and functional pore area. CONDOR 76:153-158.

Aschoff, J. (1981). Thermal conductance in mammals and birds: Its dependence on body size and circadian phase. Comparative Biochemistry and Physiology 69A:611-619.

Aschoff, J., and H. Pohl (1970). Der Ruheumsatz von Vögeln als Funktion der Tageszeit und der Körpergrösse. Journal für Ornithologie 111:38-47.

Ashby. E. (1929). Notes on the unique methods of nidification of the Australian Mallee-fowl (Leipoa ocellata) with original data supplied by Bruce W. Leake R.A.O.U. AUK 46:294-305.

Aulie, A. (1976a). The pectoral muscles and development of thermoregulation in chicks of Willow ptarmigan (Lagopus lagopus). Comparative Biochemistry and Physiology 53A:343-346.

Aulie, A. (1976b). The shivering pattern in an artic (Willow ptarmigan) and a tropical bird (Bantam hen). Comparative Biochemistry and Physiology 53A:347-350.

Aulie, A., and P. Moen. (1975). Metabolic thermoregulatory response in eggs and chicks of Willow ptarmigan (Lagopus lagopus). Comparative Biochemistry and Physiology 51A:605-609.

Bakken, G.S. (1976). A heat transfer analysis of animals: Unifying concepts and the application of metabolic chamber data to field ecology. Journal of Theoretical Biology 60:337-384.

Baltin, S. (1969). Zur biologie und ethologie des Talegalla-Hunhs (Alectura lathami, Gray) unter besonderer berucksichtigung des verhaltens wahrend der brutperiode. Zeitschrift fur Tierpsychologie 26:524-572.

Barott, H.G. (1937). Effect of temperature, humidity, and other factors on the hatch of hen's eggs and on energy metabolism of chick embryos. Technical Bulletin No. 553, United States Department of Agriculture, Washington D.C. 45pp.

Bartholomew, G.A. (1972). The water economy of seed-eating birds that survive without drinking. Proceedings of the XVth International Ornithological Congress. pp 237-254.

Bartholomew, G.A. and T.J. Cade (1963). The water economy of land birds. AUK 80:504-539

Bartholomew, G.A., and W.R. Dawson (1953). Respiratory water loss in some birds of Southeastern United States. Physiological Zoology 26:162-166.

Bartholomew, G.A. and D.L. Goldstein (1984). The energetics of development in a very large altricial bird, the Brown Pelican. Pages 347-357 in R.S. Seymour (ed) Respiration and Metabolism of Embryonic Vertebrates. Junk, Hauge.

Bech, C. (1980). Body temperature, metabolic rate, and insulation in winter and summer acclimated Mute swans (Cygnus olor). Journal of Comparative Physiology 136B:61-66.

Bellchambers, T.P. (1916). Notes on the Mallee Fowl Leipoa ocellata rosinae. South Australian Ornithologist 2:134-140.

Bellchambers, T.P. (1917). Notes on the Mallee Fowl Leipoa ocellata rosiae. South Australian Ornithologist 3:78-81.

Bennett, K.H. (1883). On the habits of the Mallee hen, Leipoa ocellata. Proceedings of the Linnaean Society of New South Wales 8:193-197.

Bernstein, M.H. (1973). Development of thermoregulation in Painted Quail, Excalfactoria chinensis. Comparative Biochemistry and Physiology 44A:355-366.

Bernstein, M.H., and K. Schmidt-Nielsen (1974). Ventilation and oxygen extraction in the crow. Respiration Physiology 21:393-401.

Birchard, G.F., and D.L. Kilgore (1980). Conductance of water vapor in eggs of burrowing and nonburrowing birds: Implications for embryonic gas exchange. Physiological Zoology 53:284-292.

Boersma, P.D., and N.T. Wheelwright (1979). Egg neglect in the Procellariiformes: Reproductive adaptations in the Fork-tailed storm-petrel. CONDOR 81:157-165.

Booth, D.T. (1985). Crop and gizzard contents of two Malleefowl. EMU (in press).

Bradley, S.R., and D.R. Deavers (1980). A re-examination of the relationship between thermal conductance and body weight in mammals. Comparative Biochemistry and Physiology 65A:465-476.

Breitenbach, R.P., and T.S. Baskett. (1967). Ontogeny of thermoregulation in the Mourning dove. Physiological Zoology 40:207-217.

Brickhill, J. (1983). Malleefowl a remarkable bird with an uncertain future. Australian Natural History 21:147-151.

Bucher, T.L. (1981). Oxygen consumption, ventilation and respiratory heat loss in a Parrot, Bolborhynchus lineola, in relation to ambient temperature. Journal of Comparative Physiology B 142:479-488.

Bucher, T.L. (1985). Ventilation and oxygen consumption in Amazona viridigenalis. Journal of Comparative Physiology B 155:269-276.

Bucher, T.L., and M.C. Barnhart (1984). Varied egg gas conductance, air cell gas tensions and development in Agapornis roseicollis. Respiration Physiology 55:277-289.

Burt, W.H. (1943). Territoriality and home range concepts as applied to mammals. Journal of Mammalogy 24:346-352.

Cade, T.J., C.A. Tobin, and A. Gold (1965). Water economy and metabolism of two estrildine finches. Physiological Zoology 38:9-33.

Cain, B. (1972). Cold hardiness and the development of homeothermy in young Black-bellied tree ducks. Wilson Bulletin 84:483-485.

Calder, W.A. (1964). Gaseous metabolism and water relations of the Zebra finch, Taeniopygia castanotis. Physiological Zoology 37:400-413.

Calder, W.A. (1968). Respiratory and heart rates of birds at rest. CONDOR 70:358-365.

Calder, W.A., and J.R. King. (1974). Thermal and caloric relations of birds. Pages 259-413 in D.S. Farner and J.R. King (eds) Avian Biology Vol.4. Academic Press New York.

Calder, W.A., and K. Schmidt-Nielsen. (1967). Temperature regulation and evaporation in the Pigeon and Roadrunner. American Journal of Physiology 213:883-889.

Campbell, A.J. (1901). Nests and eggs of Australian birds pt.2. Sheffield, England. pp 698-708.

Carey, C. (1979). Increase in conductance to water vapor during incubation in eggs of two avian species. Journal of Experimental Zoology 209:181-186.

Chapman, T.E., and A.L. Black (1967). Water turnover in chickens. Poultry Science 46:761-765.

Chapman, T.E., and L.Z. McFarland (1971). Water turnover in the Coturnix quail with individual observations on a Borrowing owl, Petz conure, and Vulturine fish eagle. Comparative Biochemistry and Physiology 39A:653-656.

Chapman, T.E., and D. Mihai (1972). Influence of sex and egg production on water turnover in chickens. Poultry Science 51:1252-1256.

Chappel, M.A. (1980). Thermal energetics of chicks of arctic-breeding shorebirds. Comparative Biochemistry and Physiology 65A:311-317.

Clark, G.A. (1960). Notes on the embryology and evolution of the Megapodes (AVES: GALLIFORMES) Postilla 45:1-7.

Clark, G.A. (1964a). Ontogeny and evolution in the Megapodes (AVES: GALLIFORMES). Postilla 78:1-37.

Clark, G.A. (1964b). Life histories and the evolution of Megapodes. Living Bird 3:149-167.

Clark, L. (1982). The development of effective homeothermy and endothermy by nestling Starlings. Comparative Biochemistry and Physiology 73A:253-260.

Crawford, E.C., and R.C. Lasiewski (1968). Oxygen consumption and respiratory evaporation of the Emu and Rhea. CONDOR 70:333-339.

Davies, S.J.J.F. (1982). Behavioural adaptations of birds to environments where evaporation is high and water is in short supply. Comparative Biochemistry and Physiology 71A:557-566.

Dawson, T.J., R.M. Herd, and E. Skadhauge (1983). Water turnover and body water distribution during dehydration in a large arid-zone bird, the Emu, Dromaius novaehollandiae. Journal of Comparative Physiology B 153:235-240.

Dawson, T.J., D. Read, E.M. Russell, and R.M. Herd (1984). Seasonal variation in daily activity patterns, water relations and diet of Emus. EMU 84:93-102.

Dawson, W.R. (1962). Evolution of temperature regulation in birds. Pages 45-71 in J.P. Hannon and E.G. Viereck (eds) Proceedings Symposia on Arctic Biology and Medicine II. Comparative Physiology of temperature regulation.

Dawson, W.R. (1976). Physiological and behavioural adjustments of birds to heat and aridity. Proceedings 16th International Ornithological Congress. pp 455-466.

Dawson, W.R., and G.A. Bartholomew (1968). Temperature regulation and water economy of desert birds. Pages 357-393 in G.W. Brown (ed). Desert Biology Vol I. Academic Press N.Y.

Dawson, W.R., and A.F. Bennett (1973). Roles of metabolic level and temperature regulation in the adjustment of Western plumed pigeons (Lophophaps ferruginea) to desert conditions. Comparative Biochemistry and Physiology 44A:249-266.

Dawson, W.R., and A.F. Bennett. (1981). Field and laboratory studies of the thermal relations of hatchling Western gulls. Physiological Zoology 54:155-164.

Dawson, W.R., and C.D. Fisher (1969). Responses to temperature by the Spotted nightjar (Eurostopodus guttatus). CONDOR 71:49-53.

Dawson, W.R., and J.W. Hudson (1970). Birds. Pages 223-310 in G.C. Whittow (ed) Comparative Physiology of Thermoregulation. Vol 1 Academic Press, New York.

Dawson, W.R., A.F. Bennett, and J.W. Hudson. (1976). Metabolism and thermoregulation in hatchling Red-billed gulls. CONDOR 78:49-60.

Dawson, W.R., J.W. Hudson, and R.W. Hill. (1972). Temperature regulation in newly hatched Laughing Gulls (Larus atricilla). CONDOR 74:177-184.

Degen, A.A. (1977). Fat-tailed Awassi and German mutton merino sheep under semi-arid conditions. 1. Total body water, its distribution and water turnover. Journal of Agricultural Science 88:693-698.

Degen, A.A., B. Pinshow, P.U. Alkon, and H. Arnon (1981). Tritiated water for estimating total body water and water turnover rate in birds. Journal of Applied Physiology 51:1183-1188.

Degen, A.A., B. Pinshow, and P.U. Alkon (1982). Water flux in Chukar partridges (Alectoris chukar) and a comparison with other birds. Physiological Zoology 55:64-71.

Degen, A.A., B. Pinshow, and P.U. Alkon (1983). Summer water turnover rates in free-living Chukar and Sand partridges in the Negev desert. CONDOR 85:333-337.

Degen, A.A., B. Pinshow, and P.J. Shaw (1984). Must desert Chukars (Alectoris chukar sinaica) drink water? Water influx and body mass changes in response to dietary water content. AUK 101:47-52.

Department of Lands, South Australia (1982). Calperum Management study Draft.

Drent, R.H. (1970). Functional aspects of incubation in the Herring Gull. Behaviour supplement 17:1-132.

Drent, R.H. (1973). The natural history of incubation. Pages 262-311 in D.S. Farner (ed). Breeding biology of birds. National Academy of Science, Washington D.C.

Drent, R.H. (1975). Incubation. Pages 333-421 in D.S. Farner and J.R. King (eds). Avian Biology Vol V. Academic Press, New York.

Drent, R.H, and B. Stonehouse. (1971). Thermoregulatory responses of the Peruvian penguin, Spheniscus humboldti. Comparative Biochemistry and Physiology 40A:689-710.

Earle, R.A. (1982). Environmental and physiological factors governing avian breeding in Acacia savanna. Occassional Bulletin No. 2, p. 22. The Zoological Society of South Africa.

Ellis, H.I. (1980). Metabolism and solar radiation in dark and white herons in hot climates. Physiological Zoology 53:358-372.

Eppley, Z.A. (1984). Development of thermoregulatory abilities in Xantus' murrelet chicks Synthliboramphus hypoleucus. Physiological Zoology 57:307-317.

Farrell, D.J., and D. Balnave (1977). The in vivo estimation of body fat content in laying hens. British Poutry Science 18:381-384.

Finnlund, M., R. Hissa, J. Koivusaari, E. Merila, and I. Nuuja (1985). Eggshells of Artic terns from Finland: Effects of incubation and geography. CONDOR 87:79-86.

Fisher, C.D., E. Lindgren, and W.R. Dawson (1972). Drinking patterns and behaviour of Australian desert birds in relation to their ecology and abundance. CONDOR 74:111-136.

Forsman, E.D., E.C. Meslow, and H.M. Wright (1984). Distribution and biology of the Spotted owl in Oregon. Wildlife Monographs 87:1-64.

Freeman, B.M. (1962). Gaseous metabolism in the Domestic chicken. II Oxygen consumption in the full-term and hatching embryo with a note on a possible cause for 'death in shell'. British Poultry Science 3:63-72.

Freeman, B.M. (1964). The emergence of the homeothermic-metabolic response in the Fowl (Gallus domesticus). Comparative Biochemistry and Physiology 13:413-422.

Freeman, B.M. (1966). The effects of cold, noradrenaline and adrenaline upon the oxygen consumption and carbohydrate metabolism of the young Fowl (Gallus domesticus). Comparative Biochemistry and Physiology 18:369-382.

Freeman, B.M. (1967). Some effects of cold on the metabolism of the Fowl during the perinatal period. Comparative Biochemistry and Physiology 20:179-193.

Freeman, B.M. (1970a). Thermoregulatory mechanisms of the neonate Fowl. Comparative Biochemistry and Physiology 33:219-230.

Freeman, B.M. (1970b). Some aspects of thermoregulation in the adult Japanese quail (Coturnix coturnix japonica). Comparative Biochemistry and Physiology 34:871-881.

Frith, H.J. (1955). Incubation in the Mallee Fowl (Leipoa ocellata, MEGAPODIIDAE). Proceedings XI International Ornithological Congress. pp 570-574.

Frith, H.J. (1956). Temperature regulation in the nesting mounds of the Mallee-fowl Leipoa ocellata Gould. C.S.I.R.O. Wildlife Research 1:79-95.

Frith, H.J. (1957). Experiments on the control of temperature in the mounds of the Mallee-fowl, Leipoa ocellata Gould (MEGAPODIIDAE). C.S.I.R.O. Wildlife Research 2:101-110.

Frith, H.J. (1959a). Breeding of the Mallee Fowl, Leipoa ocellata Gould (MEGAPODIIDAE). C.S.I.R.O. Wildlife Research 4:31-60.

Frith, H.J. (1959b). Incubator birds. Scientific American 201:52-58.

Frith, H.J. (1962a). Conservation of the Mallee Fowl, Leipoa ocellata Gould (MEGAPODIIDAE). C.S.I.R.O. Wildlife Research 10:33-51.

Frith, H.J. (1962b). The Mallee-fowl, The bird that builds an incubator. Angus and Robertson, Sydney. 136pp.

Gleeson, T.T. (1979). Foraging and transport costs in the galapagos marine iguana, Amblyrhynchus cristatus. Physiological Zoology 53:549-557.

Gordon, M.S. (1982). Animal Physiology. Principles and Adaptations. 4th ed. MacMillan. New York. 699pp.

Gould, J. (1865). Hand book to the birds of Australia Vol II. London pp 155-166.

Grant, S.G., T.N. Pettit, H. Rahn, G.C. Whittow, and C.V. Paganelli (1982). Water loss from Laysan and Black-footed Albatross eggs. Physiological Zoology 55:405-114.

Green, B., and I. Eberhard (1983). Water and sodium intake, and estimated food consumption, in free-living Eastern quolls, Dasyurus viverrinus. Australian Journal of Zoology 31:871-880.

Grigg, G.C., C.R. Drane, and G.P. Courtice (1979). Time constants of heating and cooling in the Eastern water dragon Physignathus lesueruii and some generalizations about heating and cooling in reptiles. Journal of Thermal Biology 4:95-103.

Grubbs, D.E. (1980). Tritiated water turnover in free-living desert rodents. Comparative Biochemistry and Physiology 66A:89-98.

Haftorn, S. (1983). Egg temperature during incubation in the Great tit Parus major, in relation to ambient temperature, time of day, and other factors. Fauna norv. Ser C, Cinclus 6:22-38.

Haftorn, S. (1984). The behaviour of an incubating female Coal tit Parus ater in relation to experimental regulation of nest temperature. Fauna norv. Ser. C, Cinclus 7:12-20.

Hails, C.J. (1983). The metabolic rate of tropical birds. CONDOR 85:61-65.

Hamilton, H.L. (1965). Lillie's Development of the Chick, An introduction to Embryology. Holt, Rinehart and Winston, New York. 624pp.

Hayworth, A.M., and W.W. Weathers (1984). Temperature regulation and climatic adaptation in Black-billed and Yellow-billed magpies. CONDOR 86:19-26

Herreid, C.F., and B. Kessel (1967). Thermal conductance in birds and mammals. Comparative Biochemistry and Physiology 21:405-414.

Hill, R.W. (1972). Determination of oxygen consumption by the use of the paramagnetic oxygen analyser. Journal of Applied Physiology 33:261-263.

Hill, R.W., and D.L. Beaver.(1982). Inertial thermostability and thermoregulation in broods of Redwing blackbirds. Physiological Zoology 55:250-266.

Hill, R.W., D.L. Beaver, and J.H. Veghte (1980). Body surface temperatures and thermoregulation in the Black-capped chickadee (Parus atricapillus). Physiological Zoology 53:305-321.

Hissa, R., S. Sarrela, H. Rintamäki, H. Lindén, and E.Hohtola (1983). Energetics and development of temperature regulation in Capercaillie Tetrao urogallus. Physiological Zoology 56:142-151.

Hoiby, M., A. Aulie, and O.B. Reite (1983). Oxygen uptake in Fowl eggs incubated in air and pure oxygen. Comparative Biochemistry and Physiology 74A:315-318.

Holleman, D.F., and R.A. Dieterich (1973). Body water content and turnover in several species of rodents as evaluated by the tritiated water method. Journal of Mammalogy 54:456-465.

Howell, T.R. (1979). Breeding biology of the Egyptian plover, Pluvianus aegyptius. University of California Publications in Zoology 113:1-76.

Hoyt, D.F., and H. Rahn (1980). Respiration of avian embryos - A comparative analysis. Respiration Physiology 39:255-264.

Hoyt, D.F., R.G. Board, H. Rahn, and C.V. Paganelli (1979). The eggs of the Anatidae: Conductance, pore structure, and metabolism. Physiological Zoology 52:438-450.

Hoyt, D.F., D. Vleck, and C.M. Vleck (1978). Metabolism of avian embyros: Ontogeny and temperature effects in the Ostrich. CONDOR 80:265-271.

Hudson. J.W. and S.L. Kimzey (1966). Temperature regulation and metabolic rhythms in populations of the House sparrow, Passer domesticus. Comparative Biochemistry and Physiology 17:203-217.

Hudson, J.W., W.R. Dawson, and R.W. Hill. (1974). Growth and development of temperature regulation in nesting Cattle egrets. Comparative Biochemistry and Physiology 49A:717-741.

Johnston, D.W. (1971). The absence of brown adipose tissue in birds. Comparative Biochemistry and Physiology 40A:1107-1108.

Kendeigh, S.C. (1939). The relation of metabolism to the development of temperature regulation in birds. Journal of Experimental Zoology 82:419-438.

Kendeigh, S.C. (1940). Factors affecting length of incubation. AUK 57:499-513.

Khaskin, V.V. (1961). Heat exchange in birds' eggs on incubation. Biophysics 6:97-107.

King, J.R., and D.S. Farner (1961). Energy metabolism, thermoregulation and body temperature. Pages 215-288 in A.J. Marshall (ed) Biology and Comparative Physiology of Birds. Vol. 2. Academic Press, New York.

King, J.R. and D.S. Farner (1964). Terrestrial animals in humid heat: Birds. pages 603-624 in D.B. Dill (ed.) Handbook of Physiology Section 4, Adaptations to the Environment. American Physiological Society, Washington D.C.

Koskimies, J. (1962). Ontogeny of thermoregulation and energy metabolism in some gallinaceous birds. Bologna. Universita. Instiyuo Zoologico. Ricerche di Zoologia Applicata alla Caccia. Suppl. Vol.4 pp 149-160.

Koskimies, J., and L. Lahti (1964). Cold-hardiness of the newly hatched young in relation to ecology and distribution in ten species of European ducks. AUK 81:281-307.

Kreitzer, J.F. (1972). The effect of embryonic development on the thickness of the egg shells of Coturnix quail. Poultry Science 51:1764-1765.

Lack, D.L. (1968). Ecological adaptations for breeding in birds. Methuen and Co., London.

Lasiewski, R.C. (1969). Physiological responses to heat stress in the Poorwill. American Journal of Physiology 217:1504-1509.

Lasiewski, R.C., and G.A. Bartholomew (1966). Evaporative cooling in the Poor-will and Tawny frogmouth. CONDOR 68:253-262.

Lasiewski, R.C., and R.S. Seymour (1972). Thermoregulatory responses to heat stress in four species of birds weighing approximately 40 grams. Physiological Zoology 45:106-118.

Lasiewski, R.C., A.L. Acosta, and M.H. Bernstein (1966). Evaporative water loss in birds - 1. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. Comparative Biochemistry and Physiology 19:445-457.

Lasiewski, R.C., W.R. Dawson, and G.A. Bartholomew (1970). Temperature regulation in the Little papuan frogmouth, Podargus ocellatus. CONDOR 72:332-338.

Lasiewski, R.C., S.H. Hubbard, and W.R. Moberley (1964). Energetic relationships of a very small passerine bird. CONDOR 66:212-220.

Lasiewski, R.C., W.W. Weathers, and M.H. Bernstein (1967). Physiological responses of the Giant hummingbird, Patagona gigas. Comparative Biochemistry and Physiology 23:797-813.

Lee, P., and K. Schmidt-Nielsen (1971). Respiratory and cutaneous evaporation in the Zebra finch: Effect on water balance. American Journal of Physiology 220:1598-1605.

Lewis, F (1939). The breeding habits of the Lowan in Victoria. EMU 39:56-62.

Lundy, H. (1969). A review of the effects of temperature, humidity, turning, and gaseous environment in the incubator on the hatchability of the hen's egg. Pages 143-176 in T.C. Carter and B.M. Freeman (eds). The fertility and hatchability of the hen's egg. Oliver and Boyd, Edinburgh.

McArthur, A.J. (1981). Thermal resistance and sensible heat loss from animals. Journal of Thermal Biology 6:43-47.

McFarland, D.J., and E. Baher (1968). Factors affecting feather posture in the Barbary Dove. Animal Behaviour 16:171-177.

MacFarlane, W.V., and B. Howard (1972). Comparative water and energy economy of wild and domestic animals. Symposia Zoological Society of London 31:261-296.

McGlip, J.N. (1948). Sightings of birds in Murray mallee. South Australian Ornithologist 16:48-49.

McMullan, R.A. and L.L. Eberhardt (1953). Tolerance of incubating pheasant eggs to exposure. Journal of Wildlife Management 17:322-330.

McNab, B.K. (1966). An analysis of the body temperature of birds. CONDOR 68:47-55.

McNab, B.K. (1970) Body weight and the energetics of temperature regulation. Journal of Experimental Biology 53:329-348.

McNab, B.K. (1980a). On estimating thermal conductance in endotherms. Physiological Zoology 53:145-156.

McNab, B.K. (1980b). Food habits, energetics, and the population biology of mammals. American Naturalist 116:106-124.

McLennon, H. (1906). The Lowan. Its haunts and habits. The Australasian 15/12/1906. p 1440.

Maloney, J.E. (1984). The development of the respiratory system in placental mammals. Pages 373-387 in R.S. Seymour (ed) Respiration and Metabolism of Embryonic Vertebrates. Dr. W. Junk Publishers, Dordrecht, Neverlands.

Marder, J., and R. Berstein (1983). Heat balance of the Partridge Alectoris chukar exposed to moderate, high, and extreme thermal stress. Comparative Physiology and Biochemistry 74A:149-154.

Marshall, W. (1939). The Brush-Turkey and 'turning'. EMU 38:489-491.

Mattingley, A.H.E. (1908). Thermometer-bird or Mallee-fowl (Leipoa ocellata) Part I. EMU 8:53-61.

Medway, W., and M.R. Kare (1957). Water metabolism of the Domestic fowl from hatching to maturity. American Journal of Physiology 190:139-141.

Metcalfe, J., M.K. Stock, and R.L. Ingermann (1984). The effect of oxygen on growth and development of the chick embryo. Pages 205-219 in R.S. Seymour (ed). Respiration and metabolism of embryonic vertebrates. Dr. W. Junk Publishers, Dordrecht, Netherlands.

Misson, B.H. (1977). The relationships between age, mass, body temperatureand metabolic rate in the neonatal Fowl (Gallus domesticus). Journal of Thermal Biology 2:107-110.

Moreng, R.E., and R.L. Bryant (1956). The resistance of the chicken embryo to low temperature exposure. Poultry Science 35:735-757.

Morris, D.W. (1984). Rodent population cycles: Life history adjustments to age-specific dispersal strategies and intrinsic time lags. Oecologia 64:8-13.

Morrison, P.R., and W.J. Tietz (1957). Cooling and thermal conductivity in three small Alaskan mammals. Journal of Mammology 38:78-86.

Nagy, K.A. (1983). The doubly labeled water ($^{3}HH^{18}O$) method: A guide to its use. UCLA Publication No. 12-1417 pp45.

Nagy, K.A., and D.P. Costa (1980). Water flux in animals: Analysis of potential errors in the tritiated water method. American Journal of Physiology 283:R454-R465.

Neumann, R., J.W. Hudson, and R.J. Hock (1968). Body Temperatures. Part II. Birds. Pages 334-343 in P.L. Alterman (ed) Metabolism. Federal Society of Experimental Biology, Bethesda, Md.

Nice, M.M. (1962). Development of behaviour in precocial birds. Transactions of the Linnaean Society of New York 4:1-247.

Nicol, S.C. (1978). Rates of water turnover in marsupials and eutherians: A comparative review with new data on the Tasmanian devil. Australian Journal of Zoology 26:465-473.

Nomoto, S., W. Rautenberg, and M. Iriki (1983). Temperature regulation during exercise in the Japanese quail (Coturnix coturnix japonica). Journal of Comparative Physiology B 149:519-525.

Norton, D.W. (1972). Incubation schedules of four species of Calidridine sandpipers at Barrow, Alaska. Condor 74:164-176.

Odum, E.P. (1942). Muscle tremors and the development of temperature regulation in birds. American Journal of Physiology 136:618-622.

Ohmart, R.D., T.E. Chapman, and L.Z. McFarland (1970). Water turnover in Roadrunners under different environmental conditions. AUK 87:787-793.

Oliphant, L.W. (1983). First observations of brown fat in birds. CONDOR 85:350-354.

Packard, M.J., and G. C. Packard (1984). Comparative aspects of calcium metabolism in embryonic reptiles and birds. Pages 155-180 in R.S. Seymour (ed). Respiration and metabolism of embryonic vertebrates. Dr. W. Junk Publishers, Dordrecht, Neverlands.

Paganelli, C.V. (1980). The physics of gas exchange across the avian eggshell. American Zoologist 20:329-338.

Paganelli, C.V., and H. Rahn (1984). Adult and embryonic metabolism in birds and the role of shell conductance. Pages 193-204 In R.S. Seymour (ed). Respiration and metabolism in embryonic vertebrates. Dr. W. Junk publishers, Dordrecht, Neverlands.

Paganelli, C.V., R.A. Ackerman, and H. Rahn (1978). The avian egg: In vivo conductance to oxygen, carbon dioxide, and water vapor in late development. Pages 212-218 in J. Piiper (ed). Respiratory functions in birds, adult and embryonic. Springer-Verlag Berlin.

Paganelli, C.V., A. Olszowka, and A. Ar (1974). The avian egg: Surface area, volume, and density. CONDOR 76:319-325.

Palokangas, R., and R. Hissa (1971). Thermoregulation in young Black-headed gull (larus ridibundus L.). Comparative Biochemistry and Physiology 38A:743-750.

Pembrey, M.S., M.H. Gordon, and R. Warren (1895). On the response of the chick before and after hatching to changes of external temperature. Journal of Physiology 17:331-348.

Peters, J.P., and D.D. Van Slyke (1946). Quantitative clinical chemistry, Vol I. 2nd ed. Williams and Wilkins Co. Baltimore.

Pettit, T.N., G.S. Grant, G.C. Whittow, H. Rahn, and C.V. Paganelli (1982a). Embryonic oxygen consumption and growth of Laysan and Black-footed albatross. American Journal of Physiology 242:R121-R128.

Pettit, T.N., G.S. Grant, G.C. Whittow, H. Rahn, and C.V. Paganelli (1982b). Respiratory gas exchange and growth of Bonin petrel embryos. Physiological Zoology 55:162-170.

Porter, W.P. (1969). Thermal radiation in metabolic chambers. Science 166:115-117.

Purdue, J.R., and H. Haines (1977). Salt water tolerance and water turnover in the snowy plover. AUK 94:248-255.

Rahn, H. (1982). Comparison of embryonic development in birds and mammals: Birth weight, time, and cost. Pages 124-137 in C.R. Taylor et. al. (eds) A Companion to Animal Physiology. Cambridge University Press, New York.

Rahn, H. (1984). Factors controlling the rate of incubation water loss in bird eggs. Pages 271-288 In R.S. Seymour (ed) Respiration and Metabolism of embryonic vertebrates. Dr. W. Junk publishers, Dordrecht, Neverlands.

Rahn, H., and A. Ar (1974). The avian egg: Incubation time and water loss. Condor 76:147-152.

Rahn, H., and A. Ar (1980). Gas exchange of the avian egg: Time, structure, and function. American Zoologist 20:477-484.

Rahn, H., C.V. Paganelli, and A. Ar (1974). The avian egg: Air-cell gas tension, metabolism and incubation time. Respiration Physiology 22:297-309.

Randall, W.C. (1943). Factors influencing the temperature regulation of birds. American Journal of Physiology 139:56-63.

Ricklefs, R.E. (1967). Relative growth, body constituents, and energy content of nestling Barn swallows and Red-winged black birds. AUK 84:560-570.

Ricklefs, R.E. (1974). Energetics of reproduction in birds. Pages 152-296 in R.A. Paynter Jr (ed) Avian Energetics. Publication of the Nuttal Ornithological Club No. 15. Cambridge, Massachusetts.

Ricklefs, R.E., and F.R. Hainsworth (1968). Temperature regulation in nestling Cactus Wrens: The development of homeothermy. CONDOR 70:121-127.

Ricklefs, R.E., and D.D. Roby (1983). Development of homeothermy in the diving petrels Pelecanoides urinatrix exsul and P. georgicus, and the Antarctic prion, Pachyptila desolata. Comparative Biochemistry and Physiology 75A:307-311.

Ricklefs, R.E., and S.C. White (1981). Growth and energetics of chicks of the Sooty tern (Sterna fuscata) and Common tern (S. Hirundo). AUK 98:361-378.

Rintamaki, H., S. Saarela, A. Marjakangas, and R. Hissa (1983). Summer and winter temperature regulation in the Black grouse Lyrurus tetrix. Physiological Zoology 56:152-159.

Robinson, D.E., G.S. Campbell, and J.R. King (1976). An evaluation of heat exchange in small birds. Journal of Comparative Physiology. 105:153-166.

Roby, D.D., R.E. Ricklefs (1984). Observations on the cooling tolerance of embryos of the Diving petrel Pelecanoides georgicus. AUK 101:160-161.

Romanoff, A.L. (1930). Biochemistry and biophysics of the developing hen's egg. I. Influence of humidity. Cornell University Agricultural Experiment Station Memoir 132:1-27.

Romanoff, A.L. (1936). Effect of different temperatures on the pre-natal and post-natal development of the chick. Poultry Science 15:311-315.

Romanoff, A.L. (1941). Development of homeothermy in birds. Science 94:218-219.

Romanoff, A.L. (1960). The avian embryo. The Macmillan Company, New York. pp1305.

Romanoff, A.L. (1967). Biochemistry of the avian embryo. John Wiley and Sons, New Yory. pp 398.

Romanoff, A.L., and A.J. Romanoff (1949). The avian egg. John Wiley and Sons, New York. pp 918.

Romanoff, A.L., and A.J. Romanoff (1972). Pathogenesis of the avian embryo. Wiley-Interscience, New York. pp 57-90.

Romijn, C. (1954). Development of heat regulation in the chick. Proceedings 10th World Poultry Congress, Edinburgh. pp. 181-184.

Romijn, C., and W. Lokhorst (1951). Foetal respiration in the Hen. The respiratory metabolism of the embryo. Monographiae Biologicae 2:187-197.

Romijn, C., and W. Lokhorst (1955). Chemical heat regulation in the chick embryo. Poultry Science 34:649-654.

Romijn, C., and W. Lokhorst. (1956). The caloric equilibrium of the chicken embryo. Poultry Science 35:829-834.

Rooke, I.J., S.D. Bradshaw, and R.A. Langworthy (1983). Aspects of water, electrolyte, and carbohydrate physiology of the Silvereye, Zosterops lateralis (Aves). Australian Journal of Zoology 31: 695-304.

Ross, J.A. (1919). Six months record of a pair of Mallee-fowls. EMU 18:285-288.

Schawn, M.W., and D.D. Williams (1978). Temperature regulation in the common raven of interior Alaska. Comparative Biochemistry and Physiology 60A:31-36.

Schoener, T.W. (1968). Sizes of feeding territories among birds. Ecology 49:123-141.

Scholander, P.F., V. Walters, R. Hock, and L. Irving (1950a). Body insulation of some arctic and tropical mammals and birds. Biological Bulletin 99:225-236.

Scholander, P.F., R. Hock, V. Walters, F. Johnson, and L. Irving (1950b). Heat regulation in some arctic and tropical mammals and birds. Biological Bulletin 99:237-258.

Scholander, P.F.,R. Hock, V. Walters, and L. Irving (1950c). Adaptations to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biological Bulletin 99:259-271.

Seymour, R.S. (1984). Patterns of lung aeration in the perinatal period of Domestic Fowl and Brush Turkey. In R.S. Seymour (ed). Respiration and Metabolism in Embryonic Vertebrates. Dr. W. Junk Publishers, Dordrecht, Neverlands.

Seymour, R.S. and R.A. Ackerman (1980). Adaptations to underground nesting in birds and reptiles. American Zoologist 20:437-447.

Seymour, R.S. and H. Rahn (1978). Gas conductance in the eggshell of the mound-building Brush Turkey. Pages 243-246 in J. Piiper (ed)., Respiratory functions in birds, adult and embryonic. Springer-Verlag, Berlin, Heidelberg, New York.

Simpson, G.S., A. Roe, and R.C. Lewontin (1960). Quantitative Zoology. Harcourt, Brace and World. New York. 440pp.

Siri, W., and J. Evers (1962). Tritium exchange in biological systems. Pages 71-80 in: Tritium in the Physical and Biological Sciences. Vienna, Symposium of International Atomic Energy Agency, Vol 2.

Skadhauge, E. (1972). Salt and water excretion in xerophilic birds. Symposium Zoological Society of London 31:113-131.

Skadhauge, E. (1981). Osmoregulation in birds. Springer-Verlag, Berlin, Heildeberg, New York.

Skadhauge, E., and S.D. Bradshaw (1974). Saline drinking and cloacal excretion of salt and water in the Zebra finch. American Journal of Physiology 227:1263-1267.

Sokal, R.R., and F.J. Rohlf (1969). Biometry. W.H. Freeman. San Francisco. 776pp.

Sotherland, P.R., G.C. Packard, T.L. Taigen, and T.J. Boardman (1980). An altitudinal cline in conductance of Cliff swallow (Petrichelidon pyrrhonota) eggs to water vapor. AUK 97:177-182.

Spiers, D.E., R.A. McNabb, and F.M. McNabb (1974). The development of thermoregulatory ability, heat seeking activities, and thyroid function in hatchling Quail (Coturnix coturnix japonica). Journal of Comparative Physiology 89:159-174.

Streit, B. (1982). Water turnover rates and half-life times in animals studied by use of labelled and non-labelled water. Comparative Biochemistry and Physiology 72A:445-454.

Sturkie, P.D. and W.J. Mueller (1976). Reproduction in the female and egg production. Pages 302-330 in P.D. Sturkie (ed)., Avian Physiology. Springer-Verlag, New York, Heidelberg, Berlin.

Tazawa, H. (1973). Hypothermal effect on the gas exchange in the chicken embryo. Respiration Physiology 17:21-31.

Tazawa, H., and M. Mochizuki (1978). Oxygen transport in chicken embryos under hypothermal exposure. Respiration Physiology 32:325-334.

Tazawa, H., and S. Nakagawa (1985). Response of egg temperature, heart rate and blood pressure in the chick embryo to hypothermal stress. Journal of Comparative Physiology B 155:195-200.

Thomas, D.H. (1982). Salt and water excretion by birds: The lower intestine as an integrator of renal and intestinal excretion. Comparative Biochemistry and Physiology 71A:527-535.

Thomas, D.H., and J.G. Phillips (1975). Studies in avian adrenal steroid function II. Chronic adrenalectomy and the turnover of $[^3H]_2O$ in domestic ducks (Anas platyrhynchos L.). General and Comparative Endocrinology 26:404-411.

Thomas, D.H., B. Pinshow, and A.A. Degen (1984). Renal and lower intestinal contributions to the water economy of desert-dwelling phasianid birds: Comparison of free-living and captive Chukars and Sand partridges. Physiological Zoology 57:128-136.

Thompson, M.B. (1983). The physiology and ecology of the eggs of the pleurodiran tortise Emydura macquarii (Gray), 1831. PhD. Thesis, The University of Adelaide.

Tracy, C.R. (1972). Newton's law: Its applicability for expressing heat loss from homeotherms. Bioscience 22:656-659.

Trost, C.H. (1972). Adaptations of Horned larks (Eremophila alpestris) to hot environments. AUK 89:506-527.

Tyler, C. and F.H. Greake (1953). Studies on eggshells. I. The determination of membrane,-, pore,-, and matrix-protein. Journal of the Science of Food and Agriculture 4:261-266.

Untergasser, G., and J.S. Hayward (1972). Development of thermoregulation in ducklings. Canadian Journal of Zoology 50:1243-1250.

Utter, J.M., and E.A. Le Febvre (1973). Daily energy expenditure of Purple martins (Progne subis) during the breeding season: Estimates using D_2O^{18} and time budget methods.

Vanderstoep, J., and J.F. Richards (1969). The changes in egg shell strength during incubation. Poultry Science 49:276-285.

Visschedijk, A.H.J. (1968). The air space and embryonic respiration 1. The pattern of gaseous exchange in the fertile egg during the closing stages of incubation. British Poultry Science 9:173-184.

Visschedijk, A.H.J. (1980). Effects of barometric pressure and abnormal gas mixture on gaseous exchange of the avian embryo. American Zoologist 20:469-476.

Visschedijk, A.H.J., A. Ar, H. Rahn, and J. Piiper (1980). The independent effects of atmospheric pressure and oxygen partial pressure on gas exchange of the chicken embryo. Respiration Physiology 39:33-44.

Vleck, C.M. (1981a). Energetic cost of incubation in the zebra finch. CONDOR 83:229-237.

Vleck, C.M. (1981b). Hummingbird incubation: Female attentiveness and egg temperature. Oecologia 51:199-205.

Vleck, C.M., and G.J. Kenagy (1980). Embryonic metabolism of the Fork-tailed storm petrel: Physiological patterns during prolonged and interrupted incubation. Physiological Zoology 53:32-42.

Vleck, C.M., D.F. Hoyt, and D. Vleck. (1979). Metabolism of avian embryos: Patterns in altricial and precocial birds. Physiological Zoology 52:363-377.

Vleck, C.M., D. Vleck, and D.F. Vleck (1980b). Patterns of metabolism and growth in avian embryos. American Zoologist 20:405-416.

Vleck, D.J.A. (1978). The energetics of activity and growth: The energy cost of burrowing by pocket gophers and energy metabolism during growth and hatching in the Emu, Rhea, and Ostrich Eggs. Ph. D. dissertation University of California, Los Angeles.

Vleck, D., C.M. Vleck, and D.F. Hoyt (1980a). Metabolism of avian embyros: Ontogeny of oxygen consumption in the Rhea and Emu. Physiological Zoology 53:125-135.

Vleck, D., C.M. Vleck, and R.S. Seymour (1984). Energetics of embryonic development in the megapode birds, Mallee Fowl Leipoa ocellata and Brush Turkey Alectura lathami. Physiological Zoology 57:444-456.

Walter, A., and M.R. Hughes (1978). Total body water volume and turnover rate in fresh water and seawater adapted Glaucous-winged gulls, Larus glaucescens. Comparative Biochemistry and Physiology 61A:233-237.

Wangensteen, O.D., D. Wilson, and H. Rahn (1970). Diffusion of gases across the shell of the hen's egg. Respiration Physiology 11:16-30.

Weathers, W.W. (1977). Temperature regulation in the Dusky munia, Lonchura fuscans (Cassin) (Estrildidae). Australian Journal of Zoology 25:193-199.

Weathers, W.W. (1979). Climatic adaptations in avian standard metabolic rate. Oecologia 42:81-89.

Weathers, W.W. (1981). Physiological thermoregulation in heat-stressed birds: Consequences of body size. Physiological Zoology 54:345-361.

Weathers, W.W., and K.A. Nagy (1980). Simultaneous Doubly labeled water ($^{3}HH^{18}O$) and time-budget estimates of daily energy expenditure in Phainopepla nitens. AUK 97:861-867.

Webb, D.R. and J.R. King (1983). An analysis of the heat budgets of the eggs and nest of the White-crowned sparrow, Zonotrichia leucophrys, in relation to parental attentiveness. Physiological Zoology 56:493-505.

Wekstein, D.R., and J.F. Zolman (1967). Homeothermic development of the young chick. Proceedings of the Society of Experimental Biology and Medicine 125:294-297.

Wekstein, D.R., and J.F. Zolman (1969). Ontogeny of heat production in chicks. Federation Proceedings 28:1023-1028.

West, G.C. (1965). Shivering and heat production in wild birds. Physiological Zoology 38:111-120.

Wetmore, A. (1921). A study of the body temperature of birds. Smithsonian Miscellaneous Collections 72:1-52.

White, F.N., and J.L. Kinney (1974). Avian incubation. Science 186:107-115.

Williams, J.B. (1985). Validation of the doubly labeled water technique for measuring energy metabolism in starlings and sparrows. Comparative Biochemistry and Physiology 80A:349-353.

Williams, J.B., and K.A. Nagy (1984). Validation of the doubly labeled water technique for measuring energy metabolism in Savannah Sparrows. Physiological Zoology 57:325-328.

Williams, J.B., and R.E. Ricklefs (1984). Egg temperature and embryo metabolism in some high-latitude Procellariiform birds. Physiological Zoology 57:118-127

Willoughby, E.J. (1969). Evaporative water loss of a small xerophilous finch, Lonchura malabarica. Comparative Biochemistry and Physiology 28:655-664.

Withers, P.C. (1983). Energy, water, and solute balance of the Ostrich Struthio camelus. Physiological Zoology 56:568-579.

Withers, P.C., E.N. Louw, and J. Henschol (1980). Energetics and water relationships of Namib desert rodents. South African Journal of Zoology 15:131-137.

Zar, J.H. (1974). Biostatistical Analysis. Prentice Hill Inc. New Jersey. 8620pp.