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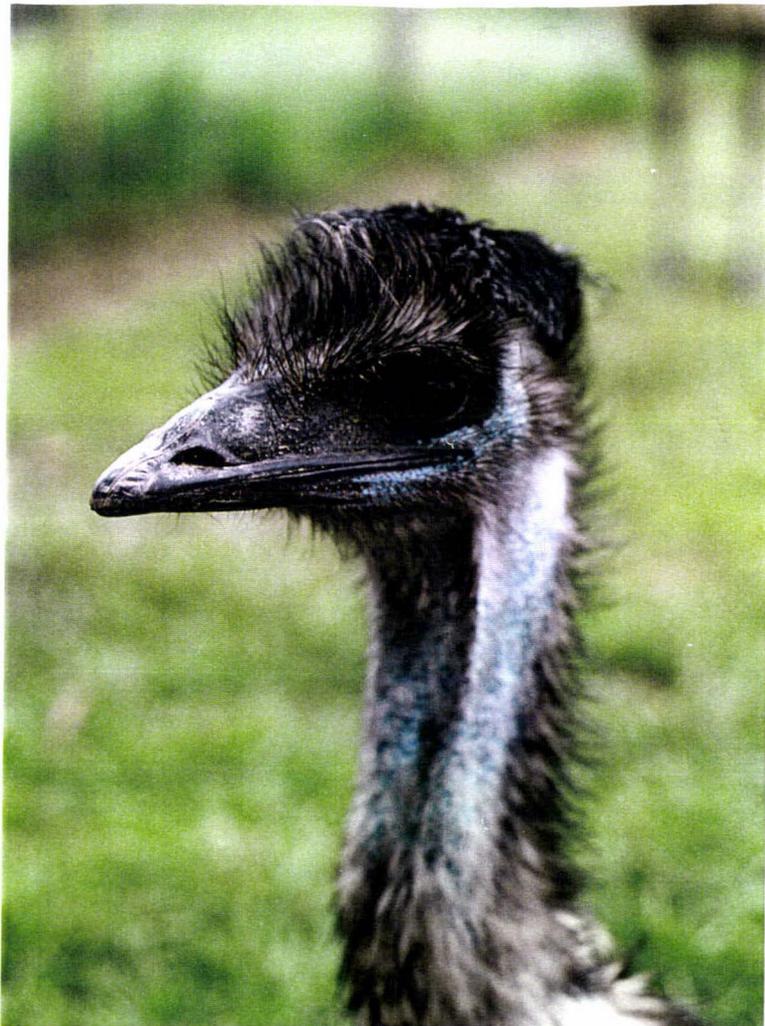
**PRODUCTION AND INCUBATION
IN FARMED EMU
(*Dromaius novaehollandiae*)**

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1996

**A thesis presented in partial fulfilment
of the requirements for the the degree of
Masters of Science in Ecology
at Massey University,
Palmerston North, New Zealand.**

I would like to dedicate this thesis to the memory of my Grandfather, Theo Zurcher,
for whom I had deep respect and admiration,
and
to Willie, Albert, Map and Kate.



“In the confrontation between the stream and the rock,
the stream always wins -
not through strength but by perseverance”

- *H. Jackson Brown, Jr.*

Abstract

The breeding, egg laying, incubation, chick survival and growth of emu (*Dromaius novaehollandiae*) were studied in a farmed population. Eggs were laid every 3 - 5 days between May to October with a peak in July. Birds laid in vegetation, or where absent, near fence lines or by artificial shelters. Clutch size was highly variable (range: 2 - 45) between individuals, and between seasons, and variability increased with the age of the hen. The fate of 578 artificially incubated eggs were recorded. Fertility levels were high (90%) but hatching success was lower. Embryonic mortality was greatest during the first trimester with a second smaller peak at the end of incubation. 434 chicks hatched, representing 68% of all eggs set and 83% of fertile eggs. Weight loss for the entire incubation period was 12.5% and was not correlated with embryo mortality. X-ray and ultrasound equipment were unsuccessful in determining egg fertility. Natural incubation was studied in two emu nests. Egg temperatures averaging 34.1°C and 31.7°C were lower and more variable than those used in artificial incubators. Eggs hatched after 51 - 54 days. In one nest, deserted eggs cooled to 12.2°C hatched when incubated artificially. Rates of egg turning varied, and two thirds of all egg turns were 90° or less, and only 12% were turned between 158.5° - 202.5°. Water loss during incubation was 10% of the initial egg weight and was greatest at the end of incubation. Males lost up to 30% of body weight during incubation. Survival, sex ratios and growth rates were determined for emu chicks hatching from 637 artificially incubated New Zealand eggs, and 105 eggs imported from Canada for incubation under class 1 quarantine conditions. Survival rates to three months were high (88%). Mortality due to hatch-related problems was restricted to the first week of rearing, and to weeks 8 - 12 when bone deformities became evident. Sex ratios were 50:50. The chicks lost weight after hatching but thereafter grew exponentially. There was no significant difference between male and female hatch weights, or rates of growth, but females grew faster and were heavier up to 18 months. Most birds that grew significantly slower from three weeks of age died within three months. The genetic identity and development of twin emu was described. DNA analysis indicated the twins were identical.

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CHAPTER ONE

General Introduction



General Introduction

Emu *Dromaius novaehollandiae*, large flightless cursorial birds endemic to Australia, are the only extant species in the family Dromaiidae (Cracraft 1973). A second species *D. diemenianus* (dwarf emu), sometimes regarded as a sub-species, is now extinct. Emu belong to the order Struthioniformes which includes three species of cassowary (*Casuarius casuris*, *C. bennetti* and *C. unappendiculatus*) now confined to Australasia; the African ostrich (*Struthio camelus*); two species of the South American rhea (*Rhea americana* and *Pterocnemia pennata*); and four species of the endemic New Zealand kiwi (the Northern brown kiwi *Apteryx mantelli*, the Southern brown kiwi *A. australis*, the great spotted kiwi *A. haastii*, and the little spotted kiwi *A. owenii*). Collectively, they are referred to as ratites by virtue of their raft-like sternum, which lacks a keel.

The first commercial emu farm was established in Western Australia in 1970 solely for harvesting emu leather (O'Malley 1989). Although that farm closed in 1973 it showed commercial leather could successfully be produced from emu. In 1976 a second farm was established by the Commonwealth Government to promote enterprises for Aboriginal communities, at Wiluna. In 1987 the Western Australian Government authorised the sale of 500 emu chicks bred at Wiluna, thus allowing the development of commercial emu farming in western Australia. Emu farms have now been established throughout eastern Australia and Tasmania, and have spread to other countries including Canada, U.S.A. and France (O'Malley 1994).

In New Zealand commercial emu farming is a rapidly growing industry, and in the early 1990s the status of emu switched from that of an exotic zoo animal to a highly valued commercial asset. This created a boom in the number of farmed emu in New Zealand. Fueled by high demand for breeding stock which outstripped supply, the price of emu rose steeply, and birds were intensively bred in pairs with eggs being artificially incubated. In an attempt to increase national flock numbers and genetic bloodlines, a small number of eggs were imported from Canada before sanctions were imposed by the Ministry of Agriculture and Fisheries (New Zealand), in November 1995. A major attraction of these large birds is their high productivity which can exceed 20 chicks per hen per year (O'Malley 1989) and the variety of emu products obtainable (Frapple

1994). These products include high quality, low fat meat, fine leather, and an oil that is highly prized by the cosmetic industry (Marshall 1994).

Emu are highly mobile and seasonally nomadic (Davies 1975, Dawson *et al* 1984), and a broad picture of the food preferences of wild emu has been provided by the work of Davies (1978) in Western Australia. Knowledge of emu social organisation is based largely on studies by Davies (1976), Curry (1979), and Coddington and Cockburn (1995). The social bonds may involve monogamy, polyandry and successive polyandry. Little information is available regarding lifetime pair bonding and currently there is little evidence that pairs re-form in successive breeding seasons (Davies and Curry 1978). Survivorship, growth rates and plumage sequences are unknown for young emu in the wild. Curry (1979) has provided most information on the development of young emu reared in captivity, but knowledge is incomplete.

The general conditions required for artificially rearing emu chicks have been described by Beutal *et al* (1983), Minnaar & Minnaar (1993), and O'Malley (1994), but information regarding the growth patterns of chicks and young emu is limited (O'Malley 1996). The hatching success of emu eggs under artificial incubation is highly variable (Beutal *et al* 1983, Potter 1994), but obtaining reliable hatching data from commercial farms is often difficult because of issues of commercial sensitivity (Deeming 1993). Very little is known about ratite embryology, especially in the emu (Minnaar and Minnaar 1993), making determination of the time of embryo death, and identification of the causes of mortality, difficult.

Behavioural studies of captive emu are important from ecological and agricultural standpoints. In view of the optimistic long term commercial prospects for emu farming, a knowledge of the breeding and incubation behaviour of captive emu in New Zealand is important for developing farm management programmes leading to optimal production and hatching success of emu eggs. At the start of this study emu productivity was low, and hatching success of eggs artificially incubated averaged just 30%. Three major reasons for this were identified: (i) the majority of New Zealand's breeding stock were young birds, and the consensus opinion among farmers was that older birds were more productive; (ii) little information was available either in New

Zealand or internationally on the commercial breeding of emu; and (iii) no research at all had been undertaken on developing farm management practices for emu in the New Zealand environment. Therefore, the primary aim of this study was to describe the egg laying behaviour, incubation and productivity of farmed emu in New Zealand.

The results of this thesis are presented in six chapters. *Chapter 2* investigates the egg laying behaviour of captive emu in relation to both seasonality, and egg laying site preference and re-use. Clutch size, the effect of egg sequence on egg size, fertility, and hatchability are also discussed. The determination of fertility levels and subsequent hatching success under artificial incubation is discussed in *Chapter 3*, with emphases on determining the stage of embryonic mortality, and early detection of fertility. The internal egg temperatures, and the frequency and magnitude of egg turning during natural incubation are determined in *Chapter 4*, and interpreted in light of the preceding results on artificial incubation conditions and hatching success. Findings on the post-hatching survival of artificially reared emu chicks, and the pattern of mortality of chicks up to three months of age are presented in *Chapter 5*. The sex ratios of the chicks and their rate of growth and development are also detailed. *Chapter 6* reports on a rare event of twinning in farmed emu, and on the genetic identity, and growth of the twin chicks. Finally, results from the preceding chapters are summarised in *Chapter 7* and used to formulate recommendations on the future captive management of farmed emu in New Zealand conditions.

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CHAPTER TWO

The breeding season and egg laying behaviour of emu



The breeding season and egg laying behaviour of captive emu

Abstract

Captive emu (*Dromaius novaehollandiae*) bred through the austral winter and spring, with egg laying beginning in May and ending in November. Seventy percent of eggs were laid from mid June mid to August. Laying was fairly synchronous, with a broad peak in July. Most (72.8%) birds preferred to lay near or in clumps of vegetation and, where this was not available, close to a fence line or an artificial shelter (98.4%). Only 2.8% of eggs were laid in open pasture and 72.0% of eggs were covered with vegetation after laying. The birds normally laid about 20 eggs in succession, one every 3 to 5 days (average: 4.4 ± 0.5 days). Clutch size was highly variable (range: 2 - 45) both between individual birds, and between laying seasons. Variability in clutch size, but not average clutch size, increased markedly with the age of the hen. The average egg laying season during 1994 was 68 ± 10 days (range: 15 - 124 days) and 83 ± 9 days (range: 23 - 140) in 1995. There was no significant difference in initial egg weights of New Zealand eggs laid in 1994 and 1995, but Canadian eggs imported in 1995 were significantly smaller than those produced in New Zealand in the same season. Chick gender could not be predicted based on egg size, as eggs producing male chicks were similar in size to those producing female chicks. Fertility did not vary with egg sequence, but the very last egg laid in a clutch was significantly less likely to hatch.

Key words: emu, *Dromaius novaehollandiae*, egg laying, clutch size, eggs, behaviour.

Introduction

Emu breed in winter throughout their range in Australia (Davies 1976). Mating and egg laying occurs mostly in the wet months, starting in late April, reaching a peak in early August (mid winter) and continuing through until September (Marchant and Higgins 1990). This pattern of reproductive activity can not be explained by "classical" mechanisms of seasonal breeding where increasing day length is the dominant

environmental cue (Marshall 1936), and emu are members of a comparatively small group of birds that are short-day breeders (O'Malley 1994, Cockrem 1995).

The general breeding biology of emu in the wild has been described by Davies (1968, 1976) and Coddington and Cockburn (1995), and Curry (1979) investigated the growth and behaviour of young emu within family groups. Beutal *et al* (1983) and Buttemer *et al* (1988) contributed experimental data obtained from artificial and natural incubation experiments, respectively.

Although extremely limited, preliminary evidence suggests that emu are opportunistic breeders, with a reproductive strategy suited to the semi-arid environment of Australia where rainfall, and therefore food supply, is both patchy and unstable. There is, however, little evidence of breeding responses to climatic factors (Marchant and Higgins 1990) although breeding is aborted completely during drought conditions (Davies 1976, K. Venn *pers. comm.*).

In the wild emu eggs are laid on the ground, often near vegetation (Gaukrodger 1925). They are usually laid in a shallow unlined depression, and are covered with plant material until shortly before incubation begins. Re-use of nesting sites in subsequent seasons has not been established. In captivity egg laying sites have not been studied, although Minnaar and Minnaar (1993) found that females selected multiple areas in an apparently random manner during the breeding season. Neither the breeding season, defined as the period during which eggs are laid (Baker 1938), nor egg laying patterns have been reported for captive farmed emu. There is little comparative information about egg laying patterns, nest site selection, clutch sizes, and reproductive age in wild emu although the timing of egg laying is said to be variable (Marchant and Higgins 1990).

This study presents detailed information on egg laying behaviour of domesticated emu in New Zealand over two consecutive breeding seasons. The study investigates egg laying site preference and the degree of site re-use and predictability - important considerations on commercial emu farms where eggs are gathered manually for artificial

incubation. The breeding season of emu in captivity, and the interval between eggs in a clutch are reported. Clutch size, the effect of egg sequence on egg size, fertility, and hatchability are also discussed. Comparative data on egg size are presented for eggs imported from Canada.

Methods

Observations of domesticated emu were carried out at an emu farm near Colyton (40°14'S, 175°39'E), in the lower North Island of New Zealand. Daily patterns of egg laying were recorded over two breeding seasons, between May and October 1994, and May and November 1995. All eggs laid were removed for artificial incubation, either on the evening they were laid or early the following morning. Where egg laying intervals were regular, breeding pens were surveyed for eggs by the emu farmer every three days. In pens where egg-laying was erratic, searches were carried out daily.

In 1994, 11 pairs of emu (two - four years old) were observed regularly, with four pairs used as focal birds for behavioural observations. When possible, egg laying behaviour was observed. Breeding bird numbers increased to 18 pairs ($\sigma^{\circ}:\text{♀}$) and 1 trio ($\sigma^{\circ}:\text{♀}\text{♀}$) in 1995. When several females were expected to lay on the same evening, observations were divided between all birds using a 5 minute scan sampling technique. Once a female began egg laying, *ad libitum* sampling was used to record egg laying behaviour in detail. The time and duration of egg laying, and the extent of egg covering by the male were noted.

The position of each egg in the pen was recorded and distances from the nearest fence posts and vegetation were measured. To minimise disturbance, egg positions were noted (and marked in the pens when necessary) when the eggs were collected, and then a number of egg positions were measured together later. In the 1995 breeding season egg positions were marked on maps of individual pens as the eggs were located and collected. The general condition of the egg (i.e. clean, wet, muddy or shell-less), egg laying date, and date of egg collection were recorded. Using a white pencil, all eggs were labelled with the number of the female and a unique egg number. Eggs were

weighed (± 1 g) using electronic scales, and length and width measured (± 0.1 mm) with Vernier calipers.

Clutch size was defined as the total number of shelled (viable) eggs produced by an individual female in one breeding season. A few eggs contained yolk and albumen surrounded only by shell membranes or, in rare cases, a very brittle layer of thin white shell. As these could not be artificially incubated they were considered non-viable. Some eggs were laid with only a thin pale green shell but were deemed viable because usually they were found intact and could be successfully incubated artificially.

In 1995, 105 eggs were imported to New Zealand from Canada and incubated artificially under quarantine conditions (*Chapters 3 and 5*). The eggs were stored in Canada for 2 - 24 days before importation. Once in New Zealand, the eggs were weighed and measured immediately before artificial incubation. Original fresh weights of these eggs were unavailable. Egg laying dates and parentage were known, but clutch sizes and other breeding data were not available for comparative analysis.

Statistical analyses

Data were analysed using Student's *t* tests, linear regression analysis, analysis of variance, chi-squared analysis (Bailey 1981), and general linear model statistical analyses (Sokal and Rohlf 1982) as appropriate. The software packages Minitab (Version 9.0 for win), SigmaPlot (Version 3.10 for win) and Excel (Version 5.0) were used.

Egg measures are reported as means \pm standard errors unless otherwise indicated. A two sample sign test for non-normally distributed data was used to analyse the duration of egg laying season in subsequent years. Differences in clutch size and egg laying season in first time breeders (female emu laying eggs in their first season, regardless of age) and experienced breeders (female emu who had produced eggs in a previous breeding season) were investigated respectively using an unpaired *t* test and a two sample sign test. Productivity of birds over time was tested in two ways. The non-parametric Wilcoxon test for matched pairs was used to assess whether clutch size

increased with time, and a parametric Student's *t* test was used to determine whether experienced breeders laid a greater number of eggs than first time breeders. For these comparisons, clutches were only used from egg-laying females present in both years of the study.

Results

Breeding season

Egg laying in 1994 occurred between 22 May and 1 October, and in 1995 between 4 May and 3 November. The total number of eggs produced over the two breeding seasons were normally distributed (Figure 2.1). In both years the number of eggs laid increased steadily from May to late June - early July before gradually declining through August and September. First time breeders (generally two year old birds) followed a similar pattern, although their first eggs were often later than those of experienced breeders.

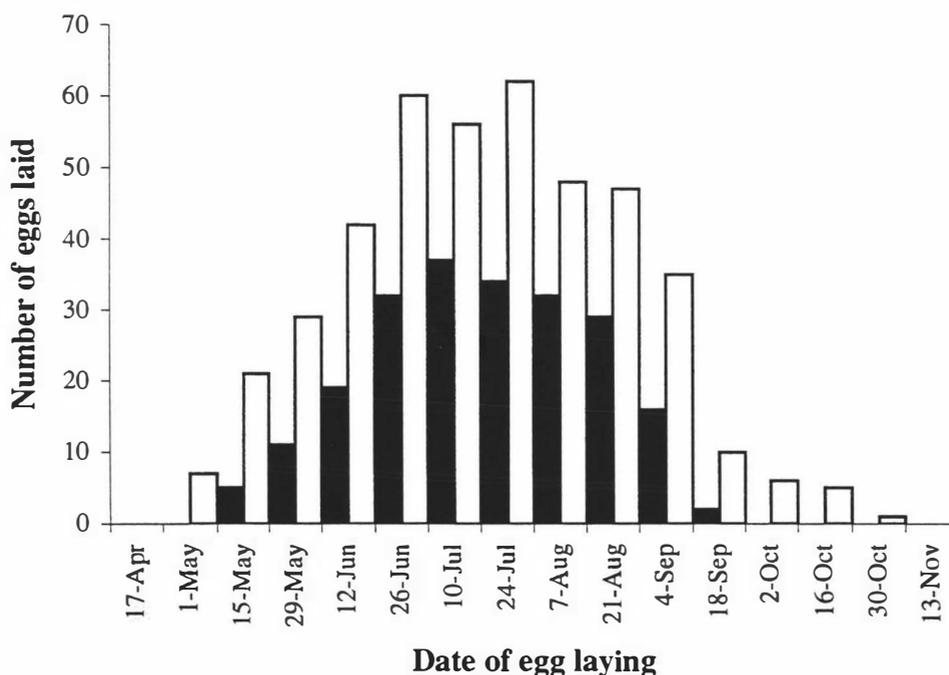


Fig 2.1 Distribution of egg laying dates (two week intervals) for farmed emu during the 1994 (■) and 1995 (□) breeding seasons, at Colyton, New Zealand.

Egg laying sites

Egg laying sites were consistent in some birds but unpredictable in others. Some birds were adept at hiding their eggs in vegetation making egg location difficult. Most birds laid their eggs near or in clumps of vegetation and, where this were not available, close to a fence post, corner of the pen, or artificial shelter (Table 2.1). Mature breeders and young birds with consistent laying patterns generally laid their eggs in two or three main sites, and these were well scattered around the pen. Once egg laying patterns and nest sites were established, egg laying was generally random within those areas. Some birds laid 3 - 5 eggs in one area before changing but this was not always consistent. Older, highly productive birds (i.e. laying 30+ eggs per year) tended to lay in a single site which was re-used over successive seasons.

In some circumstances it was difficult to determine the re-use of egg laying sites during subsequent breeding seasons, because in seven pens a large proportion of the vegetation was suddenly removed by the farmer. In two pens where this occurred clutch sizes were considerably reduced. When vegetation, or haybales were added to several pens these were often used as nest sites. Interesting, when trees and shrubs were absent in pens, areas of straw were the preferred laying sites, but when straw and vegetation were both present, eggs were most frequently laid among the plants.

Table 2.1 Egg laying site preferences in captive emu, at Colyton, New Zealand.

| Egg laying site | Vegetation present in pen | Vegetation absent in pen | Vegetation absent Haybales present | Total |
|---------------------------------|---------------------------|--------------------------|------------------------------------|-------|
| Vegetation | 321 (72.8%) | - | - | 321 |
| Fenceline or Artificial shelter | 106 (24.0%) | 127 (98.4%) | 14 (20.9%) | 247 |
| Open pasture | 14 (3.2%) | 2 (1.6%) | 2 (3.0%) | 18 |
| Haybales | - | - | 51 (76.1%) | 51 |
| Total | 441 | 129 | 67 | 637 |

On 12 occasions over the two seasons shell-less eggs were laid with the yolk and albumen surrounded only with a shell membrane. All of these eggs were laid in the open and remained uncovered. The same birds laid their normal, viable eggs in vegetation and left them well covered, suggesting that the female knew when a defective egg had been produced.

Egg laying behaviour

Observations of birds laying a total of 119 eggs showed that egg laying behaviour varied considerably between females. Before laying, females conspicuously paced the fence line for periods of 10 minutes to over 2 hours. Once a nest site was selected, the female crouched on her hocks and, when laying in vegetation, shuffled forward into it. The birds never sat down completely during egg laying but placed their heads near the ground and commonly swayed. Immediately before egg laying, the female's back was held vertical, the cloaca tilted downwards, and frequent contractions were clearly visible. During laying, the male remained between 0.5 - 4.0 m from the female. The duration of egg laying from crouching to the appearance of the egg was 7 - 15 minutes.

In six cases (5% of all observed egg laying events) where birds were laying along the fence line next to the adjacent pen, egg laying was disrupted by the neighbouring birds. Aggressive pecking and kicking through the fence by neighbouring females caused laying birds to become stressed and to stop egg laying. Disrupted birds resumed pacing for up to 40 minutes, and later resumed egg laying in another area of the pen.

New laid eggs were wet and shiny, but dried rapidly. Following laying the female walked away from the site, preening frequently. The male then looked at the egg and in 72% (n = 86) of cases, spent up to 15 minutes concealing it with leaves, twigs and grass. Some males then returned to the egg repeatedly, apparently rechecking the egg and its covering. On only eleven (9.2%) occasions did the female participate actively in covering the egg.

Egg laying times and intervals

Mature breeding emu mostly laid their eggs in the late afternoon and early evening and the time of egg laying could be predicted to within 40 minutes, but first time breeders were not so consistent.

The average interval between the eggs in a clutch was 3 - 4 days (62% of intervals, n = 309, range: 2 - 33). The shortest interval between viable eggs was 2 days and this often resulted in a smaller than average egg being laid. In all instances where eggs were laid one day apart, they were shell-less, suggesting that they had not remained in the shell gland for sufficient time to gain a shell. Egg intervals did not differ with the age of the breeding female, nor with the number of eggs produced (Figure 2.2).

In four clutches, intervals of 26 - 30 days between eggs were recorded. This was not correlated with month of egg laying or the number of eggs previously laid, as large intervals between eggs occurred near the start, middle and end of clutches of various sizes. Long intervals between eggs were more common during the 1994 season but the reasons are unclear. Following extended breaks in the egg laying cycle, the intervals between subsequent eggs in the clutch were highly variable. In a small number of birds (n = 11) the interval between subsequent eggs increased, indicating the approaching end of egg production for the season. In general, egg laying ended abruptly and unpredictably.

Of nine breeding females seven produced eggs over a longer period of time in 1995 than in 1994 (\bar{x} = 89 days and 72 days, respectively), although seasonal changes in egg laying duration (the time from when the first egg in a clutch is laid, until the last is laid), was not significantly different between the two seasons ($t_{0.05[7]} = 0.988$; $p = 0.352$). First time breeders (n = 11) produced eggs for an average of 76.2 ± 9.6 days, while experienced breeders (n = 18) laid for 77.9 ± 9.5 days. These periods were not significantly different ($t_{0.05[7]} = 0.124$; $p = 0.93$).

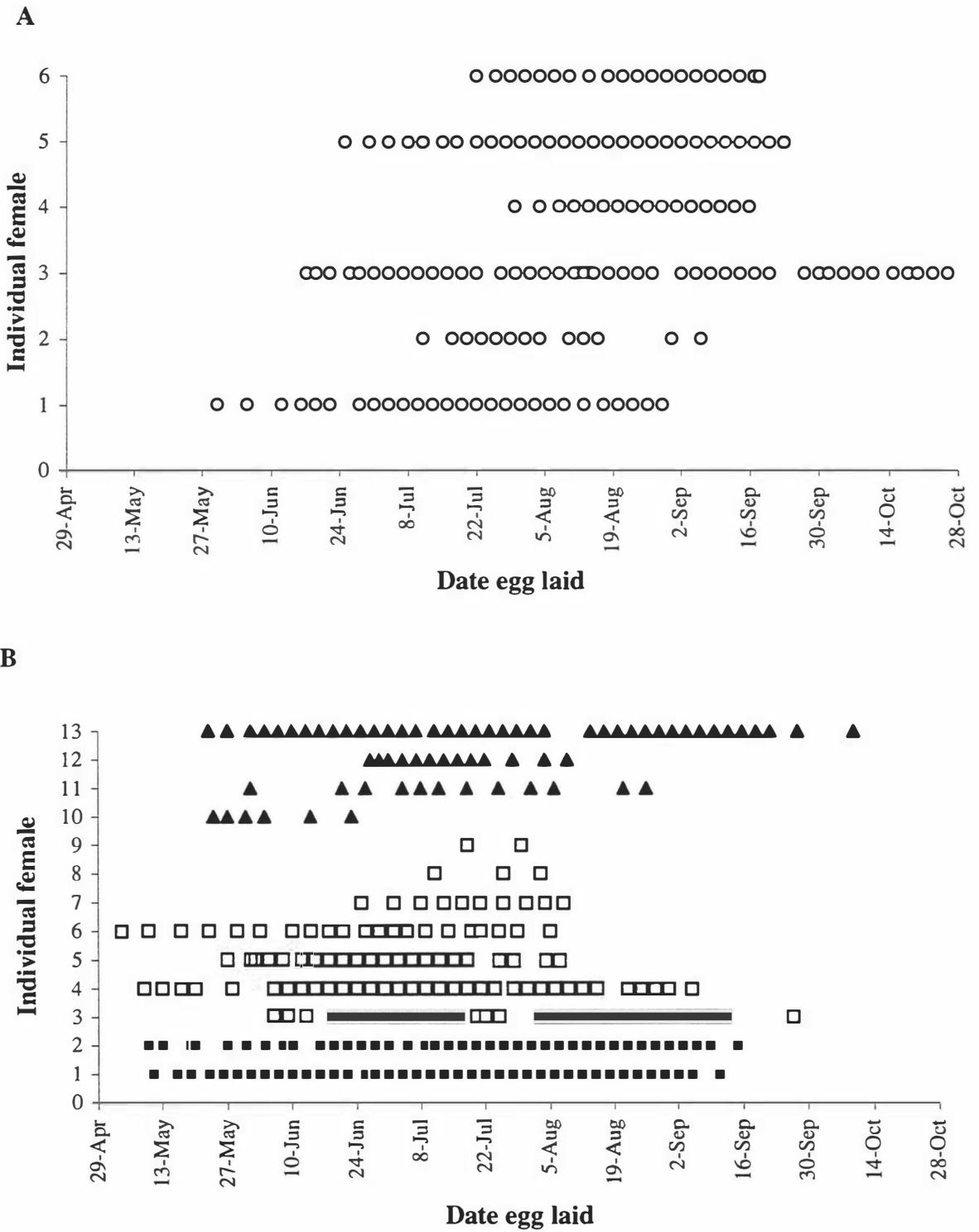


Fig 2.2 Egg laying intervals and clutch size for: **A** 2 year old birds, and **B** birds aged 3 (■), 4 (□) and 5+ (▲) years in 1995.

Clutch size

A total of 637 eggs were laid over the two breeding seasons by 20 females. In 1994, 214 eggs were produced by 11 breeding females, resulting in an average clutch size of 19.5 ± 2.2 (range: 9 - 31) eggs. This increased to 22.3 ± 3.1 (range: 2 - 45) eggs per female in the subsequent season when 19 breeding females laid a total of 423 eggs.

Emu were paired up at two years of age. Fifty seven percent ($n = 14$) of these birds bred successfully, averaging 22 eggs per female in their first breeding season. On average two year old emu laid later in their first season. Unfortunately it was difficult to obtain the clutch sizes and breeding success of these birds in the following year as many had been sold and moved elsewhere. It was, therefore, not possible to determine the proportion which did not lay as two year olds but subsequently produced eggs as three year olds.

The clutch sizes of individual females that did not leave the study farm varied between seasons (Table 2.2). For productive females present in both years of the study, clutch sizes showed no consistent increase with age ($z = 0.357$, Prob ($|z| > 0.357$) = 0.72). There was also no significant difference in the number of eggs laid by first time breeders and experienced breeders ($t_{0.05[8]} = 0.539$; $p = 0.594$). In fact, females laying for the first time produced more eggs on average ($\bar{x} = 22.36 \pm 3.31$) than experienced breeding birds ($\bar{x} = 19.94 \pm 2.86$). The average number of eggs laid per female each year did not increase with age but there was more variability in clutch size in older birds (Figure 2.3).

Table 2.2 Variation in individual clutch size at Colyton, New Zealand, during two successive breeding seasons (1994 and 1995).

| Female | Age [#] | 1994 | 1995 | Variation |
|--------|------------------|------|------|-----------|
| I29 | 2 | 18* | 39 | + 21 |
| A26 | 3 | 15 | 2 | - 13 |
| P21 | 3 | 17 | 3 | - 14 |
| P22 | 3 | 23 | 22 | - 1 |
| S27A | 3 | 16 | 21 | + 5 |
| S27 | 3 | 31 | 34 | + 5 |
| O13 | 3 | 30 | 32 | + 2 |
| O01 | 4 | 9* | 6 | - 3 |
| O02 | 4 | 15 | 12 | - 3 |
| V08 | 25+ | 27 | 40 | +13 |

year of age in 1994

* excluding 3 soft-shelled eggs

* excluding 5 soft-shelled eggs

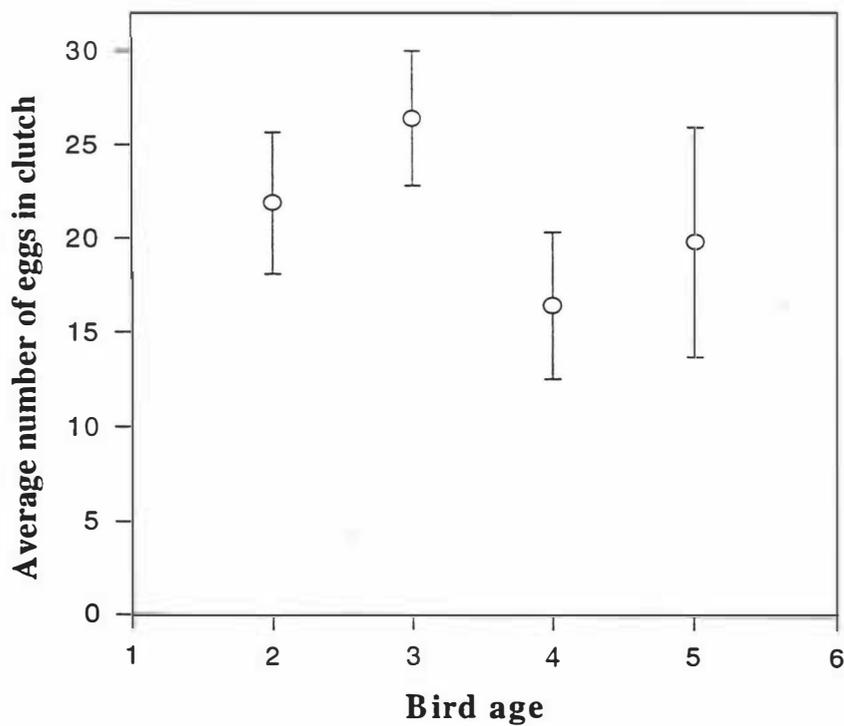


Fig 2.3 Average clutch size in relation to age of the female in 1994 and 1995 at Colyton, New Zealand.

Egg measurements

There was no significant difference ($t_{0.05[629]} = 1.82$; $p = 0.070$) in initial egg weights between 1994 and 1995 so data from the two years were combined. Egg width and length were slightly greater in 1995, and slightly more variable in 1994 (Table 2.3). The average initial weight of all New Zealand eggs was 580.80 ± 2.54 g ($n = 637$, range: 335 - 724 g). New Zealand eggs laid on the study farm in 1995 were significantly larger than those imported from Canada ($t_{0.05[526]} = 2.54$; $p = 0.012$). There was no significant difference in initial egg weight of eggs producing males and those producing females (585.24 ± 4.21 g and 580.95 ± 5.28 g, respectively; $t_{0.05[355]} = 0.64$; $p = 0.52$).

Table 2.3 Measurements (\pm S.E.) of New Zealand eggs in 1994 and 1995 at Colyton, and imported Canadian eggs.

| Egg measurements | New Zealand 1994 | New Zealand 1995 | Canada 1995 |
|--------------------|----------------------------------|----------------------------------|----------------------------------|
| Initial weight (g) | 575.02 ± 4.66 (335 - 705) | 583.78 ± 3.00 (401 - 724) | 566.84 ± 5.82 (385 - 694) |
| Length (mm) | 131.59 ± 0.50 (107 - 152) | 132.83 ± 0.36 (111 - 149) | 132.32 ± 0.62 (110 - 145) |
| Width (mm) | 88.02 ± 0.27 (74 - 97) | 88.55 ± 0.19 (78 - 96) | 86.85 ± 0.33 (77 - 94) |

Birds that bred in both 1994 and 1995 tended to produce larger eggs in 1995 but the difference was not significant (586.9 ± 22.2 g and 607.2 ± 12.1 g, respectively; $t_{0.05[18]} = 1.48$; $p = 0.09$). First time breeders ($n = 11$) had significantly smaller eggs (546.7 ± 17.2 g) than those laid by experienced breeders (587.7 ± 10.4 g, $n = 18$; $t_{0.05[27]} = 2.17$; $p = 0.019$) but this difference was confounded by the effect of bird age. However, the median egg weight for eight first time two year old breeders was 525.6 ± 16.1 g, significantly smaller than eggs produced by older (3+ years) first time breeders ($\bar{x} = 602.8 \pm 29.3$ g; $t_{0.05[9]} = 2.44$; $p = 0.019$), suggesting the primary factor influencing egg size was age. This was confirmed by comparing all two year old breeders with older breeding females.

Egg size from two year old breeding birds was significantly smaller ($\bar{x} = 525.6 \pm 16.1$ g) than those produced by older birds ($\bar{x} = 590.7 \pm 9.1$ g), regardless of breeding experience ($t_{0.05[24]} = 3.63$; $p = 0.001$).

Each female tended to lay eggs of a particular shape and dimension (Figure 2.4). These differences enabled the maternity of eggs laid by the trio (1 σ :2 ϕ) to be ascribed with reasonable certainty.

Often, a pattern was evident in the change of egg size with egg sequence, with egg size tending to increase over the first five eggs laid. Subsequent decreases and increases occurred in cycles of six to eight eggs. Weight differences between the smallest and largest eggs in a clutch ranged from 19 to 230 g.

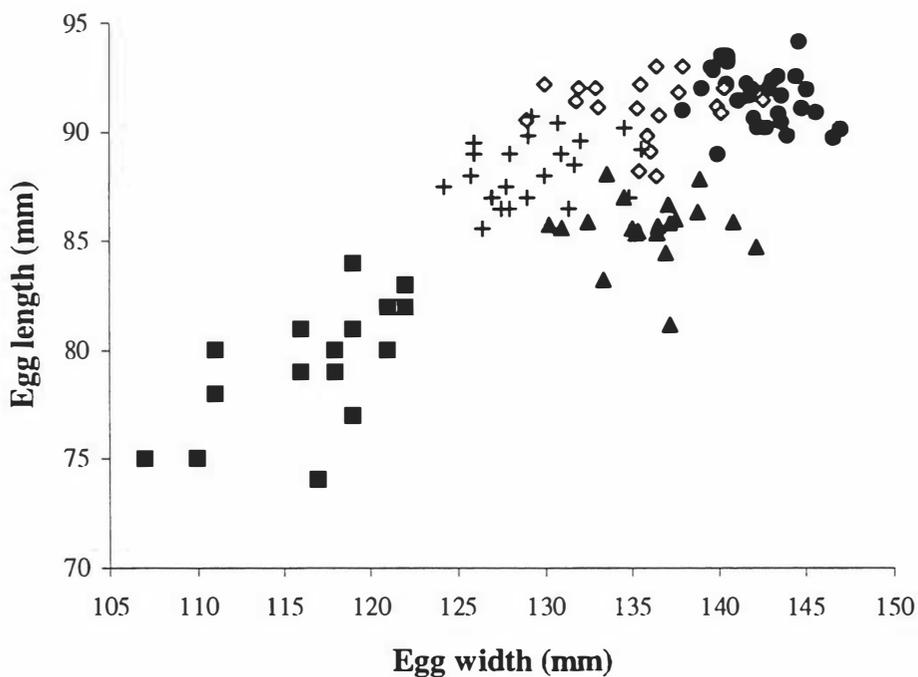


Fig 2.4 Egg measurements from three individual breeding females (● O725, $n = 40$; ▲ 9946, $n = 29$; ■ I29, $n = 18$) and two females in a trio (◇ P22, $n = 22$; + = S27A, $n = 21$) at Colyton, New Zealand, in 1995.

Egg sequence and hatching success

To determine the effect of egg sequence on hatching success, the first, middle and last eggs of each clutch were selected and the outcome determined. The first and middle eggs did not differ much in hatchability but the last egg in a clutch was significantly less likely to hatch ($\chi^2 = 9.100$, $df = 2$, $p = 0.05$). When hatching success was calculated for randomly selected eggs laid in the first, middle, and last third of a clutch, eggs laid in the middle tended to have higher hatching success than those at either end, but only the very last egg laid had a significantly lower probability of hatching.

Interestingly, fertility did not vary with egg sequence, or decline as the breeding season progressed. There was no significant decline in levels of fertility in individual breeding females through time ($z = 0.674$, $\text{Prob}(|z| > 0.674) = 0.50$), nor in hatching success of eggs in individual clutches in subsequent seasons ($z = 1.376$, $\text{Prob}(|z| > 0.376) = 0.17$). There was also no indication of egg weight affecting embryo mortality directly (Figure 2.5) although unhatched fertile eggs were heavier than fertile eggs which successfully hatched (*Chapter 3*).

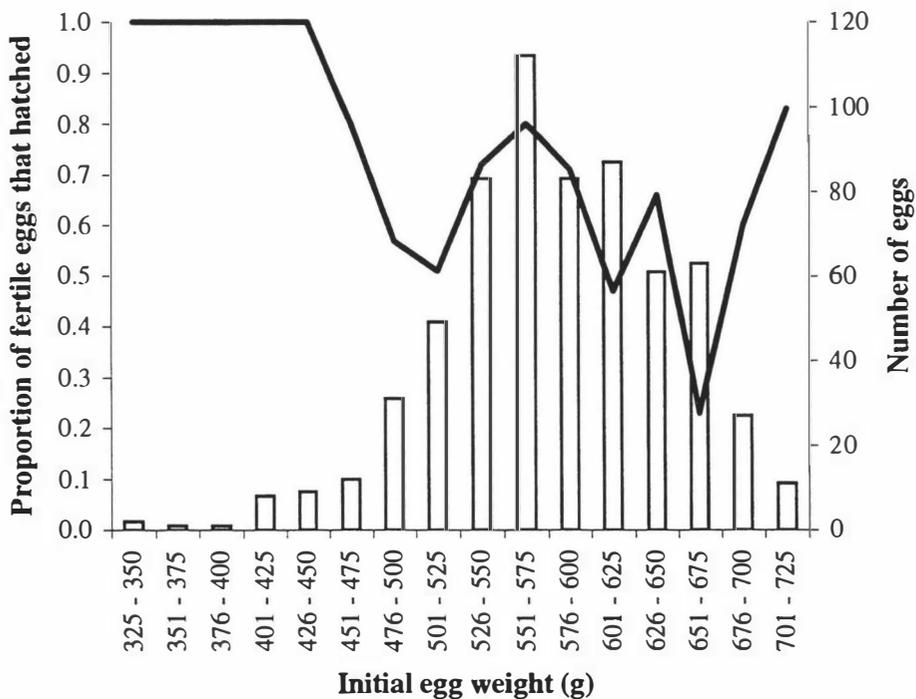


Fig 2.5 Proportion of fertile eggs that hatched in relation to initial egg weight (line), and the number of eggs in each weight category (bar).

Discussion

Breeding season

The egg laying season for captive emu in 1994 was similar to that reported for free-living emu (late April to September (Marchant and Higgins 1990, O'Malley 1994)), although in 1995 laying continued until the end of October. In both years the peak in egg laying occurred in July, one month earlier than the August peak reported by Marchant and Higgins (1990). It is common for the breeding season to be longer in captive than in wild birds, but the difference observed here is less pronounced than has been reported in other ratites such as the ostrich (*Struthio camelus*, Jarvis *et al* 1985b), rhea (*Rhea americana*, Bruning 1974) and kiwi (*Apteryx* spp, in the wild: McLennan 1988, Potter 1989; in captivity: Cockrem *et al* 1992).

The age of first breeding in emu is generally two years. During this study only slightly more than half of all paired two year old females laid eggs in their first season. This may reflect current farm management practices and it would be useful to compare the production of first time breeders that are forced paired (as was the case here) with those in communal pens where they can chose their mate. The youngest female known to lay eggs was 16 months of age, and the youngest male to produce fertile eggs was 12 months old (*unpubl. data*). The oldest productive bird (producing 35 - 40 eggs per year) was at least 25 years old, exceeding by at least five years the productive lifespan of captive female emu reported by Jensen *et al* (1992). The productive lifespan of free living emu is unknown.

Egg laying sites

Eggs were laid in a variety of sites, apparently randomly by some birds but in predictable places by others. It was not clear whether this variation in the predictability of egg laying sites related more to the age of the bird or to the design of the pen, but the use of multiple, randomly selected sites in farmed emu may be maintained by the continual removal of eggs for artificial incubation. The selection of a new nesting site by the female when her eggs disappear, and the concealing of eggs with vegetation by the male, may be of adaptive value against nest predators in the wild.

Small shrubs should be planted in breeding pens to reduce the number and scatter of laying sites. Dummy eggs can also be left out to encourage emu to relay in the same site. Birds can be induced to lay in straw nest sites and even artificial shelters (*unpubl. data* (from farms elsewhere in New Zealand)) if nesting vegetation is completely absent from pens. In pens containing just pasture and no other form of shelter or nesting refuge, eggs are most likely to be laid in the corners of the pen.

Egg laying behaviour

Once compatible breeding pairs are established they should be kept together in the same breeding pen in successive years to maximise predictability in egg laying sites. Where inter-pair disturbance is high, black shade cloth should be fitted along fence lines between adjacent pens to act as a visual barrier to reduce egg laying disruptions.

The timing of egg laying in captive emu was fairly predictable although there was individual variation. Mature females laid their eggs at a similar time of the day on each occasion, usually during late afternoon - early evening, and egg laying events could often be predicted to within ± 30 minutes.

Preferred egg laying sites should be noted and records kept for each pair on the time interval between eggs and the time of day egg laying occurs. This can lower pair disturbance by reducing the amount of time spent searching for eggs and the amount of time eggs remain outside thereby minimising the chance of eggs becoming wet, damaged or contaminated with bacteria that can reduce hatchability.

Intervals between eggs

The average interval of 3 - 4 days between successive eggs was longer than those previously recorded (Beutal *et al* 1983, Buttemer *et al* 1988). Buttemer *et al* (1988) recorded an average egg laying interval of 2.9 days in captivity, with the interval declining as breeding progressed (4 days decreasing to 3 then 2). This general three day laying cycle was also reported by Minnaar and Minnaar (1993), although they did not report the daily intervals between eggs in a clutch. An interval of two days has been reported in wild emu (Davies 1976).

A similar change occurs in kiwi, with the intervals between eggs in a clutch in wild kiwi (McLennan 1988, Potter 1989) being greater than those recorded in captive birds (Cockrem *et al* 1992). The extended egg laying season and increased clutch sizes seen in some captive ratites is probably a consequence of a regular food supply throughout the year.

A small proportion (1.7%) of all eggs produced in both seasons were laid without shells. Most of these eggs were laid only one to two days after the previous egg in the clutch, suggesting they had spent insufficient time in the oviduct. In most cases where eggs were laid after a short interval of two days they were smaller than the average egg size of the clutch. Also, they often had a pale green shell indicating that not all of the shell layers had been laid down.

Clutch size

The number of eggs laid by domesticated birds is often extremely variable (Romanoff and Romanoff 1949). In captivity, individual emu can be prolific layers, producing up to 45 eggs per breeding season. Reports from elsewhere state that emu can regularly lay 60+ eggs although the frequency of this is unknown (L. Baldwin, *pers. comm.*). The captive emu averaged 12+ more eggs per clutch than wild emu (Buttemer *et al* 1988), probably because in captivity eggs are removed as they are laid. Records of emu clutch sizes in the wild are limited, but Buttemer *et al* (1988) and Coddington and Cockburn (1995) reported clutch size ranged from 7 - 11 eggs in three nests, and Curry (1979) recorded clutch sizes of 6, 4, 2 and 1 eggs from four unincubated nests. These very small numbers of eggs recorded by Curry (1979) may be due to incomplete clutch formation. Buttemer *et al* (1988) found that the male initiated incubation before clutches were complete, usually after the sixth egg was laid. Captive males do not generally incubate a second clutch after the first, but Coddington and Cockburn (1995) states that females routinely engage in successive polyandry and will often lay two, or even three, clutches for natural incubation. Under natural incubation in captivity, the number of eggs produced decreases with each successive clutch (Marchant and Higgins, 1990).

Clutch size also tends to be larger in other ratites when they are held in captivity (ostrich: Hurxthal 1979, Stuart 1982, Jarvis *et al* 1985a; kiwi: McLennan 1988, Potter 1989, and Cockrem *et al* 1992). For both wild and captive birds clutch size may be dependent on the abundance of fresh vegetation in the preceding summer months (Davies and Curry 1978). In Western Australia this is directly correlated with rainfall.

In a classic study of four egg-producing breeds of domestic chicken, Romanoff and Romanoff (1949) found the frequency distribution curve of individual egg production was not symmetrical, but skewed towards low production. This skewness was typical of all egg-producing breeds. A similar trend was found in this study. A common view is that the number of eggs laid per pair increases with age (Minnaar and Minnaar 1993) and O'Malley (1989) stated that emu in their first laying year, at about 20 months of age, will lay an average of 9 to 10 eggs each, while second year birds can average over 20 eggs per bird with some individuals laying as many as 50 eggs. This view is not supported by our data, where no significant increase in productivity occurred until birds were 5+ years old. What did happen, however, was that clutch sizes became increasingly variable with age. Rather than laying small first clutches, young birds tended not to lay at all. Possibly first time breeders should be run in groups and allowed to choose their own mates.

Throughout New Zealand, clutch sizes ranged from 2 - 45 eggs and, overall, the mean number of eggs laid per female was 23 eggs per female per year (Appendix ii). The average national egg laying period, calculated from 58 clutches of eggs laid all around New Zealand, was 85 ± 5 days (range: 12 - 188 days). Marchant and Higgins (1990) reported little variation in clutch size, with a mean of nine eggs, and a maximum of 24 eggs.

Egg measurements

Eggs laid during this study tended to be narrower and lighter than those noted by Beutal *et al* (1983) and Marchant and Higgins (1990), but similar to those described by Curry (1979). The few data available on the size of eggs in wild emu (Marchant and Higgins 1990) suggest that wild eggs may be larger than eggs laid in captivity. Geographical

variation in emu egg size is unknown, but Davies and Curry (1978) concluded that seasonal differences may be large. Whether this holds for captive emu around New Zealand would be interesting to test. Welty and Baptista (1988) claim younger birds often lay both smaller eggs and smaller clutches than older birds. This was confirmed in part, with younger birds laying significantly smaller eggs than those produced by older birds, but in this study clutch size showed no consistent increase with age or breeding experience.

Differences in egg size between wild and captive birds occur in other ratites. Both Bertram and Burger (1981) and Keffen and Jarvis (1984) showed wild ostrich eggs were, on average, smaller than those of domesticated birds, and kiwi eggs laid in captivity (Reid 1981, Goudswaard 1986) are lighter than those laid in the wild (McLennan 1988, Potter 1989).

Ar *et al* (1996) reported that both exceptionally small and exceptionally large eggs of captive ostrich tend to have decreased hatchabilities. There was also a significant negative correlation between initial egg weight and the need for chicks to receive hatching assistance (Ar 1996). We found little evidence for size mediated mortality during the study, although unhatched fertile eggs tended to be heavier than hatched fertile eggs (*Chapter 3*).

Factors affecting egg production

Emu vary widely in the date when they first lay and laying date is not a good predictor of the total number of eggs that will be produced. Also, individual birds were variable across seasons in their date of first laying. The start of egg laying in previous seasons did not appear to reflect the onset of egg production in subsequent seasons. Wind, rain, and sudden changes in environmental temperature and humidity may have a temporary effect on the reproductive activity of the hen. Abrupt changes in management - both environment and nutrition, or a severe scare, may prematurely end egg production for the remainder of the breeding season.

Egg laying in emu may also be influenced by the broodiness of the male and can lead to a potential decrease in egg production. Some farmers state that once a male begins sitting on a nest he stops mating with his mate. Some males also appeared more broody than others, as indicated by their early responses to the four clutches of dummy eggs placed in pens to induce natural incubation (*Chapter 4*). Near the end of the breeding season it was difficult to induce males to begin sitting and this may have been related to bird age, nutrition, or endocrinology. Martin *et al* (1994) found levels of testosterone, responsible for stimulating mating behaviour, to be considerably reduced at the onset of natural incubation. The trigger that releases incubation in male emu is not known and a number of factors, such as the accumulation of eggs, a reduced photoperiod, or change in environmental temperatures have been suggested (Davies 1976). The first factor may be disregarded as in two instances during this study males began sitting on only one egg (*unpubl. data*), but further research in to the factors triggering natural incubation is required.

The process of domestication has already produced enforced changes in captive emu breeding behaviour. Through selective breeding, the provision of adequate nutrition, and breeding management regimes, such as the pairing of breeding birds, egg numbers are increasing and it is not known what long term effect this will have on the female.

The ability of a female emu to lay a large number of eggs depends to a large extent on her physical condition because egg formation constitutes a significant drain on bird strength and resources (Jensen *et al* 1992). The body weight of emu changes with both age and season (*unpubl. data*). Underweight birds can not endure long and persistent egg laying cycles (Minnaar and Minnaar 1993), and breeding performance is impaired in excessively over weight birds (chickens, *Gallus gallus*: Scott *et al* (1982), ostriches: Angel (1993), and kiwi: Goudswaard (1989)). Male emu are very rarely allowed to sit and incubate eggs and although the males still show an increase in body weight at the beginning of each breeding season (*unpubl. data*), this is not lost during the natural incubation of the eggs.

Conclusions

In conclusion, younger birds tended to lay smaller eggs and this was directly related to age rather than breeding experience. Data presented here dispute the widely held view that clutch size increases with age and breeding experience, and although there was no consistent increase in clutch size, clutch variability increased with age. Interestingly, in this study, first time breeders tended to produce more eggs on average than experienced breeding birds. The finding that egg size did not appear to affect mortality directly, also does not lend support to the contention that extremely small eggs and extremely large eggs are less likely to hatch.

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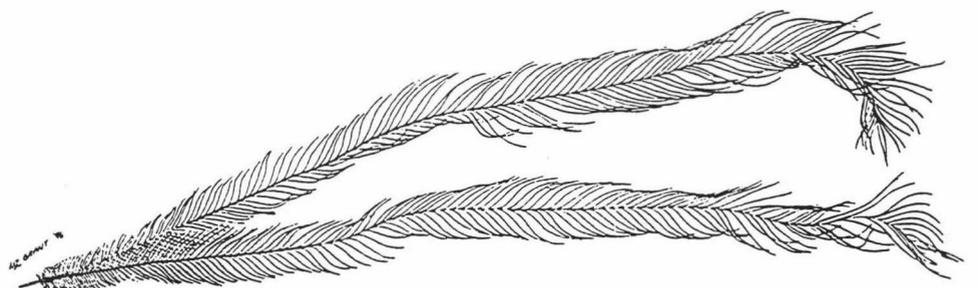
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CHAPTER THREE

Artificial incubation of emu eggs



Artificial incubation of emu eggs

Abstract

Over two seasons 637 eggs from domesticated emu (*Dromaius novaehollandiae*) were incubated artificially and the fate of 578 of these recorded. Fertility levels were high (90.3%), but 88 fertile eggs contained embryos that died before hatching (16.9% of fertile eggs set). Embryonic mortality was greatest (55.7%, n = 49) during the first trimester of artificial incubation with a second smaller peak in mortality occurring at the end of incubation. Development was variable and ranged from a small blood streak and membrane development, to chicks that died at point of hatch. A total of 434 chicks successfully hatched, representing 68.1% of all eggs set (n = 637) and 83.1% of known fertile eggs (n = 522). Weight losses during incubation were measured in order to assess the effect of this on embryo viability. The average total weight loss for the entire incubation period was 12.5% and did not appear to be correlated with embryo mortality. X-ray and ultrasound equipment was used in an attempt to determine emu egg fertility at an early stage, but both methods proved unsuccessful.

Key words: emu, *Dromaius novaehollandiae*, fertility, embryonic mortality, artificial incubation, chicks, X-ray, ultrasound, growth.

Introduction

The hatching success of emu eggs under artificial incubation is highly variable (Bowthorpe and Voss 1968, Curry 1979, Birchard *et al* 1981, Beutal *et al* 1983), but is usually low. For instance, in artificially incubated fresh eggs and in eggs that had initially been incubated by sitting birds Potter (1994) recorded success ranging from 46 to 76% over five seasons. Although no rigorous comparisons were made he concluded that the male emu appeared to be more successful than the artificial incubator at triggering embryo development. Burger and Bertram (1981) showed that most ostrich (*Struthio camelus*) eggs in incubators had lower hatching success and produced a greater proportion of weak chicks than those incubated under birds, suggesting that current artificial incubation methods may not always lead to maximum hatching success.

Reported levels of hatching success obtained by New Zealand emu farmers in 1993 averaged just 30% (*unpub. data*), and it was widely believed by farmers that this reflected low levels of fertility. Obtaining reliable hatching data from commercial emu farms is often difficult because of issues of commercial sensitivity (Deeming 1993). As a result, major inconsistencies (including inaccurate estimates of fertility and hatching success under artificial incubation), exist in the accumulated data (Button 1993). Moreover, very little is known about ratite embryology (Deeming 1995a), especially in the emu (Minnaar and Minnaar 1993), making determination of the time of embryo death, and identification of the causes of mortality, difficult.

One major factor influencing hatchability is the amount of water lost during incubation (Lundy 1969). This also affects chick size (Tullett and Burton 1982, Burton and Tullett 1985). The total amount of weight lost, together with the metabolic water produced at the end of incubation, determines water and ion concentrations in hatchling tissues (Ar and Rahn 1980). This is important because too much water loss leads to dehydration of the chick (Deeming 1993), but failure to lose enough water results in excess water storage in tissues and eventually oedema (Davis and Ackerman 1986, Davis *et al* 1988). Embryonic development in emu eggs is difficult to observe because the dark green shell prevents conventional egg candling. There is a real need to monitor foetal development in order to allow the early detection of embryo mortality and other incubation problems.

This study was designed to address lack of information about artificial incubation in emu by: (i) accurately determining fertility levels of eggs and documenting their hatchability under known incubation parameters; (ii) assessing at what stages of incubation embryos die; (iii) assessing how changes in water loss affect embryo viability and determining the level of water loss during artificial incubation that is consistent with high hatching success in emu eggs; and (iv) testing several methods of detecting fertile eggs at an early stage of incubation.

Methods

Egg collection and storage

Observations were made during a study of emu production and egg development at an emu farm near Colyton (40°14'S, 175°39'E), situated in the lower North Island of New Zealand. During the 1994 and 1995 breeding seasons enclosures were surveyed for eggs by the emu farmer approximately every three days. All eggs found were removed and labelled individually with a white pencil. Eggs were weighed (± 1 g) using electronic scales, and maximum length and maximum width measured (± 0.1 mm) with Vernier calipers. Unless eggs were clean and dry when collected they were fumigated for 10 minutes in potassium permanganate and formaldehyde. The eggs were then stored for two days in 1994, and for about 4 days (range: 2 - 11 days) in 1995, in foam-lined boxes at $14 \pm 1^\circ\text{C}$ and hand-turned 180° three times per day. Eggs were incubated in a Dominion incubator, and two Hatch-rite MP II - 168 incubator units, regulated at $35.5 \pm 0.2^\circ\text{C}$ with a relative humidity of approximately 30 and $50 \pm 3\%$ depending on the amount of weight lost over the first 10 days of incubation.

Egg weight loss

A trial with freshly laid eggs showed that weight loss during short periods of storage was negligible. The initial weights of the eggs were therefore determined immediately before placing them in the incubator. Eggs were subsequently weighed during incubation at day 5, 10, 20 and 45, and the total weight loss calculated. Eggs were turned 180° automatically at 2 hour intervals. Eggs which decomposed noticeably during incubation were removed and examined for embryonic development. Later, at day 30 - 32, embryonic development was checked by placing a thin metal rod lengthwise across the eggshell to detect internal rocking movements.

At day 45 - 48 the eggs were transferred to a Dominion incubator converted into a hatcher. The hatcher was kept at 34.5°C and free water was placed in a tray at the bottom to maintain humidity at about 50%. Hatching assistance was provided by the farmer at day 50 - 51 regardless of whether it was required. To do this a small hole was made through the shell into the air sac, and the beak located. If the chick was malpositioned, the hole was enlarged, or another made, until the beak was encountered.

Unhatched eggs were drilled open after day 56 of incubation and the contents examined for evidence of embryonic development.

After hatching, the umbilical area of each chick was liberally disinfected with a 7% (wt:vol) iodine solution to minimise bacterial contamination. Once the chicks were dry, they were weighed on an electronic balance before being placed in a brooder measuring 0.9 x 2.3 m. The brooder box was lined with non-slip flooring and contained two heat lamps which maintained a temperature of about 24°C.

Hatching success, hatchability and fertility

Hatching success was calculated from the number of live chicks hatched divided by the total number of eggs laid over the season. In contrast, hatchery management is evaluated by the hatchability obtained. Hatchability is measured as the ratio of the number of eggs that hatched to the number of viable fertile eggs at the start of incubation (Osborn and Kuehler 1989). In this study fertility was determined using only those eggs where an accurate outcome was known.

Embryos

All eggs were weighed after incubation to full term and before being opened. Because the demand for intact emu egg shells (for decorative carving and other craft purposes) is high, a large proportion of eggs opened for analysis had only a small hole drilled into the blunt end. Insulation tape was placed over the eggshell to prevent the egg cracking and a cone-shaped, graduated drill bit was used to make a hole approximately 1.5 cm in diameter.

When eggshells could be destroyed, unhatched eggs were opened by making a circumferential cut around the blunt end of the egg using an electric hand drill with a small circular saw bit attached. Only the top quarter of the shell was removed so the embryo was contained in the remainder of the shell. Development, and orientation of the embryo relative to the aircell, were determined. The egg contents were then gently shaken or tipped out on to a clean white tray and, if an embryo was not immediately

obvious, examined closely to determine fertility. Badly decomposed eggs were abandoned.

Where egg contents were shaken out, it was impossible to divide the yolk and albumen fluids so a combined weight of these was obtained for each egg. Where possible attempts were made to measure these separately. In cases where chicks had died before yolk sac absorption, the yolk sac was removed and weighed independently. Fully internalised yolk sacs were dissected out and weighed from chicks that died at point of hatch although, if yolk was present throughout the digestive tract, it was difficult to determine the amount of yolk already utilized by the chick. Chick weight and sex were also recorded.

Embryo body length was measured both *in situ* (from rump to crown) and along the back when the body was carefully straightened out. Body width, head length and width, wing length (from the top of the humerus to the carpal joint) and leg length (both tibiotarsus and tarsometatarsus) were also recorded. The diameter of the eye was measured in some instances. From all embryos notes were made on prominent developmental features and any abnormalities in embryo structure or position were documented. All embryos were labelled and initially preserved in 5% formalin before being stored in 70% ethanol. Intact chicks were placed in 10% formalin.

Embryonic mortality

Embryos were categorised in three groups based on their stage of development and estimated age of death. These were determined using unpublished data supplied by M. Minnaar (*pers. comm.*) on embryonic growth rates and physical characteristics of emu embryos during incubation. Eggs were deemed infertile (INF) when there was no sign of embryonic development and the yolk and albumen were completely separate and clear. Evidence of embryonic development included tissues such as the blastoderm, extra-embryonic membranes, and small embryos up to about 50 mm in size. Main features included a soft beak, legs, toes (lacking toenails) and prominent eyes. Chicks dying before, and up to, an estimated age of 3 weeks constituted early embryonic death (EED). Mid-embryonic death (MED) included mostly developed but small (90 - 150

mm) embryos, aged 3 - 5 weeks, possessing feathers, toenails, and the chorioallantoic membrane completely surrounding the inner shell. Late embryonic death (LED) included embryos that were fully developed but died before internal pipping, and 'dead in shell' chicks where the bird had pipped the shell internally (and possibly externally) but died before emerging. These categories were grouped together because the method of hatching assistance given by the farmer usually made it impossible to distinguish between these. Live chicks (CH) were those that successfully hatched.

Determination of egg fertility

A stethoscope (Top Master cardiologist, 3M-NZ Limited) was used for two weeks in the 1994 breeding season to determine fertility by listening for sounds within the egg. A total of eight eggs were radiographed at different stages during incubation, using a portable X-ray machine at the study farm (settings: 50 - 60 kV and 10 mAs). These settings were established after two unhatched eggs, which had been incubated for 54 days, were x-rayed in the Department of Veterinary Clinical Sciences, Massey University.

A third attempt to determine egg fertility was made using a Toshiba ultrasound machine in the Department of Veterinary Clinical Sciences, Massey University, with an egg incubated for 53 days. Coupling fluid (sonigel) was used between the ultrasound transducer and the shell surface to reduce ultrasonic reflection between the shell - air interface.

Statistical analyses

Data were analysed using Student's *t* tests, linear regression analysis, analysis of variance, and general linear model statistical analyses (Sokal and Rohlf, 1982) as appropriate. The software packages Minitab (Version 9.0 for win), SigmaPlot (Version 3.10 for win) and Excel (Version 5.0) were used.

Results

Fertility

Of a total 637 eggs produced by the emu hens in 1994 and 1995, 578 were available for examination and of these 522 (90.3%) were fertile (Table 3.1).

Table 3.1. Productivity and hatching success in the 1994 and 1995 emu breeding seasons at Colyton, New Zealand. See text for definition of terms.

| | 1994 | 1995 | Total |
|----------------------------|-------|-------|-------|
| Number of breeding females | 11 | 19 | 30 |
| Total eggs laid | 214 | 423 | 637 |
| Total eggs examined | 168 | 410 | 578 |
| Infertile eggs | 9 | 47 | 56 |
| Embryos | 20 | 68 | 88 |
| Live chicks hatched | 139 | 295 | 434 |
| Hatching success | 64.9% | 69.7% | 68.1% |
| Hatchability | 87.4% | 81.3% | 83.1% |
| Fertility | 94.6% | 88.5% | 90.3% |

In total 434 (83.2%) chicks hatched, and 23 (4.4%) died at point of hatch. Sixty five (12.5%) embryos died earlier. Of these, seven died at a very early stage when only a blood spot or extra-embryonic membrane was evident (Figure 3.1). The total straightened body length was found to be a more reliable estimate of embryo size than was embryo length measured *in situ* (Appendix iii). This was because *in situ* embryos varied in both body curvature and embryo orientation. Unhatched fertile eggs contained embryos from 2.2 to 55.3 mm in length (Table 3.2), as well as fully grown chicks that died immediately at point of hatch (Plate 3.1).

Average levels of fertility were high for both the 1994 and 1995 New Zealand breeding seasons (94.6%, $n = 159$, and 88.5%, $n = 363$, respectively), although considerable variability (range 0 - 100%) existed between pairs. Over the 1994/1995 seasons 83.3% of the 30 breeding pairs had fertility levels greater than 75%, and 46.6% had levels higher than 95%. Only one female (018) consistently produced infertile eggs throughout the season.

Table 3.2 Embryo measurements of unhatched fertile emu eggs after artificial incubation at Colyton, New Zealand in 1994 and 1995. See text for definition of terms.

| Measurement (mm) | Mean \pm S.E. | Range | N |
|--------------------------------|------------------|---------------|----|
| Body length (<i>in situ</i>) | 15.11 \pm 0.95 | 1.87 - 35.80 | 58 |
| Body length (straightened) | 20.88 \pm 1.32 | 2.24 - 55.30 | 58 |
| Body width | 9.55 \pm 0.50 | 1.30 - 17.60 | 58 |
| Head length | 12.24 \pm 0.90 | 3.25 - 28.70 | 46 |
| Head width | 9.22 \pm 0.52 | 2.30 - 17.90 | 46 |
| Leg length | 14.33 \pm 1.54 | 11.07 - 42.50 | 43 |
| Wing length | 4.39 \pm 0.39 | 1.60 - 11.20 | 43 |
| Eye diameter | 4.60 \pm 0.42 | 0.50 - 9.85 | 36 |

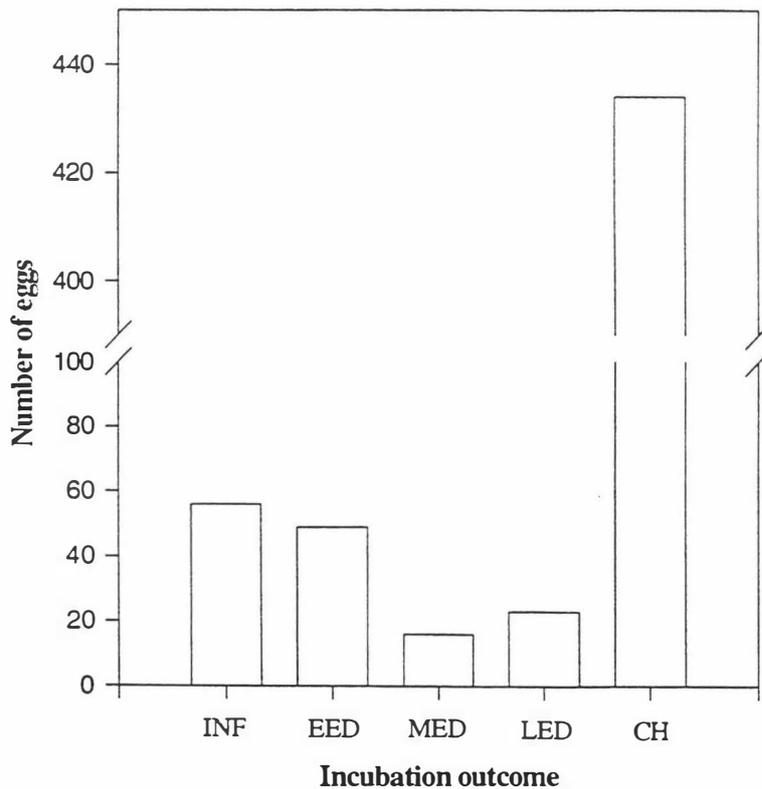


Fig 3.1. Patterns of embryonic mortality and hatchability of 578 artificially incubated emu eggs, at Colyton, New Zealand, in 1994 and 1995. INF = infertile, EED = early embryonic death, MED = mid embryonic death, LED = late embryonic death, CH = live chicks. See text for details.

A



B



Plate 3.1 Emu embryonic mortality during artificial incubation. **A** Early embryonic mortality. **B** Late embryonic mortality (yolk sac removed).

Of the 23 eggs containing embryos that died in late incubation, 11 embryos were known to have penetrated the air sac and three had externally pipped. It was difficult to determine the stage of hatching for the remaining nine chicks because the shell and membrane were broken away by the farmer before the eggs could be accurately examined. Of the 81 eggs with obvious embryonic development, four (4.9%) contained embryos that were malpositioned relative to the air sac. Such birds accounted for 3.6% of all chicks produced in 1994 and 5.4% of all chicks in 1995.

One embryo (P22-63) was very emaciated (Plate 3.2). This was estimated to have died at about day 14, but may have been older if, as indicated by its wasted state, development had progressed at a slower rate than normal. Three embryos were found with twisted necks. It is not known whether this was the cause of death, or a result of it. Some embryos showed signs of decomposition, but only six heavily so. Five eggs containing embryos which failed to hatch were cultured under aseptic conditions upon opening. All were found to be sterile and neither bacteria nor fungi was isolated.

Hatching success

Breeding success over the two seasons is summarised in Table 3.1. The hatching success of all eggs in a clutch ranged from 11.1 - 93.1%, and the variation in clutch outcomes over two seasons is plotted in Figure 3.2.

A



B



Plate 3.2 Early emu embryonic mortality during artificial incubation. **A** Unusual embryo development (P22-63). **B** Normal embryo development.

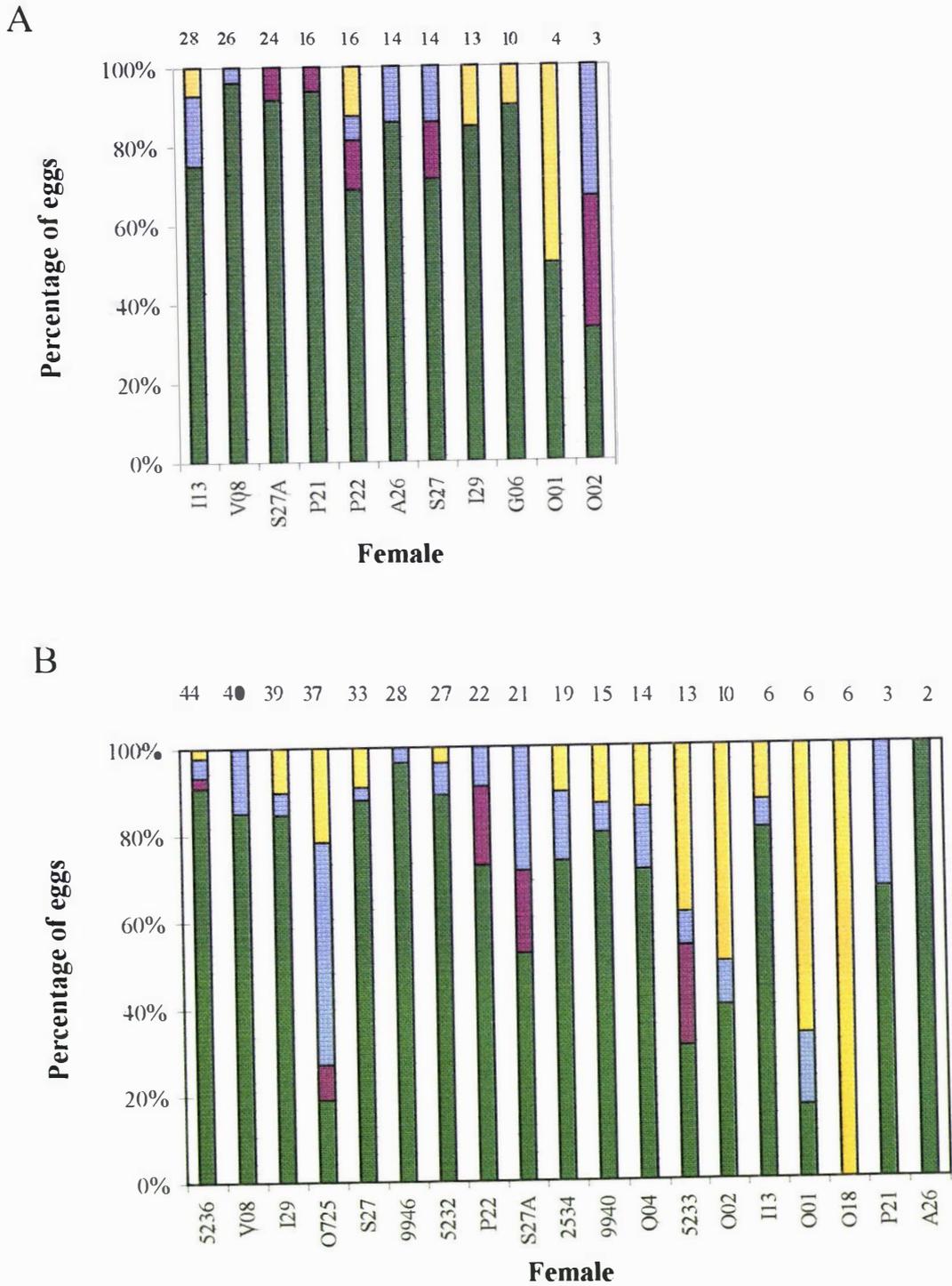


Fig 3.2 Incubation outcomes of clutches from individual female emu during: **A** 1994 and **B** 1995 at Colyton, New Zealand. Clutch sizes are indicated across the top of the figure. CH = (■), LED = (■), Embryo = (■), INF = (■).

Chick mortality at hatching

The eggs were opened by the farmer between day 50 - 51 of incubation and, in the majority of cases, the chicks were weak and premature. In four instances the vascular chorioallantoic membrane was ruptured and haemorrhaging resulted, with the chicks dying 2 - 6 hours after bleeding began. Unhatched eggs that were examined after incubation frequently contained fully developed chicks that died 2 - 5 days prior to hatching. In these birds the yolk sacs were completely or partly unabsorbed, and in five instances the navel had not even started to close. Two chicks managed to pip externally with their yolk sac and intestine emerging from the navel. Both died shortly after. Three chicks with fully absorbed yolk sacs died of unknown hatch-related problems but were unavailable for further analysis or post-mortem. The frequency with which chicks died during the later stages of incubation was apparently unrelated to gender.

Necropsies of chicks that died at point of hatch showed various amounts of yolk withdrawn into the body cavity. For these birds the cause of death was unknown and in only one instance did there appear to be a blockage due to twisting of the yolksac umbilicus, and small intestine.

Embryos were found in the eggs of the majority (83.3%) of breeding pairs (n = 30), although in one female (0725) 76% of fertile eggs failed to hatch. This bird, in her fifth year, laid for the first time in 1995 and produced 40 eggs. Of the 37 eggs of known outcome, 19 contained embryos ranging from 3.9 to 36.0 mm in length (straightened), and were estimated to have died from about day 4 to day 22 of artificial incubation. Three chicks died at point of hatch and seven hatched successfully. Infertile eggs occurred randomly throughout the clutch.

Egg weight loss

There was no significant difference in egg weight loss between the two seasons (1994: $\bar{x} = 12.40 \pm 0.13\%$ and 1995: $\bar{x} = 12.66 \pm 0.09\%$ respectively; $t_{0.05[588]} = 1.740$; $p = 0.083$). The average percent total weight loss for all eggs during artificial incubation was normally distributed around a mean of $12.56 \pm 0.11\%$ (Figure 3.3), ranging between 3.55% and 25.31% for individual eggs (n = 590). Weight loss per week remained

almost constant throughout incubation and averaged 1.54 ± 0.27 g per day or 1.68% per week.

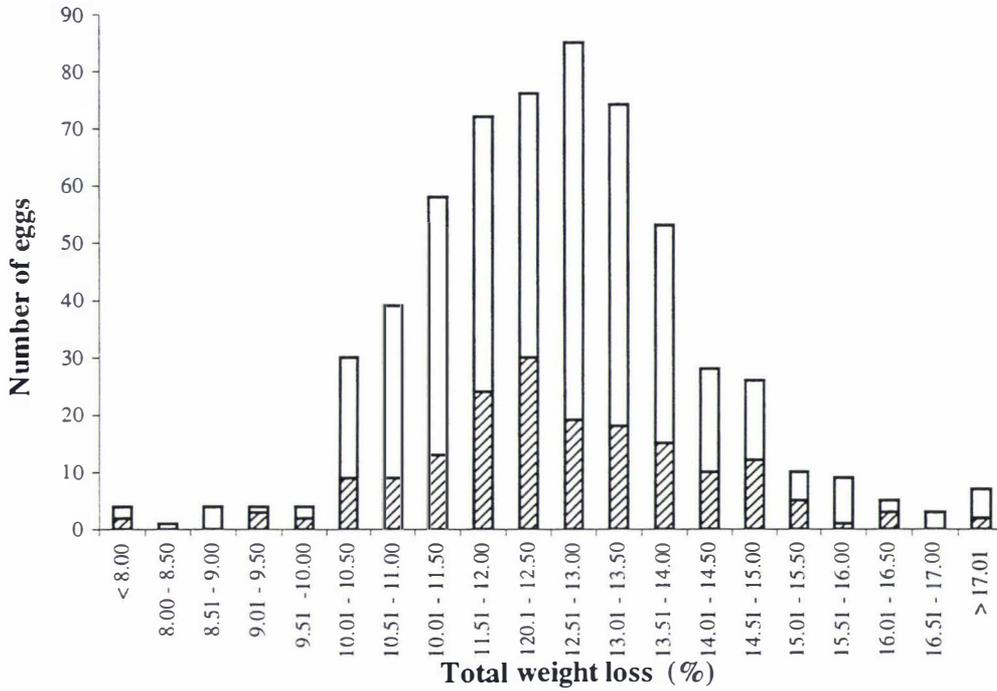


Fig 3.3 Total weight loss and hatchability of all emu eggs at Colyton, New Zealand, in 1994 and 1995. Shaded areas represent unhatched eggs.

There was no significant difference in weight loss between fertile and infertile eggs ($\bar{x} = 112.54 \pm 0.08\%$ and $\bar{x} = 12.43 \pm 0.29\%$ respectively; $t_{0.05[538]} = 0.45$; $p = 0.66$). There was, however, a difference between the initial weights of fertile eggs that hatched and fertile eggs that did not hatch, with the latter being significantly heavier (580.63 ± 2.98 g, and 598.22 ± 6.40 g respectively; $t_{0.05[507]} = 2.43$; $p = 0.016$). This difference was not reflected in the average total water lost from eggs in each group (fertile hatched: $\bar{x} = 12.53 \pm 0.09\%$; fertile unhatched: $\bar{x} = 12.78 \pm 0.22\%$; $t_{0.05[485]} = 1.09$; $p = 0.280$). Embryonic mortality was consistent across a range of average total weight losses (Figure 3.4).

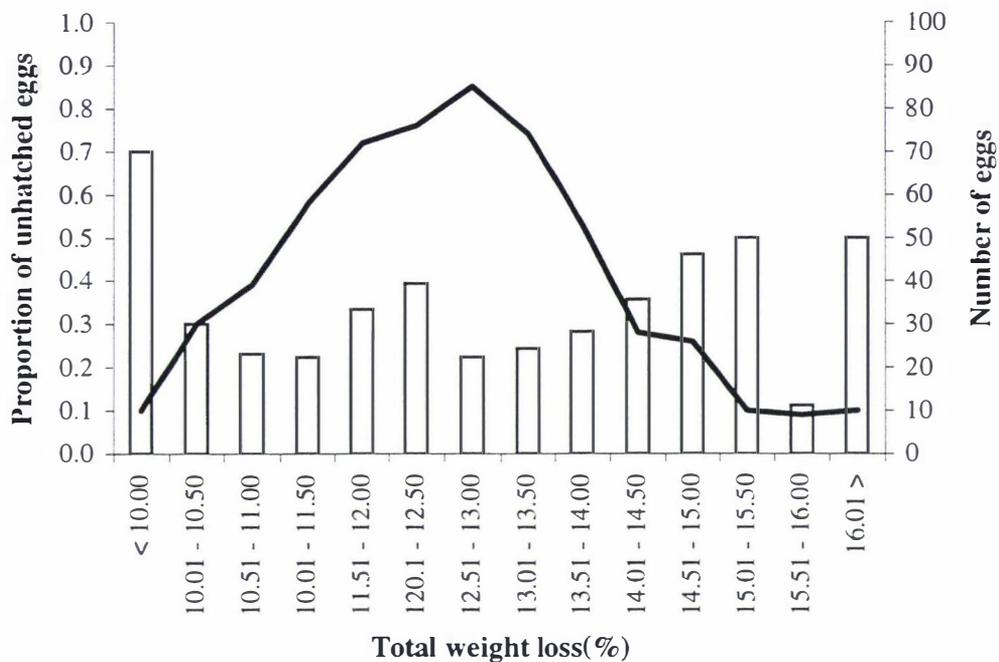


Fig 3.4 Proportion of unhatched fertile eggs (bars) and number of eggs laid (line) in relation to total egg weight loss during incubation at Colyton, New Zealand, in 1994 and 1995.

Determination of fertility

Rocking was detected in most eggs by day 32, while in a few it was not detectable until day 38 - 40. Eggs at various stages during incubation were listened to using the stethoscope, but no embryonic heartbeat could be heard at any stage. Immediately before hatching, chick breathing and movements were audible through the shell but a heartbeat was still not detectable, even after internal pipping between day 48 - 52.

Of the two eggs initially x-rayed, one contained a 62 mm embryo which was estimated to be 3 - 4 weeks of age. Although the air cell was clearly visible on the radiograph the embryo was invisible. The second egg contained a 3 mm embryo. In one egg, the contents were removed and the shell filled with water. Previously collected embryos of different sizes were individually placed inside and radiographed. The largest embryo

was fully developed and approximately 35 - 40 days old, but skeletal structures were still masked by the thickness of the shell and remained invisible.

Using ultrasound, no embryonic heartbeat was obtained from any position on the egg. The shell was found to have large differences in acoustic impedance due to the physical density of the calcium carbonate present, and produced reflections of almost 100%. The equipment was then used on human and horse subjects producing in both cases clear, accurate pictures of soft tissue and heartbeat, thus eliminating the possibility of equipment malfunction and operator error.

Discussion

Fertility

Fertility rates varied between individual emu, but on average were high. Losses due to infertility generally accounted for less than 10% of all unhatched eggs, similar to levels of infertility found in domestic poultry and ducks (Deeming 1995a). Fertility levels did not change significantly through the breeding season (*Chapter 2*), and infertile eggs were sometimes produced in a sequence of otherwise fertile eggs. Commercial farms in both Australia and America have recorded fertility levels of 78% on average, and in individual breeding pairs this can be as high as 90% (K. Venn, *pers. comm.*, H. Baldwin, *pers. comm.*).

Hastings (1994) identified infertility as the major single limiting factor to commercial production of captive ostriches. In seven out of eight ostrich breeding pairs egg infertility ranged from 30 - 60%, although some eggs may have suffered from early embryonic death. Slightly lower levels were reported by Mellet (1993) and Deeming (1995a) who stated ostrich infertility rates in South Africa and England were 30% and 22.2% respectively. The highest incidence of infertility in the ostrich was recorded at the start of breeding, but this was not the case in emu during this study. Both ostrich and emu pairs were maintained all year round and it may be more productive to separate the sexes in the non-breeding season in order to increase virility and mating effectiveness.

Hatching success

According to Landauer (1967) hatchability of eggs in domestic chickens has a genetic basis while Vince (1970) considers that it is affected by numerous environmental and nutritional factors. Very few studies have assessed the influence of egg orientation on hatching success under artificial incubation or determined optimal levels of weight loss during storage before artificial incubation of ratite eggs, and more work is necessary. Reed (1994) suggested that emu eggs laid at the beginning of the season generally had thicker shells and higher quality albumen, enabling longer egg storage periods. Our results indicated no difference in hatchability of emu eggs related to short periods of egg storage (*unpub. data*) or time of egg laying during the breeding season (*Chapter 2*).

Little scientific research has addressed hatching success of artificially incubated ratite eggs, or details of embryonic mortality. Reported levels of hatching success of artificially incubated ostrich eggs are highly variable (Bertram and Burger 1981, Jarvis *et al* 1985, Mellet 1993), ranging from 37.2% (Deeming 1995a) to 85% (Ar 1996). The success rate for artificially incubated kiwi (*Apteryx* spp) eggs is near zero when the eggs have not been naturally incubated for at least 3 weeks (Goudswaard 1986, J. McLennan *pers. comm.*) and only 29.7% of eggs removed after this period hatch successfully (Cockrem *et al* 1992).

In captive emu, Beutal *et al* (1983) recorded a hatching success of 45% for 38 eggs partially incubated artificially, while O'Malley (1989) stated artificial incubation can achieve hatching successes of 60 - 65%, with rotten eggs and infertility being the major cause of lost eggs (O'Malley 1994). These results are similar to those reported here, although the causes of hatching failure appear to differ. Bacterial contamination and infertility were not major causes of egg failure in this study. Rather, peaks in embryo mortality both early and late in incubation accounted for the failure of fertile eggs, with the most likely causal factor being sub-optimal conditions during artificial incubation.

Embryonic mortality

In all domesticated birds two peaks of mortality occur during incubation - in the first 1 - 2 weeks, and again in the last 4 - 5 days of incubation (Romanoff and Romanoff 1972,

Carey 1980, Deeming and Ayres 1995). In this study 4.4% of all eggs laid over both seasons contained fully developed embryos that died prior to hatching, with considerably greater mortality at the start of incubation than at the end. This contrasts with patterns found in ostriches (Deeming 1995a) and other birds, where mortality levels were greater during the later stages of artificial incubation. Early mortality may reflect embryonic viability, poor egg handling and storage conditions prior to incubation, and incorrect temperatures during incubation. Clearly there is a need for further research into the causes of early embryo mortality.

Webb (1987) showed high temperatures are more deleterious to avian embryos than low temperatures, and normal embryonic growth and survivorship diminishes with increase durations of exposure, especially in the early stages of development. Consequently, this would lead to higher early embryonic deaths during the initial stages of incubation. A major period of embryonic growth occurs in the second half of incubation (Freeman and Vince 1974, Hoyt 1987) associated with an increase in embryonic heat and circulation (Hoyt 1987, Vleck and Vleck 1987), and embryos may be able partially to regulate their own temperature. This may include the ability to maintain egg temperatures different to that in the incubator, allowing the embryo to accommodate heat stress for a period before death occurs.

Deficiencies in essential nutrients such as vitamin B₂ and B₁₂ also result in embryonic death (Hermes 1989a). B₁₂ deficiency increases embryo mortality in the intermediate stages of incubation with embryos exhibiting oedema, short beaks, curled toes and poor leg muscle development (Hermes 1989b). Some of these symptoms were evident in a number of emu chicks in this study that died either immediately before, or after, hatching with extremely pale blotchy thin legs but large fluid-filled stomachs.

Most chicks that died immediately before or after hatching were very weak with partially absorbed yolk sacs. The most likely cause of this was probably too vigorous intervention in the hatching process. Many emu farmers in New Zealand assist chicks to hatch in the belief that they will die if the shell is not cracked for them. Hatching assistance is to be discouraged because it disrupts the normal hatching sequence and affects post hatching development (Deeming 1995b, Ar 1996). Deeming and Ayres

(1995) noted that out of eight ostrich chicks helped to hatch by breaking the shell, six died within 10 days and the remaining two had poor rates of growth. If hatching assistance is deemed essential (e.g. the chick is in a breech position, or appears trapped) it is preferable to give limited aid by removing only a small part of the shell.

In rhea (*Rhea americana*), Dolensek and Bruning (1978) suggest that if the chick is not given enough opportunity to struggle naturally within the egg, it may be unable to fully withdraw the yolk sac. In many cases, New Zealand emu farmers experiencing this problem were assisting chicks out of their shell soon after (and in some cases before) external pipping, and hatching was completed rapidly (in 1 - 2 hours; Appendix ii). Other farmers who provided manual hatching assistance over a period of one to two days reported a lower incidence of incompletely-absorbed yolk sacs (*unpubl. data*). This may have been a result of the extra time given for the chick to fully absorb the yolk sac (*Chapter 4*). Post-hatching mortality is generally greatest 12 - 48 hours after hatching, perhaps reflecting damage caused through hatching assistance (*Chapter 5*).

Hatching success may be improved if eggs are not transferred to the hatcher until after they are internally pipped (Reed 1994). Usually 24 - 48 hours elapse between internal pipping and external pipping of the shell. During this time the vascular membrane network is closing down and the yolk sac is absorbed internally. Embryonic metabolism reaches a plateau and large amounts of heat are produced by the embryo. Jarvis *et al* (1985) found ostrich embryos began to produce significant amounts of heat towards the end of incubation, and in four eggs, shell surface temperatures reached 37°C despite being artificially incubated at 35°C. As shell surface temperatures were not monitored in the hatcher, it was difficult to determine if overheating was a problem during the eggs' period in the hatcher, although there was evidence of this in 1994 when small hatchers were used. To reduce late embryonic deaths as a result of heat stress, the hatcher should be treated like an incubator and, when at full capacity, temperatures should be reduced to offset the heat gain from surrounding eggs. This will reduce the chance of chicks dying from hyperthermia.

Egg water loss

Patterns in embryonic mortality have been closely linked to the degree of variation in weight loss from an egg during incubation (Deeming 1995a, Ar 1996), reflecting both the water vapour conductance of the shell and the initial weight of the egg (Ar and Rhan 1980, Ar *et al* 1996). In chicks suffering late embryonic death, mortality has been associated with excessive water loss rather than a failure of the developmental process (Jensen *et al* 1992). Chicks dying at point of hatch, or immediately after it, did not show significant deviations in weight loss because in most eggs weight loss was maintained within regular limits of 10 - 14%.

In a study examining embryonic mortality in ostrich eggs, Ley *et al* (1986) found a number of embryos died late in development. Most died from suffocation before internal pipping into the air cell. Ley *et al* (1986) believed that this was due to insufficient water loss during incubation resulting in weak, oedematous, chicks. Ar *et al* (1996) confirmed this, and found that embryonic mortality rose through incubation depending on the specific water vapour conductance of the eggs. When artificial incubation parameters were adjusted to achieve a total weight loss of 13%, both hatching success and chick quality increased and the proportion of chicks requiring hatching assistance was reduced. Manipulation of incubation parameters was not possible in this study, so we could not test this in emu. However, embryonic mortality appeared consistent across a range of average weight losses from 12 to 15%, and our measured weight loss in emu eggs during artificial incubation averaged about 1.54 g/day, or 12.56% overall, for eggs incubated at 35.5°C in two incubators with 30 and 50% humidity respectively.

Deeming (1995a) found that the hatching success of ostrich eggs was inversely related to the initial weight of the egg and the initial weight of ostrich eggs that subsequently hatched was significantly lower than that of unhatched eggs. Deeming (1995a) also noted that eggs that hatched lost less weight during incubation than those failing to hatch. A similar result was obtained in this study but embryonic mortality was not related to egg size (*Chapter 2*), nor was it a result of water loss during incubation. Synder and Birchard (1982) suggested that there may be a period during incubation

when embryos are especially sensitive to water loss. In the domestic chicken total water loss was not a good predictor of hatching success (Synder and Birchard 1982), and embryos were particularly sensitive to water loss during early incubation, consistent with results obtained by Simkiss (1980). Ar (1996) concluded that in ostrich eggs, the average daily water loss does not have to be constant, providing total water loss is appropriate (about 13%). Analyses of the effects of egg shape and egg volume on the amount of water lost from naturally incubated emu eggs would be valuable (*Chapter 4*).

Embryo position

Most embryos were found to lie across the egg, at right angles to its long axis. They lay on their left side, partly sunk into the top of the yolk. In larger embryos (150+ mm long) the body sits deeper in the yolk than does the head. Many chicks that fail to hatch lie in the normal hatching position, with the rump of the embryo located away from the air cell and the base of the neck adjacent to the air cell. The neck is curved strongly, the right foot next to the maxilla, and the left foot beside the neck. In the most frequent malposition the chick's head lay in the small pointed end of the egg rather than the large blunt end (described in chickens by Robertson (1961a) as Malposition II). This position is not always lethal for chickens (Robertson 1961b), and Byerly and Olsen (1936) showed 46.1% of chickens in this position were still able to hatch. Three emu chicks successfully hatched from this position, but with difficulty.

Minnaar and Minnaar (1993) noted that emu chicks in torpedo-shaped eggs are more prone to take up malpositions. In this study there were a number of long foot-ball shaped eggs, but egg shape did not appear to be the main cause of malpositioned chicks here. M. Minnaar (*pers. comm.*) did not consider the incidence of breech chicks to be a problem in emu and suggested malpositions are a result of poor incubation practices, possibly relating to turning regimes.

Although the incidence of malpositioned emu chicks was extremely low (both in this study and at a national level; Appendix ii), the major reason given by farmers for providing hatching assistance was to prevent a "large number of breech chicks dying in the shell". The infrequent occurrence of these chicks, however, does not justify

hatching assistance, and mortality at point of hatch and just after hatching is substantially greater as a result of this interference. Further observations of both the position of embryos during hatching and of full-term embryos which fail to hatch will enable an accurate description of the hatching sequence in the emu to be developed. Variations in embryo position are probably due to continuous movements during development and a number of different orientations can occur. Because the stage at which the embryo assumes the correct hatching position is unknown, it is important to accurately define what constitutes a malposition in emu chicks.

Conclusions

It will be some time before the New Zealand emu industry becomes fully commercial, but identified problems in production, such as embryonic mortality and low hatching success, should be dealt with immediately. In New Zealand the rarity and value of emu eggs influences incubation practices significantly. At present, hatchability of an individual egg and survival of the chicks takes priority over careful manipulative experimental studies that could determine optimum incubation parameters for maximum hatching success.

As infertility does not appear to be a major concern in the majority of captive farmed emu in New Zealand, educating farmers regarding embryo detection, and subsequently improving hatching success of fertile eggs, requires further attention. Poor understanding by farmers of the pattern of embryo development in the emu, especially during hatching, may contribute to poor hatch results. Both radiography and ultrasound proved unsuccessful at detecting emu egg fertility at an early stage of incubation. Candling of eggs shows a progressive increase in dark shadowing within the egg and is considered to be an important method of assessing development during incubation even though fine details are not easily seen (Deeming 1995b). There are currently no studies reporting on observations of emu eggs during candling using infra-red light, and detailed observations are needed to better understand the hatching process.

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CHAPTER FOUR

Natural incubation of emu eggs



Natural incubation of emu eggs

Abstract

Incubation temperatures, rates of egg water loss, and egg-turning behaviour throughout incubation were studied telemetrically in two emu (*Dromaius novaehollandiae*) nests in September and October 1995. Male 1 maintained an average central egg temperature of 34.1°C with a range of 32.3 - 35.2°C and hatched all three viable eggs between day 51 - 54. Male 2 maintained an average egg temperature of 31.7°C (25.6 - 35.4°C) until day 38 when he deserted the nest. On that day the internal egg temperatures fell to 12.2°C before his three viable eggs were transferred to an artificial incubator (35.5°C) where they hatched at 52 - 54 days. Rates of egg turning varied throughout incubation and between the two nests. Male 1 turned his eggs on average 8.5 times per day (range: 3 - 12), while Male 2 averaged 13 turns per day (range: 7 - 16). About two thirds of all egg turns were between 67.5° and 112.5° or less, and only 12% were turned between 158.5° and 202.5°. Eggs were most commonly turned between 09:00 and 18:00 hrs, and turning was minimal between 23:00 and 02:00 hrs. The average interval between turns differed between nests (180 ± 13 min and 105 ± 6 min). The maximal interval between turns was 17 hr 40 min. The average rate of water loss from the eggs increased from 0.51 g/day to 1.13 g/day over the incubation period and the total average water loss over the full incubation period was equal to $9.6 \pm 0.5\%$ of the initial egg weight of 553.3 ± 16.1 g. During incubation Male 1 lost 30% of his body weight, about 150 g per day. Once incubation ceased, weight gain was rapid and ranged from 157 g to 457 g/day.

Key words: emu, *Dromaius novaehollandiae*, telemetry, natural incubation, internal egg temperature, egg turning degree, egg turning frequency.

Introduction

The incubation strategy of the emu (*Dromaius novaehollandiae*) is unusual in that, unlike the greater rhea (*Rhea americana*) (Bruning 1974) and the ostrich (*Struthio camelus*) (Bertram and Burger 1981, Swart *et al* 1987), the male is adapted to complete

his incubation vigil entirely alone (Handford and Mares 1985). Emu are also remarkable in being winter breeders, and the male neither eats nor drinks (Davies 1976, Curry 1979) throughout the eight week (Buttemer *et al* 1988) incubation period.

Emu eggs are currently incubated commercially using techniques adapted from those used for poultry. Under artificial conditions, attempts are made to maintain incubator temperatures within quite narrow limits, and in poultry this is generally consistent with high hatchability (Deeming 1987). In emu, however, hatchability varies widely (O'Malley 1989, Potter 1994). For birds in the wild, incubation temperatures do vary but hatching success remains high (Freeman and Vince 1974). Further research into natural incubation is required because present artificial incubation techniques may not be appropriate for optimum hatching success of emu eggs.

Telemetric data obtained by Buttemer *et al* (1988) indicated temperatures of two naturally incubated artificial emu eggs increased steadily from 32° to 34°C during the first 10 days of incubation, and remained at 34°C for the next 15 days, before rising to a plateau of 36°C from day 35 until hatching time. Using a single telemetric egg, Curry (1979) recorded internal egg temperatures reaching 37.4°C during incubation (average 36.3°C), but did not report a change in core temperature as incubation progressed. Both studies refute Fleig's (1973) statement that the emu incubation temperature is 35°C and presumably constant. Very few studies report continuous recording over most of the incubation period for more than one nest of the same species (Burger and Williams 1979, Haftorn 1983). This is important because considerable variation in incubation temperature can exist over the period of incubation, and between nests (Buttemer *et al* 1988).

Very little information is available regarding the frequency and magnitude of egg turning in emu, although casual nest observations indicate eggs are turned anywhere from two to nine times per day (Bowthorpe and Voss 1968, Davies 1976, O'Malley 1994).

In light of this, the three objectives for this study were (i) to measure continuously the core temperature in emu eggs throughout natural incubation; (ii) to record the frequency and magnitude of egg turning under natural incubation, and (iii) to determine the subsequent hatching success of viable eggs under natural incubation. This was coupled with detailed observation of the incubating males' behaviour. Construction and calibration of the telemetry "smart" eggs is discussed and egg temperatures obtained by this technique are compared with those reported elsewhere. Weight losses over the course of incubation are reported for both the eggs and the incubating males.

Methods

Eggs and study site

The study was undertaken on an emu farm in Colyton (40°14'S, 175°39'E), situated in the lower North Island of New Zealand, between September 1995 and November 1995. Supplementary data were obtained during a pilot study of a naturally incubating male emu late in the 1994 breeding season. Breeding emu were kept in pairs in enclosures ranging from 40 - 60 x 12 m in size, containing predominantly pasture with a mixture of native and introduced plants which formed nesting refuges. During the two breeding seasons, pens were regularly surveyed for eggs by the emu farmer (*Chapter 2*), and all eggs found were collected and labelled for artificial incubation (*Chapter 3*). Unhatched dummy eggs were placed in four pens, in sites of frequent egg laying to induce natural incubation by the resident males. Three days later a second egg was added to the first to simulate natural clutch formation, and in two pens, a third egg was added. All eggs were placed in vegetation and the birds were left relatively undisturbed.

Telemetric "smart" eggs

The smart eggs were two emu eggs whose contents had been replaced with telemetry equipment using methods similar to Rowe (1978), and McLennan and McCann (1991). Paraffin wax was used to fill the remainder of the shells because it has thermal properties close to that of egg albumen (Varney and Ellis 1974). One egg was split longitudinally into equal halves and contained a small thermal transmitter (SirTrack) embedded in the centre of the shell. This monitored temperatures to within $\pm 0.2^{\circ}\text{C}$.

The second egg was sawn at the pointed end and contained a central thermal transmitter and another beneath the shell surface. Both eggs contained four orientation transmitters which monitored the degree of egg rotation to within $45 \pm 22.5^\circ$. The end cap was weighted with lead to prevent rolling bias, and the eggs were rejoined using silicon glue. An air space was not left. The completed eggs weighed 612 g and 657 g, well within the reported normal range (335 - 724 g) for natural eggs (Marchant and Higgins, *Chapter 2*). Both were immediately accepted by the incubating males.

The portable receiving and recording equipment was located in an observational hide situated between 20 and 50 m from the incubating birds. Daily equipment checks and behavioural observations were possible with minimal disturbance to the incubating males. Once the smart eggs were placed in the clutch, internal egg temperature and egg turning regimes were scanned at 10 minute intervals and the hourly mean and standard deviation calculated. Each transmitter was sampled in sequence for 20 seconds and data signals were recorded using an ATS R2100 receiver connected to an ATS DCCII data-logger. A SirTemp decoder connected to the receiver converted the signals (45 pulses/minute) into a digital readout enabling continuous recording of egg temperature (Plate 4.1). Ambient temperature and humidity were measured using a thermograph and a hygograph positioned just above ground level at a site 20 m from the nests.

In the early stages of incubation several days of data were lost because of receiving problems in the data-logging equipment. The problem was solved by running a coaxial cable out to the more distant nest where the inner and outer cores of the cable were separated into a 'T' junction and buried around the nest. Ellis and Varney (1973) used a loop aerial, in methods similar to this, and suggested the telemetry egg should be within 2 m of a receiving aerial for optimal reception. Once the new antenna was in place, and initial recording and down-loading problems were solved, the system worked well.

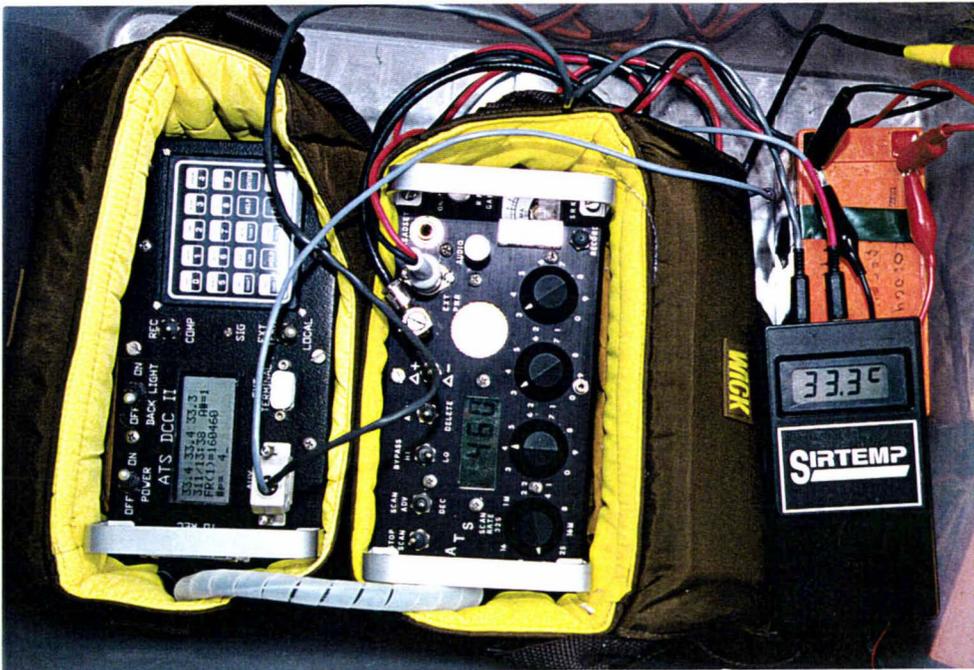


Plate 4.1 ATS DCCII data-logger (left) and a SirTemp decoder (right) connected to an ATS R2100 receiver (centre). The system is powered by a 12 volt sealed lead acid gel battery. The datalogger display shows the temperature of transmitter 460, the current Julian day, and the time. The receiver display shows the current transmitter, and the decoder shows the egg temperature.

The smart eggs were capable of providing accurate, detailed egg temperature and egg rotation data without the need for long periods of field observation. The telemetry system was calibrated in a constant temperature water bath and verified using certified mercury-in-glass thermometers, after use in the field. A further check was completed using a forced air 'Hatch-rite' incubator and supplementary mercury thermometers.

Incubation time

Initial dummy clutches, which had been used to induce the males to sit, were removed after five days of incubation and replaced with experimental clutches, comprising three natural eggs, one smart egg and one sand-filled dummy egg. Incubation time was counted from the date of replacement until all eggs hatched. Due to the high value of

viable emu eggs in New Zealand and the general reluctance of emu farmers to supply them, only six viable eggs (three per clutch) were obtained for the study.

Weight changes and nest microclimate

Egg weights were obtained within 24 hours of laying using electronic field scales (± 1.0 g), and then weighed weekly throughout incubation. Nest temperature and humidity were occasionally measured using a portable "Hanna" thermo-hygrometer with a relative humidity and temperature sensitivity of $\pm 2\%$ and 0.1°C , respectively. Embryo development was determined by placing a thin metal rod length-wise across the eggshell to detect internal rocking movements.

Approximately two weeks before incubation both males were weighed. Neither bird was weighed during incubation in order to minimise disturbances. Both birds were weighed immediately after incubation ceased but unfortunately the weight gain of both males could be monitored for only four weeks following incubation, after which time the birds were removed from the study farm. Adult body weights before and after incubation were determined by herding the male emu down to an enclosed deer holding pen where they were weighed in a walk-in crush on electronic scales (± 100 g).

Statistical Analyses

Data were analysed using Student's *t* test, linear regression analysis, analysis of variance, and general linear model statistical analyses (Sokal and Rohlf, 1982) where appropriate. The mean and standard errors are given unless stated otherwise. The software packages Minitab (Version 9.0 for win), Excel (Version 5.0) and SigmaPlot (Version 3.10) were used.

Results

Incubation time

Three year old Male 1 commenced incubation of an experimental clutch on 20 September 1995, and 5 year old Male 2 began on 28 September 1995. Male 1 remained at the nest for 59 days, including the 5 day preliminary incubation of the dummy clutch.

The second nest was abandoned by Male 2 after 38 days of natural incubation. His eggs were then transferred to an artificial incubator (*Chapter 3*) for the remainder of the incubation period, and hatched successfully after a total incubation period of 52 - 54 days.

Nest site

Each incubating male gathered near-by vegetation and dropped it around his body forming a nest platform 0.8 - 1.5 m in diameter, on which the clutch was incubated. Nest appearance and structure differed notably between the two nest sites. The nest of Male 1 contained an 8 - 12 cm thick mat of dried plants in a natural shallow depression, and was in direct sunlight. In contrast, the nest of Male 2 was in a poorly drained, shaded area partially surrounded by large shrubs. This nest site was relatively damp with only a small, muddy layer of vegetation forming the nest platform.

Incubation behaviour

During incubation Male 1 rarely left the nest uncovered for more than a few minutes, and seldom changed position when on the nest, aside from head and neck movements. Mostly he alternated between sleeping with his head and neck withdrawn in the familiar S-curve, and being alert, with his head held vertically or neck stretched horizontally along the ground (Plate 4.2). Male 2's nest site was fairly close to the front of the pen where he was subject to considerable disturbance. He frequently abandoned the nest, often taking up to 30 minutes to return to incubate the clutch. Disturbance probably influenced his subsequent desertion of the nest at day 38.

A



B



Plate 4.2 Male 1 incubating **A** at the nest in September 1995, and crouching **B** at the nest in October 1995, at Colyton, New Zealand.

Incubation temperatures

Over the first 30 days of incubation the core temperature of the telemetric egg in nest 1 increased from 32.6°C to an average of 34.1°C (Figure 4.1).

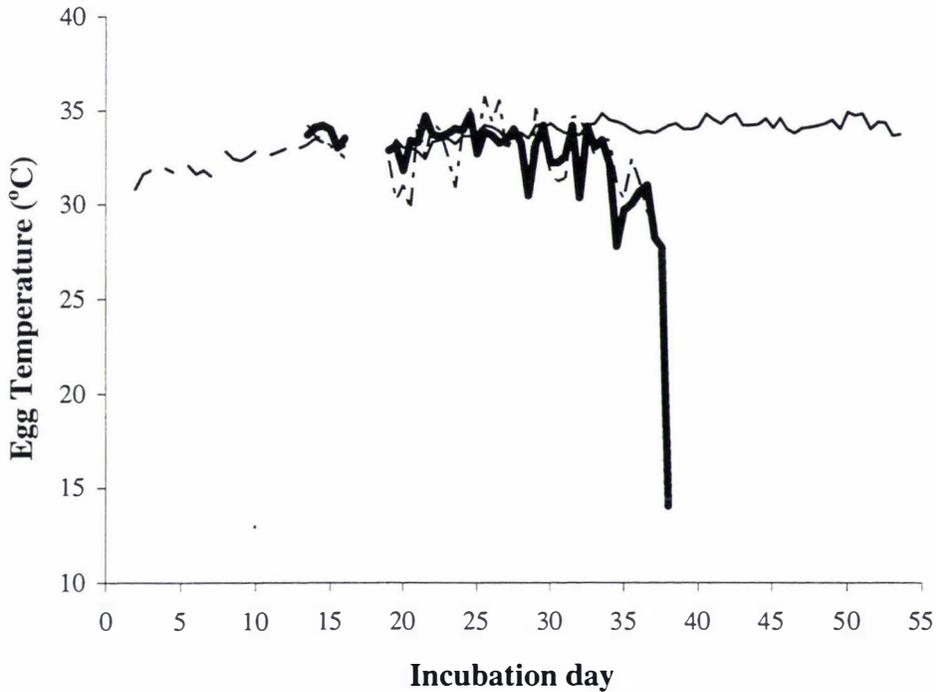


Fig 4.1 Mean daily temperature of two telemetric emu eggs over the course of natural incubation in two emu nests during 1995 at Colyton, New Zealand (Male 1: — central egg temperature, Male 2: — central and --- shell egg temperature).

The incubation temperatures recorded in nest 2 were considerably more variable over the entire incubation period, reflecting the longer periods Male 2 spent off the nest. In the week prior to deserting the nest the variability in egg temperatures increased sharply (Figure 4.2).

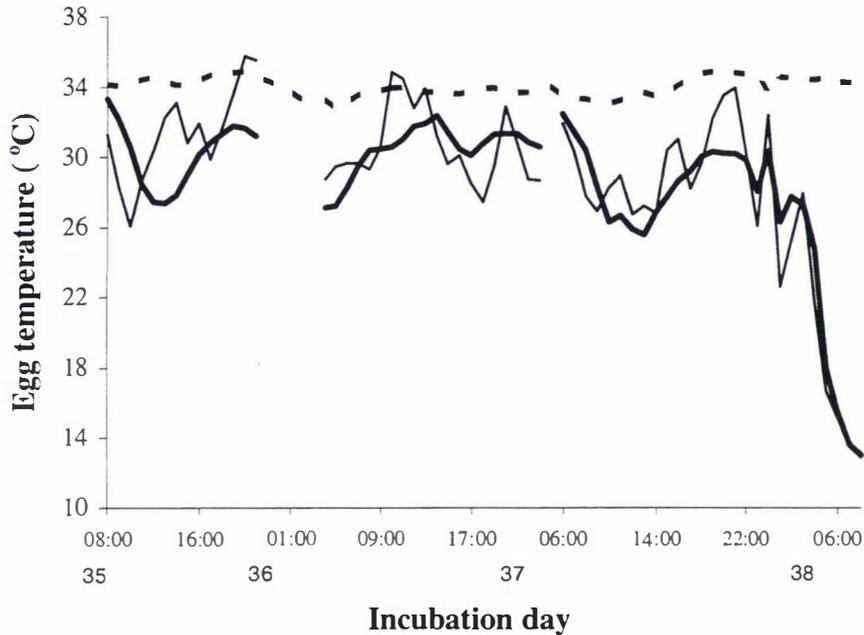


Fig 4.2 Average hourly internal temperatures of two telemetric eggs, three days before nest desertion by Male 2 at Colyton, New Zealand in 1995. (Male 1: --- central egg temperature, Male 2: — central and — shell egg temperature).

Egg turning

Before incubation, the males regularly covered the dummy eggs with vegetation, but did not appear to turn them. Active egg turning began three to six hours after the males started sitting. Both females remained fairly close to the nest site, but neither were ever seen turning the eggs even when the males were temporarily off the nest. Males turned the eggs infrequently, either by shuffling his body on the nest or by turning the eggs with his bill.

Shuffling of the body enabled all the eggs to be turned at once. The incubating birds did this by alternately moving their folded legs forward, causing their body to rock slightly. Turning the eggs with the bill occurred when the male returned after brief excursions from the nest. Once standing at the nest, the male would examine the eggs with his bill, one foot placed on either side of the eggs. He then crouched in a squatting position (resting on the metatarsus) above the eggs. In most cases (78% of all egg turning

observations) eggs were turned at this point. The ventral side of the bill was used to pull the most distant egg towards the back of the nest and under the male's belly. This normally resulted in the entire clutch being turned to some degree and even in a small clutch of just five eggs, eggs were often rolled over one another. Three to eight rolls were used to rearrange the clutch. As the males lowered themselves down on to the eggs, they shuffled back and forth slightly. Once sitting, they often picked up near-by vegetation and threw it over their shoulder or back before settling down to incubate.

Rates of egg turning varied between the two nests (Table 4.1) but not throughout the recorded incubation period. No difference was observed in egg rolling behaviour between real eggs and the smart eggs, even when rocking was evident. The assumption that eggs were turned the shortest distance during each turning event, for example 90° rather than 270°, was confirmed by direct observation of egg turning behaviour. About two thirds of all egg turns ranged between 67.5° - 112.5° or less and only 12% were in the range of 180° ± 22.5° (Figure 4.3).

Table 4.1 Frequency of egg turning events per day and the interval between egg turns in two clutches of eggs naturally incubated at Colyton, New Zealand, in 1995.

| Average ± S.E. (Range) | Male 1 | Male 2 |
|---|----------------------------------|---------------------------------|
| Number of egg turns per day | 8.91 ± 0.49 (3 - 12) | 13.16 ± 0.57 (7 - 16) |
| Time interval between egg turns (hr:min) | 02:50 ± 00:13 (00:10 - 17:40) | 01:46 ± 00:06 (00:10 - 8:20) |

The eggs were most commonly turned between 09:00 and 18:00 hours. Male 2 exhibited a number of peaks in egg turning throughout this period while Male 1 most frequently turned his eggs between 11:00 and 18:00 hours (Figure 4.4).

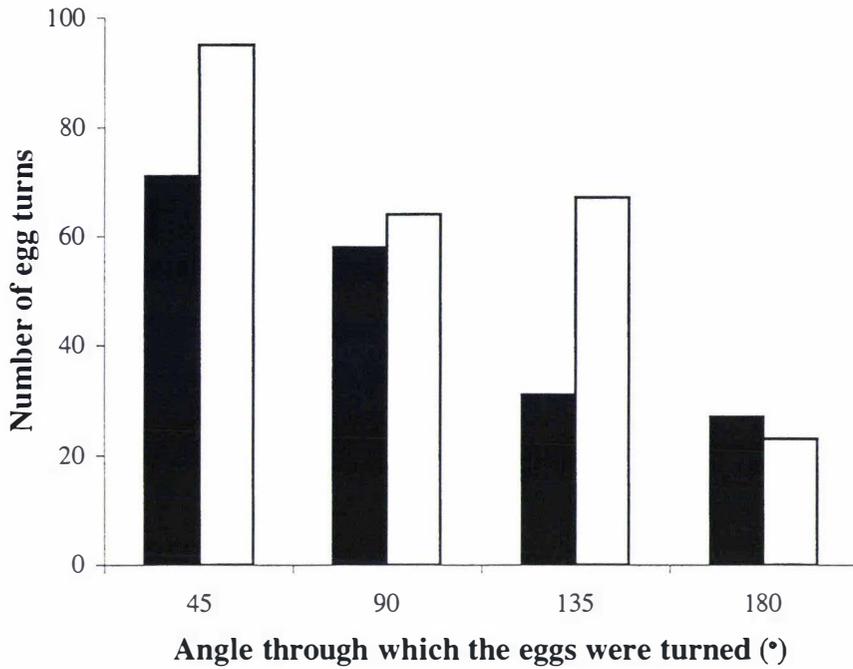


Fig 4.3 The frequency and magnitude of egg turns by Male 1 (■) and Male 2 (□) during natural incubation of two clutches of eggs at Colyton, New Zealand, in 1995 (egg orientation scanned electronically at 10 minute intervals; total number of scans = 3038).

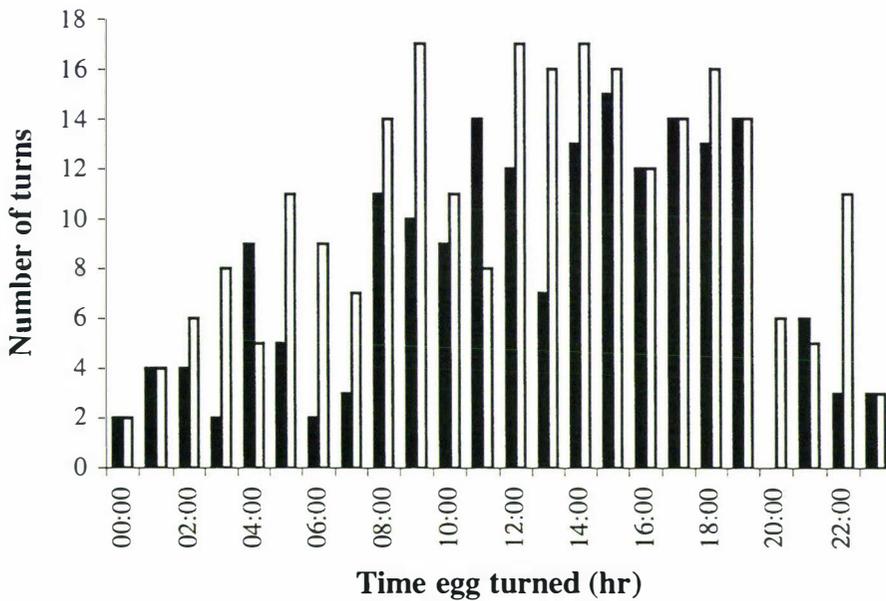


Fig 4.4 The frequency of egg turning in relation to the actual time the egg was turned by Male 1 (■) and Male 2 (□) during natural incubation of two clutches of eggs at Colyton, New Zealand, in 1995 (egg orientation scanned electronically at 10 minute intervals; total number of scans = 3038).

Nest microclimate

Throughout incubation egg temperature was always higher than ambient temperature which ranged from 2 - 26°C (average 14°C). Relative humidity ranged between 50% - 85%. Although incubation of the two nests was initiated two weeks apart, the mean daily air temperature and relative humidity did not differ significantly between them. Ambient air temperature appeared to have little effect on nest air temperatures when both males were actively incubating, and ranged from 33.4 to 35.0°C in nest 1, and 32.8 to 35.6°C in nest 2.

Embryonic movements

Movement in Male 1's eggs was detected on the 38th day of incubation, and all three were rocking vigorously by day 42. It is possible that rocking was evident at an earlier stage in nest 1, but adverse weather prevented determination of this. Rocking movements increased significantly when the eggs were left to cool, gently tapped, or whistled at. Embryonic movements were first detected in Male 2's eggs on day 34 of incubation. The morning after Male 2 abandoned his nest, the core temperature of the smart egg had dropped to 12.2°C and all three viable eggs were wet and cold. No rocking was observed, but the eggs were immediately transferred to the artificial incubator where they later hatched.

Hatching behaviour

No active communication between Male 1 and the young chicks was observed either before, or immediately after, they hatched. Of the three chicks which hatched under this male, two moved or called for up to 48 hours before hatching began. The chicks externally pipped, or began to break their shell, 14 - 22 hours before hatching (Table 4.2).

Vocalisations and laboured breathing were common during internal pipping and throughout the hatching period. The emu chicks did not chip a circle around the egg during hatching. Instead, all three (and those which hatched unassisted under artificial incubation), enlarged the preliminary external hole producing additional cracks in the shell. The initial fractures weakened the shell, and the chicks' legs pushing against the

greater intact portion of the shell eventually forced the end out. The male made no attempt to dispose of the eggshells and continued to sit on them, causing them to progressively break into smaller pieces. When the male crouched to roll the eggs, the empty shells were rolled at the same time.

Table 4.2 Egg weight loss and chick hatch weights of six emu eggs naturally incubated at Colyton, New Zealand, in 1995. Eggs from nest 2 completed the last 14 - 16 days of incubation in an artificial incubator. See text for details.

| Nest | Chick (& egg) number | Initial egg weight (g) | Total weight loss at day 50 (%) | Chick weight (g) | Incubation length (days) | Maximum time from external pipping to hatch (hrs) |
|------|----------------------|------------------------|---------------------------------|------------------|--------------------------|---|
| 1 | 1 (396) | 562 | 10.1 | 396 | 51 | 14 |
| 1 | 2 (397) | 579 | 7.6 | 397 | 53 | 20 |
| 1 | 3 (399) | 490 | 10.9 | 328 | 54 | 22 |
| 2 | 4 (411) | 605 | 8.4 | 393 | 54 | - |
| 2 | 5 (412) | 550 | 10.2 | 373 | 52 | - |
| 2 | 6 (414) | 534 | 10.3 | 332 | 53 | - |

After the three chicks from nest 1 were transferred to an artificial brooder (*Chapter 5*) all other eggs were removed. The male began feeding on vegetation surrounding the nest site within 15 minutes of egg removal. Male 1 remained near the nest for an additional 45 minutes before gradually moving away to graze in other areas of the pen. He did not return to the nest site to sleep but rather slept in vegetation near the front of the pen where the pair had slept frequently before nesting.

Egg weight change during incubation

The mean rate of water loss from the three viable emu eggs in nest 1 during the 51 - 54 incubation period was 1.05 ± 0.08 g/day. This represents a net loss of 51.45 g, or 9.43% of the initial egg weight of 543.4 ± 27.4 g. Eggs in nest 2 lost 54.24 g or 9.64% of the initial egg weight of 563.0 ± 21.5 g during incubation. Immediately before nest desertion, eggs in nest 2 lost on average 1.01 ± 0.02 g of their initial weight daily. The daily rate of water loss increased from 0.53 to 1.05 g/day ($p < 0.01$) in nest 1, and from 0.51 to 1.13 g/day in nest 2, over the incubation period.

Male weight change during incubation

Male 1 remained at the nest without feeding, drinking or defecating for the entire incubation period even though supplementary pellets were provided daily for his mate. Vegetation surrounding the nest site was gathered as nest material but was not seen to be eaten by Male 1. On a number of occasions ($n = 4$), Male 2 was observed eating supplementary food, but was never observed drinking water. The feeder was situated close to the nest site (about 2 m away), and its close proximity to the nest site, and his unsettled incubation regime, may have contributed to foraging. Weight change in the incubating males are summarised in Table 4.3.

Table 4.3 Change in the body weight of two male emu before and after natural incubation in 1995, at Colyton, New Zealand. * Body weights were determined before (-) incubating began, and after (+) incubation finished; 0 indicates weighing occurred on the day incubation finished.

| Male | Date weighed | Time (days)* see caption | Body weight (kg) | Change in body weight (kg) | Rate of change (g/day)# |
|------|---------------------------|-----------------------------|---------------------|----------------------------------|-------------------------------|
| 1 | 4 Sep | - 14 | 39.8 | - | - |
| | 13 Nov Incubation ends | 0 | 28.0 | - 11.8 | - 151 |
| | 27 Nov | + 14 | 34.4 | + 6.4 | + 457 |
| | 11 Dec | + 28 | 38.5 | + 4.1 | + 293 |
| 2 | 4 Sep | - 24 | 43.4 | - | - |
| | 10 Nov Nest deserted | - | - | - | - |
| | 13 Nov | + 3 | 37.5 | - 5.9 | - 76 |
| | 27 Nov | + 17 | 39.7 | + 2.2 | + 157 |
| | 11 Dec | + 31 | 42.0 | + 2.3 | + 164 |

- weight loss, + weight gain

Discussion

Incubation temperature

This study is the first to report near-continuous emu egg temperatures and egg turning patterns throughout incubation. The mean central egg temperatures of 34.1°C and 31.7°C recorded here in two naturally incubated emu nests are 2 - 5°C lower than those

recorded by Curry (1979) throughout incubation, and by Buttemer *et al* (1988) over the last 20 days of incubation. Incubation temperatures in other species of ratites are also variable. For example McLennan and McCann (1991) found male great spotted kiwi (*Apteryx haastii*) maintained a core egg temperature of 28° - 31.8°C. Bruning (1973, 1974) reported that both wild and captive rhea maintained an average egg temperature of 36.4°C, and Bertram and Burger (1981) found the mean temperature in the centre of ostrich eggs was 32.9°C \pm 0.7°C (range 30.8°-33°C). Thus the temperatures listed here for naturally incubating emu fall within the broad range found in other ratites, and within the range reported for birds in general (range for 37 bird species = 30.0 - 39.2°C; \bar{x} = 34.0°C) (Carey 1980)).

At the start of incubation, the developing embryo floats near the surface of the egg, regardless of egg turning (Buttemer *et al* 1988). It is therefore exposed to warmer temperatures than would be measured at the centre of the egg. Consequently, the central temperatures recorded here are probably lower than those actually experienced by the embryo in these early stages (*A. Ar pers. comm.*). It is important to note that Buttemer *et al's* (1988) internal emu egg temperatures were derived from transmitters continually floating near the upper surface of the egg. The temperatures they recorded might more accurately reflect the temperature experienced by embryos at the start of incubation, but probably overestimate temperatures experienced by the embryo from mid incubation on.

In the final stages of natural incubation we were recording the temperature of a telemetric egg among a clutch of eggs containing large embryos which, near the end of incubation, generate their own heat (Drent 1970, Hoyt *et al* 1978). This would result in differences in central temperatures between the telemetric egg and viable eggs in the clutch, which in turn might be expected to influence the behaviour of the incubating male (Franks 1967), yet this was not obviously so. Also the variation in mean daily egg temperature during incubation is not related to increased parental attentiveness (Curry (1979) and this study) nor to increases in adult brood patch temperature. Buttemer and Dawson (1989) found that the mean body temperatures of two naturally incubating emu which successfully hatched chicks averaged 37.8°C and changed little throughout incubation. Vleck *et al* (1980) showed emu embryo oxygen consumption was negligible

during the first 15 days of artificial incubation, and then increased sharply between 25 - 35 days of incubation. This suggests that embryonic heat production is responsible for the rise in temperature in the late stages of incubation, as recorded by Buttemer *et al* (1988). In this study, egg temperatures gradually increased up to day 30 and then levelled out.

The incubation period in emu is variable (Fleay 1936, Davies 1968, Bowthorpe and Voss 1968, Curry 1979) and incubation usually starts before the clutch is complete (Fleay 1936). Buttemer *et al* (1988) recorded up to three additional eggs being added to clutches up to seven days after the start of incubation, with an average incubation period of 54.5 days, similar to incubation lengths recorded by Curry (1979) and Beutel *et al* (1983). The three viable eggs incubated naturally under Male 1 produced chicks after 51, 53 and 54 days, 3 - 5 days shorter than reported elsewhere.

Incubation temperatures can vary significantly between individual nests and at different stages of incubation, as shown by Male 2's temperature regimes prior to nest desertion. Periods of high temperature may periodically speed up embryonic development, compensating for slower development at other times. Currently, under artificial incubation, temperatures of 35.6°C produce hatching chicks at about 51 days of incubation (Reed 1994) and it is well known that hotter temperatures increase embryo development and consequently reduce incubation length (Webb 1987). Presumably eggs incubated at 34°C would have a longer incubation period than the 51 - 54 days we recorded. In rhea (Vleck *et al* 1980) and ostrich (Jarvis *et al* 1985) records suggest a shorter period for naturally incubated nests, and this appears to be the case with the naturally incubated emu eggs in this study. The data presented here show emu can hatch after an incubation period similar to, but at a lower temperature than that currently used in artificial incubators.

Avian embryos appear much less resistant to overheating than to chilling (Drent 1975, Webb 1987) and Buttemer and Dawson (1988) found embryo thermal sensitivity declines substantially as incubation proceeds. Clearly avian species differ in their responses to temperature fluctuations of limited duration and emu embryos have a high

tolerance to a wide range of temperatures by day 38 of incubation, as shown by the three embryos surviving after nest desertion in this study.

Determining egg temperatures using telemetry

Egg temperatures that occur during natural incubation can be used to indicate appropriate temperature regimes in artificial incubation. Methodological problems, however, may arise in the analysis of egg temperatures under natural incubation (A. Arpers. *comm*, Webb 1987). Artificial eggs filled with wax, silicon or other compounds may not accurately mimic the heat transfer characteristics of avian eggs (Webb and King 1983). Curry (1979) used an oil filled telemetric egg, Buttemer *et al* (1988) used floating transmitters. In this study the transmitters were embedded in paraffin wax, thus, the varying emu temperatures recorded in the three studies may partly reflect different techniques in determining central egg temperatures. Equally, they may reflect natural variation in individual male emu incubation behaviour, or differences in nest site and nest micro-environment. Considerable variations were also found in studies of internal egg temperatures and incubation lengths of ostrich eggs undergoing natural incubation (Siegfried and Frost 1974, Jarvis *et al* 1985, Swart and Rhan 1988). Prior to research by McLennan (1988), Rowe (1978) also used telemetry to record kiwi incubation temperatures, which averaged 35°C at embryo depth. Rowe (1978) then verified the data using a shell filled with water and a thermal transmitter, and recorded temperatures of 35.4°C ± 0.7°C which were assumed to be a good approximation of egg temperatures recorded under the bird.

Although this study reported emu egg temperatures and turning patterns throughout incubation, continuous data collection is now needed to reduce the obvious bias in telemetry studies of unequal sampling between initial and final stages of incubation, and between day and night (Webb 1987). In this study there was no significant change in the incubation behaviour and nest attentiveness as the incubation period progressed, counter to Curry's (1979) observations. Prior to nest desertion all three viable eggs in nest 2 were rocking vigorously, yet the male still deserted. This suggests that evidence of chick development, in the form of strong embryo movement and heat generation, is insufficient by itself to maintain incubation behaviour in the male.

Egg turning

It is widely accepted that avian eggs must be turned periodically during incubation and that turning is associated with increased hatchability (Lundy 1969, Tullett and Deeming 1987). Failure to turn eggs has been known to result in the reduction in chorioallantoic membranes (Tyrell *et al* 1954) which in turn has an adverse effect on embryo oxygen exchange (Romanoff 1960), premature adhesion between the extra-embryonic membrane (Freeman and Vince 1974), poor utilization of albumen by the embryo (Tazawa 1980), and slower or abnormal embryo development (Tazawa 1980, Tullett and Deeming 1987). Both egg temperature and egg turning influence embryo development (Ar 1996), particularly during the first trimester of incubation when a large number of embryonic deaths usually occur (*Chapter 3*). This is evident from observations in ostrich (Burger and Bertram 1981), kiwi (Goudswaard 1986, McLennan *et al* 1996) and emu (Potter 1994) where hatching success is considerably greater in eggs that were initially incubated naturally.

This appears to be the first study in which transmitters have been placed in emu eggs to record turning regimes of eggs under natural incubation. The assumption that eggs were turned the shortest distance during each turning event, for example 90° rather than 270°, was confirmed by direct observation of egg turning behaviour, and hatching was successful with small, fairly infrequent egg turns. This is in direct contrast to current artificial incubation techniques, where the eggs are always turned 180°. Minnaar and Minnaar (1993) have stated that 180° rotation greatly increases hatchability in artificially incubated emu eggs but offer no supporting data. Due to electronic equipment problems in the early part of the study, initial egg turning regimes could not be continuously recorded, but where data were recorded the patterns appeared similar to those obtained during continuous recording later in incubation. It would be interesting to determine if there is a change in the frequency or degree of egg turning as incubation progresses. It is also important to monitor the position of eggs in the clutch and the effect this has on egg temperature and turning results.

In the past, research has focused mainly on optimal temperature and humidity parameters under artificial incubation (Webb 1987, Ar 1996). Egg turning also

influences the rate of embryonic development and it is possible that the degree of egg turning is more important than the frequency of egg turning. Presumably, under natural incubation, embryos must be repeatedly stimulated by slight movements and sounds made by the incubating parents, but very little research has been conducted in this area. The extent to which ostriches move their eggs during incubation has not been clearly reported (Jarvis *et al* 1985). Sauer and Sauer (1966) noted that eggs were displaced daily, usually when the bird moved while on the nest. The use of the beak in egg turning occurred only when the bird stood to re-arrange the clutch, or after a change in incubating birds (Hurxthal 1979). Kiwi eggs are disproportionately large (400 g) in relation to the body weight of the adult bird (Calder 1979, Rowe 1980) and are incubated in small burrows in which movement is restricted (Potter 1989). Consequently eggs are turned infrequently during the long incubation period of up to 85 days (Reid and Williams 1975, Goudswaard 1996). In some nests, vegetation collected by the male kiwi may build up around the egg, in some cases up to the mid-line, resulting in it becoming semi-buried during late incubation, (McLennan 1988), and this must make it difficult, if not impossible, for the egg to be turned (J. McLennan *pers comm.*). This also suggests that the kiwi egg may be turned most frequently in the early stages of incubation. When monitoring rhea nests both in the wild and in captivity, Bruning (1974) found that centrally located eggs were moved less frequently than peripheral ones. This did not occur in the emu nests but the clutch sizes were small, and differences in egg turning may arise in larger clutches.

Hatching success

In this study, six chicks hatched from six eggs. Although the sample size was small, this hatching success was considerably greater than that obtained under artificial incubation. In emu, Fleay (1936) similarly reported hatching success of 100% under natural incubation (*Chapter 3*). Curry (1979), however, reported hatching successes ranging from 27 - 78% under natural incubation by captive emu, but he did not report whether all eggs were fertile. O'Malley (1989, 1994) found natural incubation produced a hatching success of 62.3%, with abandoned nests, rotten eggs and infertility being the main causes of hatching failure. Interesting, in this study, there was also a difference in hatching success in eggs of the same clutch under the two incubation regimes. Eggs laid

both before and after naturally incubated eggs, failed to hatch under artificial incubation. All eggs were fertile, containing embryos of varying sizes, and it appears that this mortality was directly related to sub-optimal conditions in the artificial incubator.

There was no noticeable change in male incubation behaviour before chicks hatched in the nest. Curry (1979) witnessed a slight increase in parental incubation activity over the incubation period, but this was not accompanied by subsequent increases in body heat or incubation temperature. Chicks incubated naturally hatched totally unassisted and were dry with completely absorbed yolk sacs and closed navels. All were highly active under the male shortly after hatching and before their subsequent removal to the artificial hatcher. This is in direct contrast to the lethargic nature of chicks hatched under artificial conditions (*Chapter 3*).

Aggressive parental behaviour was observed between the incubating male and the first chick during the late afternoon, the day after hatching. Male 1 frequently hissed and snapped his bill towards the chick, pecking it on the body and the head if it was in reach. To prevent risk of injury, the chick was immediately removed and placed into an artificial brooder. Once the chick was removed, the male rolled the remaining eggs in the clutch and resumed incubating with no obvious concern for the missing chick. Males with young are generally aggressive towards other emu and at times towards humans (Curry 1979) but there are no other records of aggressive physical contact towards the chicks by the incubating male. Curry (1979) documented parent-chick interactions in emu and pecking was usually gentle. No other birds were present in the breeder pens, so it was not possible to determine whether male aggression increased as incubation progressed, as was recorded in incubating rhea by Bruning (1974). No aggression was shown by the male towards the female during incubation contrary to Fleay (1936), but, as a precaution, she was removed from the pen one week before hatching to prevent any aggression towards the chicks.

Hatching synchrony

In a number of bird species hatching is synchronised by auditory communication between embryos inside eggs (Vince 1969). A female emu may continue to add eggs to

a clutch up to 13 days after the male begins incubation (Fleay 1936) and in wild clutches hatching is synchronised to within four days (Fleay 1936, Davies 1976, Curry 1979), as confirmed in this study. Buttemer *et al* (1988) found all emu eggs in a naturally incubated clutch hatched within 42 hours of one another, with the last eggs laid hatching quickest. In the wild, synchronised hatching is very important for both the emu and the rhea, as the males desert the nest 24 - 36 hours after the first chick hatched (Faust 1960, Bruning 1974, Davies 1976) and all eggs not hatched during this period were abandoned. Based on Buttemer *et al's* (1988) investigation into the thermal relations of emu eggs during natural incubation, the pattern of rising incubation temperatures should facilitate hatching synchronisation for all members of the clutch. It is interesting to note that recorded egg temperatures were slightly lower over the last 48 hours before the third chick hatched. This, and the removal of other chicks as a form of hatching stimulus, may have contributed to the slightly longer incubation period and slower rate of hatching for this chick.

Egg weight loss

Without exception, avian eggs lose water throughout their incubation at a fairly consistent rate (Rahn and Ar 1974, Rhan *et al* 1976). Ar and Rahn (1980) examined egg water losses for 81 bird species and found that eggs lost on average 15% of their initial mass to the point of hatch despite substantial differences in both incubation period and fresh egg mass. Curry (1979) and Buttemer *et al* (1988) reported that naturally incubated emu eggs lost on average about 10% of their initial mass over the entire incubation term, similar to the average weight loss reported here, and that this was inversely related to humidity. Buttemer *et al* (1988) argue that this low rate of water loss is due to emu eggs having a conductance to water vapour that was 65% of that predicted for eggs of identical mass and incubation period. Bertram and Burger (1981) found water losses in ostrich eggs during the 42 days' incubation was 11-12% (\bar{x} = 162 g) of initial egg weight although considerable variation existed both between nests and between eggs. Bertram and Burger (1981) measured a lower rate of water loss in artificial incubators than that recorded under natural incubation (2.81 g/day and 3.60 g/day respectively). In this study, weight loss in artificially incubated eggs was substantially greater (*Chapter 3*) than that in naturally incubated eggs. This may have a

considerable influence on the lower levels of hatching success currently achieved in emu eggs under artificial incubation. Investigation into the effects of nest humidity on emu hatching success is required as the relative humidity of the air around the egg controls the rate of water loss from the eggs, which can significantly affect hatchability and chick quality (Tullett and Burton, 1982). It is therefore important to monitor weather, nest microclimate, parental attentiveness and other components responsible for the maintenance of egg temperatures in order to determine whether differences in measured egg temperatures are due to different environmental factors or to differences in preferred egg temperatures.

Male weight loss

Although both feeding (Bowthorpe and Voss 1968, Dawson *et al* 1984) and drinking (Fleay 1936, Dawson and Skadhauge 1983) have been reported by male emu during incubation, it is generally rare, and only Male 2 was seen eating infrequently ($n = 4$). Throughout the course of incubation, Male 1 lost 30% of his body weight, about 150 g/day. This was considerably more than the 113 g/day of body weight lost in one emu during natural incubation recorded by Buttemer and Dawson (1989). During this study, both birds were fed *ad libitum* once incubation ceased, and Male 1 rapidly gained body weight at about 450 g/day during the first two weeks, and then about 290 g/day over the next fortnight. Male 2 lost nearly half the amount of weight lost by Male 1. This was expected after his shorter incubation, but the rate of weight gain in this bird continued at a slower, more consistent rate to that of Male 1.

It appears male emu recover body condition remarkably quickly following natural incubation. As post-incubation weight gains have not previously been recorded in emu, and with the small sample sizes in the study, it is difficult to determine how typical these weight losses and subsequent gains are in incubating emu. The only information available from other ratites regarding weight changes during incubation is for kiwi. McLennan (1988) reported the weights of incubating male kiwi declined 7.5 - 23% during the duration of incubation. Potter (1989) found individual males that were weighed both before and after incubating full term on average lost 380 g or 14 - 20% of peak body weight, and regained 450 g in eight weeks after incubation.

Conclusions

In conclusion, this study shows that emu eggs under natural incubation experience lower, and more variable temperatures than those presently used in artificial incubators. The frequency and magnitude of egg turning is also less under natural incubation. Appropriate reductions during artificial incubation could significantly affect levels of hatching success. Further research should target the effects of reducing temperatures, and rate of weight loss in eggs in artificial incubators, to ones similar to those reported here during natural incubation (34°C and 10%, respectively). The effects of reducing the angle of egg turning should also be investigated.

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CHAPTER FIVE

Chick survival and growth rates in captive emu



Chick survival and growth rates in captive emu

Abstract

Survival, sex ratios and growth rates were determined for emu (*Dromaius novaehollandiae*) chicks hatching from three sets of artificially incubated emu eggs. Sets one and two comprised 214 and 423 eggs laid and incubated on the study farm in 1994 and 1995, respectively. Set three comprised 105 eggs imported from Canada and incubated under class 1 quarantine conditions. The survival rates to three months of age for these three sets of chicks were 90.6%, 85.4%, and 93.5%, respectively. Mortality due to hatch-related problems was restricted to the first week of rearing, and to weeks 8 - 12 when structural bone deformities became evident. Sex ratios of the chicks, as determined by DNA analyses, did not differ significantly from 50:50 in every set of chicks. The chicks decreased in weight for the first three to five days after hatching before beginning a sustained period of exponential growth. There was no significant difference between male and female hatch weights or rates of growth, but females tended to grow faster and were slightly heavier from one month of age. This trend continued up to 18 months of age. Most birds that died within three months showed poor rates of growth. This difference was significant from three weeks of age.

Key words: emu, *Dromaius novaehollandiae*, survival, sex ratio, growth rate, artificial incubation, mortality, bone deformities.

Introduction

Emu, like other ratites, are precocial, and the stripy brown and white young are capable of walking and feeding 5 - 24 hours after hatching (Davies 1976). At this time they leave the nest with the male and stay together as a family group without the female (Curry 1979). The period of chick dependence in the wild is unknown, but it appears to be inversely related to food availability (Marchant and Higgins, 1990). If food is plentiful the male leaves the chicks when they are 5 - 7 months old, and will breed the following season. When food is limited, however, chick growth rates slow and the male may accompany the chicks for up to 18 months, foregoing breeding (Davies and Curry

1978). Survivorship, growth rates and plumage sequences are unknown for young emu in the wild (Marchant and Higgins 1990). Curry (1979) has provided most information on the development of young emu reared in captivity, but knowledge is incomplete (Davies 1976, Dolensek and Bruning 1978).

Survival rates of emu chicks are important variables in farming yet such data are rarely recorded, partly because males with young are aggressive towards other emu, and at times towards humans (Curry 1979), and partly because of farmer reluctance. The male rarely allows the female to approach the chicks, and in captivity females and non-breeding adults are often removed from the pen before hatching because they will kill the chicks (Fleay 1936, Davies 1978, Curry 1979).

The general conditions required for rearing emu chicks successfully have been described by Beutal *et al* (1983), Minnaar & Minnaar (1993), and O'Malley (1994a), but information regarding the growth patterns of chicks and young emu is limited (Alldredge 1993, O'Malley 1996). In New Zealand, where all emu chicks are reared under artificial conditions, the farm management practices employed and the resulting hatching success and chick survival achieved vary widely. Data on the pattern of chick growth in the first few weeks of life are, therefore, required as benchmarks against which different husbandry practices may be compared. Patterns of weight change are also needed to provide a useful indicator of chick health, and gender. Davies (1976) suggested that there is a possible bias towards female chicks in emu sex ratios but information is limited. Similarly, few data exist on levels and causes of chick mortality, although Potter (1994) suggested that the most vulnerable period is from hatching to three months of age, after which chick mortality declines sharply.

This study, covering two breeding seasons, reports on the post-hatching survival of artificially reared emu chicks, and the pattern of mortality of chicks up to three months of age. The sex ratios of the chicks and their rate of growth and development are also detailed.

Methods

New Zealand eggs and chicks

The study was conducted on an emu farm at Colyton (40°14'S, 175°39'E), New Zealand, with 214 and 423 eggs laid in 1994 and 1995 respectively (*Chapter 2*). All eggs were incubated artificially (*Chapter 3*), and observations were made on the survival, sex ratio and growth of chicks that hatched. Each chick was individually marked upon hatching with a numbered plastic leg band and, 24 - 48 hours later, a microchip (Trovan) was inserted into the pipping muscle for permanent identification. Body weight was recorded when the chick completed hatching was removed to a brooder.

The brooder box was lined with non-slip matting (2.9 x 0.9 m) and contained two heat lamps which maintained a temperature of about 24°C. While in the brooder box, hatchlings were fed *ad libitum* on commercial emu starter, in the form of crumble mash, and fresh water. Once the chicks were fully active and eating (at about 7 - 10 days) they were transferred to rearing pens. These measured 1.2 x 2.5 m and although birds had unlimited access to an outside run (1.6 x 16 m) during the day they were shut inside for the night. Heat lamps maintained an indoor temperature of about 18 - 20°C. Fifteen to 20 chicks of similar age were held in each pen.

Three times a day the chicks were fed a pelletised supplementary food ration formulated at Massey University. Small gravel and water was available *ad libitum*. The chicks also ate grass growing in the outside enclosures, and occasionally were seen eating various insects. At about three months of age chicks were moved to larger pens (12 x 40 m) and remained outside permanently.

Canadian eggs and chicks

In May 1995, 105 emu eggs were imported into New Zealand from Canada and incubated under class 1 quarantine regulations. Room temperature in the quarantine unit was maintained at 16 - 18°C for newly hatched chicks but this was supplemented in the brooder boxes with heat lamps, and electric blankets. These were reduced gradually as quarantine progressed. After the 65 day quarantine period, the Canadian chicks were transported to the resident emu farm where they were released into two large outside

pens (about 2000 m²). For the first two weeks the chicks were confined to sheds at night.

The chicks were supervised for most of each day and every attempt was made to keep them well exercised, even in the confines of the quarantine unit. Initially, each chick was caught and weighed at week 1, 2, 3, 5, 7 and 10. Later they were weighed less regularly as handling difficulties and bird numbers increased. Chicks up to 4 kg were weighed (± 1 g) on small electronic scales. Larger chicks were weighed (± 100 g), with little stress to the birds, in a walk in-walk out crate. Individual emu weights and feed consumption were recorded weekly up to three weeks of age, and every two weeks thereafter. The feed conversion ratio was calculated as the amount of meal consumed (g) divided by body mass gain (g). Additional records included any medication, skeletal abnormalities, or unusual behaviour. The sex of each chick was determined by P.E. Zoogen, California, using DNA analysis of blood samples taken from the main wing vein at 4 - 8 weeks of age.

Chicks hatched in 1994 were weighed regularly as juveniles in 1995. Growth patterns were analysed for 1994 chicks up to two years of age, and for New Zealand and Canadian chicks hatched in 1995, up to 30 weeks of age. The percentage chick survival was defined as the number of chicks surviving to three months divided by the total number of chicks hatched (Button, 1993).

Statistical Analyses

Data were analysed using Student's *t* tests, linear regression analysis, analysis of variance, and general linear model statistical analyses (Sokal and Rohlf, 1982) as appropriate. Chi-squared analysis (Bailey, 1981) was used to compare the sex ratios of the chicks. The software packages Minitab (Version 9.0 for win), SigmaPlot (Version 3.10 for win) and Excel (Version 5.0) were used.

Results

Chick survivorship

Of the total 511 chicks that hatched during the two year study, 450 (88.1%) survived to three months of age. Survivorship varied between years, and was lower than that obtained from imported Canadian eggs (Table 5.1).

Table 5.1 Survival of emu chicks to three months of age from all chicks hatched at Colyton, New Zealand, during 1994 and 1995, and from imported Canadian eggs hatched in quarantine.

| | N.Z. 1994 | N.Z. 1995 | Canada 1995 |
|----------------------------|-----------|-----------|-------------|
| Eggs laid | 214 | 423 | 105 |
| Live chicks hatched | 139 | 295 | 77 |
| Number of chicks surviving | 126 | 252 | 72 |
| % chicks surviving | 90.6% | 85.4% | 93.5% |

In 1994, not all chicks were available for post-mortem. The deaths of the three chicks that were examined each appeared to be related to an unabsorbed yolk sac and subsequent yolk sac infection. Of the 43 post-hatch mortalities occurring in 1995, 67% were a result of yolk sac problems and haemorrhaging during or immediately after hatching (Table 5.2). Over both years, 66% of emu chick deaths were attributed to hatch-related problems within the first week; 28% were due to leg rotations at a later stage (about 6 - 12 weeks of age); and 6% involved other causes.

Table 5.2 Post-hatch mortalities of emu chicks from New Zealand and Canadian stock in 1994 and 1995 at Colyton, New Zealand.

| Cause of Death | New Zealand 1994 | | New Zealand 1995 | | Canada 1995 | |
|----------------|------------------|----------------|------------------|----------------|-------------|----------------|
| | Number | % of mortality | Number | % of mortality | Number | % of mortality |
| Yolk sac | 3 | 24 | 18 | 42 | 1 | 20 |
| Hatch Problems | 5 | 38 | 7 | 16 | 2 | 40 |
| Haemorrhaging | | | 4 | 9 | | |
| Leg Rotation | 5 | 38 | 11 | 26 | 1 | 20 |
| Other | | | 3 | 7 | 1 | 20 |
| Total | 13 | | 43 | | 5 | |

Two chicks under went yolk sac removal operations and, although one chick survived for 10 days, neither operation was successful. Yolk sacs dissected from 18 chicks averaged about 130 g (range: 70.6 - 206.0 g) and made up approximately 40% of the total chick weight. Two chicks were found dead in the brooder 7 and 8 days respectively after hatching. The causes of these death were unknown but were probably associated with hatching problems. Two Canadian chicks were extremely weak and hardly moved after being assisted from the shell at hatching, while the third had a twisted neck and appeared to be blind. All three were euthanased in the first week.

Four additional chicks (including a Canadian chick) died at later stages. One chick died at 79 days and on post-mortem showed a considerably enlarged liver. The reason for this was unknown. The second chick suffered a cloacal prolapse and although this was sutured back in place the chick later died. The remaining two chicks died as a result of accidents in the paddock. Seven further chicks hatched with unusual problems. One appeared to have a neurological disorder producing continuous convulsions, while a second was paralyzed in the left leg and blind. Both were euthanased at 5 and 7 days of age respectively. Two chicks were referred to as 'star-gazers' due to progressive backward deflexion of the head shortly after hatching. 'Star-gazing' has been attributed to thiamine deficiency (Minnaar and Minnaar 1993), and the symptoms in our chicks disappeared when the chicks were given a course of selenium and ceporex, placed outside in sunshine and encouraged to exercise.

Leg deformities

In older chicks the most common cause of mortality was deformity of one or other leg. In 1995, eleven chicks (25.6% of post hatch mortalities) were subsequently euthanased between 3 - 12 weeks of age due to outward twisting of the tibia. Five Canadian chicks were also affected. The total incidence of leg rotations during 1994 and 1995 was fairly low (3.6% and 3.7%, respectively). Once rotation began, however, structural change was rapid (within 24 - 48 hours), and ranged from fairly mild to severe bone deviations. Of the 11 affected chicks nine (82%) came from two breeding females (5232 and 0725; Table 5.3).

Table 5.3 The incidence of leg rotation in two clutches of New Zealand emu eggs at Colyton in 1995.

| Female | Number of fertile eggs produced | Total number of chicks produced | Number of chicks with leg rotations | Proportion of chicks affected in clutch |
|--------|---------------------------------|---------------------------------|-------------------------------------|---|
| 0725 | 22 | 7 | 3 | 43% |
| 5232 | 26 | 24 | 6 | 25% |

Sex ratios

The sex ratios of chicks that survived to three months are shown in Table 5.4. None of the groups differed significantly from a 50:50 sex ratio. Of the 27 New Zealand clutches laid in 1994 and 1995 only two differed significantly from a 50:50 sex ratio. In 1994 a four year old female produced significantly more males ($15\sigma:4\text{♀}$; $\chi^2_{0.05[1]} = 6.368$; $p < 0.05$) but the sex ratio in her 1995 clutch did not differ significantly from 50:50. The second female showed a bias towards female chicks in 1995 ($5\sigma:11\text{♀}$; $\chi^2_{0.05[1]} = 4.250$; $p < 0.05$).

Table 5.4 Sexes of emu chicks surviving to three months of age from those hatched at Colyton, New Zealand, during 1994 and 1995, and from imported Canadian eggs hatched in quarantine.

| | New Zealand 1994 | New Zealand 1995 | Canada 1995 |
|-----------------------|------------------|------------------|-------------|
| Number of males (%) | 73 (57.5) | 117 (50.6) | 23 (49.0) |
| Number of females (%) | 54 (42.5) | 114 (49.4) | 26 (51.0) |
| Total | 127 | 231 | 59 |

The sex of 24 of the 56 New Zealand chicks that died was determined. There was no significant bias in the sex of chicks that died. The sex of the two Canadian chicks that died was not determined because quarantine regulations prevented their examination.

Hatch weights

There was a significant correlation between the weight of the emu chicks at hatching and the original fresh weight of the egg (Figure 5.1). In 1994 chick hatch weights were significantly heavier and more variable than in 1995 ($t_{0.05[429]} = 3.90$; $p = 0.0001$; Table

5.5) but this did not appear to reflect egg size (*Chapter 2*). Canadian chicks hatched in 1995 were much lighter in weight than the New Zealand chicks hatched in 1995 ($t_{0.05[364]} = 3.57$; $p = 0.0004$), reflecting a difference in initial egg weights. Although female chicks tended to be slightly lighter at hatching, there was no significant difference in male and female hatch weights (380.72 ± 3.14 and 376.43 ± 4.00 , respectively; $t_{0.05[357]} = 0.85$; $p = 0.39$).

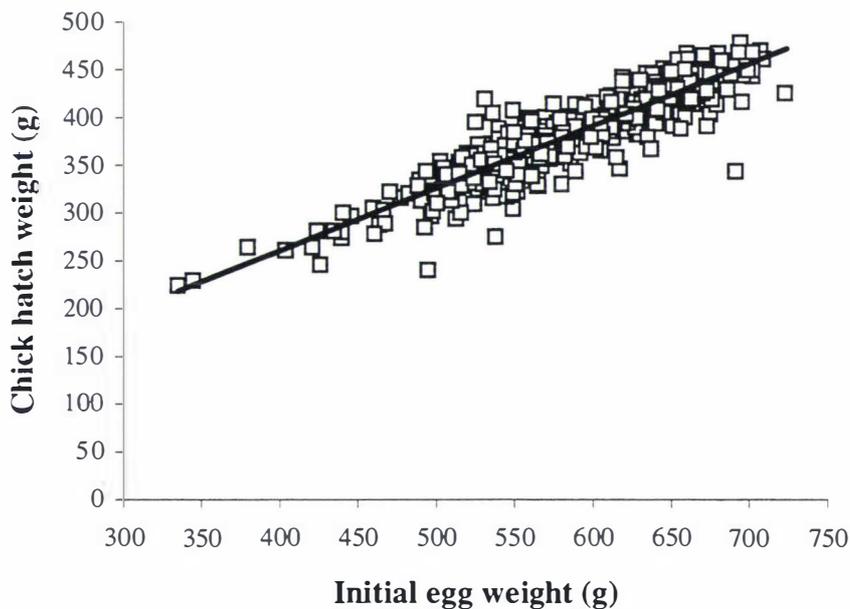


Fig 5.1 Relationship between initial egg weight and weight of emu chicks at hatching ($y = 0.6611x - 6.786$, $r^2 = 0.696$, $p < 0.001$).

Chicks that died in the first week of hatching were significantly smaller ($\bar{x} = 342.16 \pm 8.88$ g, range: 240 - 448 g, $n = 18$) than the average hatching weight of all healthy chicks ($\bar{x} = 378.80 \pm 2.21$ g, range: 224 - 478; $t_{0.05[431]} = 3.99$, $p = 0.001$). It was interesting to note there was no significant difference in hatch weights of healthy chicks ($\bar{x} = 378.09 \pm 2.27$) and those affected with leg rotations ($\bar{x} = 372.00 \pm 14.60$; $t_{0.05[404]} = 0.42$; $p = 0.67$).

Table 5.5 Average weight (\pm S.E.) at hatching for emu chicks in 1994 and 1995 at Colyton, New Zealand, and from imported Canadian eggs hatched in quarantine. The 'sex unknown' category includes chicks that were not sexed, or that died before being sexed.

| | | New Zealand 1994 | New Zealand 1995 | Canada 1995 |
|-------------|---|------------------|------------------|------------------|
| Female | Average chick hatch weight (g) \pm S.E. | 388.8 \pm 4.9 | 374.0 \pm 4.2 | 349.0 \pm 6.9 |
| | Range | 224 - 478 | 261 - 470 | 235 - 425 |
| | N | 53 | 114 | 38 |
| Male | Average chick hatch weight (g) \pm S.E. | 390.0 \pm 4.1 | 370.5 \pm 3.7 | 354.9 \pm 7.4 |
| | Range | 299 - 477 | 285 \pm 486 | 240 - 430 |
| | N | 73 | 117 | 35 |
| Sex unknown | Average chick hatch weight (g) \pm S.E. | 388.0 \pm 12.9 | 357.79 \pm 4.7 | 332.5 \pm 17.5 |
| | Range | 295 - 467 | 240 - 458 | 315 - 350 |
| | N | 13 | 64 | 2 |
| Total | Average chick hatch weight (g) \pm S.E. | 389.3 \pm 4.3 | 371.4 \pm 2.4 | 351.5 \pm 5.1 |
| | Range | 224 - 478 | 240 - 470 | 235 - 430 |
| | N | 139 | 295 | 75 |

Growth rates

All chicks lost weight after hatching as their yolk reserves were absorbed, and weight of the chick matched the initial weight of the egg at about day five. Chick weight doubled by 14 days, and again by week three in males and slightly earlier in females. The mean growth rate was exponential while it was recorded but there was a considerable increase in variability as the chicks grew older. Due to the large degree of variation in the initial weight of emu eggs, which has a significant influence on the chicks weight at hatch (Tullett and Burton 1982), the weight of the chicks was normalised as a percentage of their initial hatch weight. This reduced the amount of variability, while the pattern of growth remained the same (Figure 5.2).

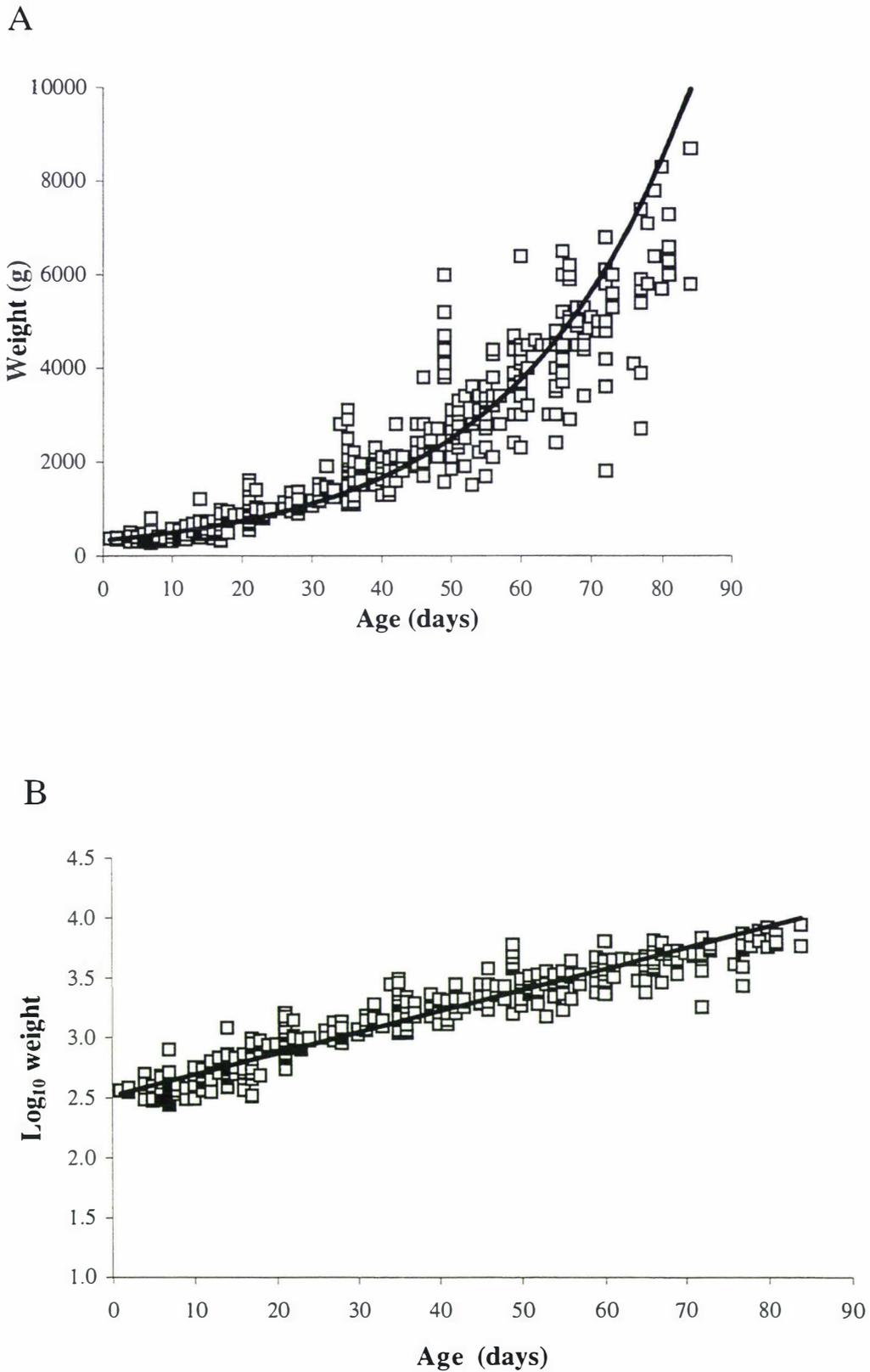


Fig 5.2 Growth rates of New Zealand chicks hatched at Colyton, New Zealand in 1995, showing **A** weight (g) ($y = 327.4e^{0.041x}$, $r^2 = 0.926$, $p < 0.05$) and **B** \log_{10} transformed to a percentage of initial egg weight ($y = 0.018x + 2.515$, $r^2 = 0.926$, $p < 0.05$).

The variation in chick size increased with age. Female chicks grew slightly faster than male chicks despite being reared under the same conditions, and by 30 weeks of age females were on average 2.5 kg heavier than males. The same trend was observed in the Canadian chicks to a slightly lesser degree. Rate of weight gain per day was initially rapid until the age of seven weeks when it reached a plateau.

Although physical symptoms of illness were unapparent in some chicks, unwell birds were easily recognised from their very slow pattern of growth (Figure 5.3). This rate of growth was significantly different by three weeks of age ($t_{0.05[241]} = 1.67$; $p = 0.011$).

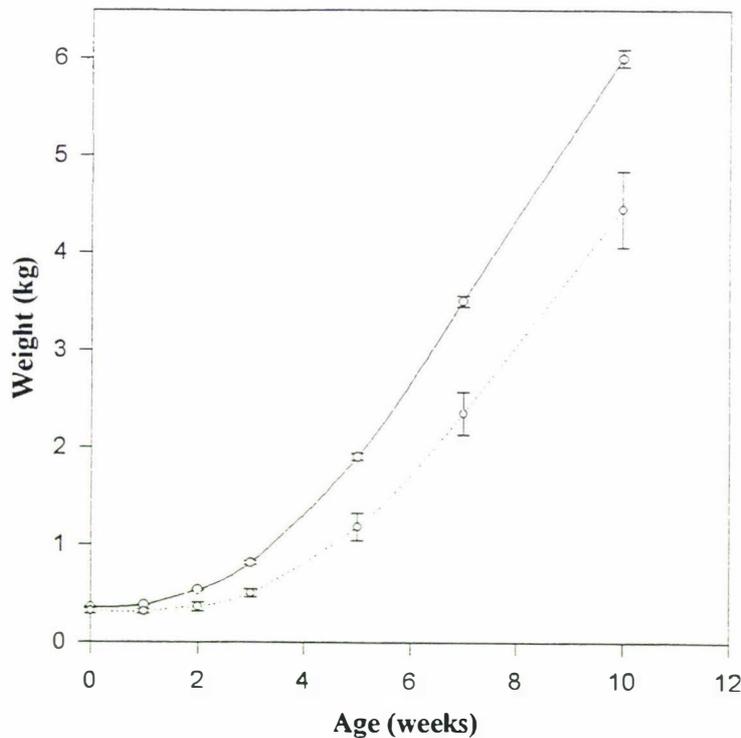


Fig 5.3 Weights of healthy (solid line) and unwell (dashed line) emu chicks during 1995 at Colyton, New Zealand.

Females also remained heavier as juveniles and variability in weight increased markedly with age (Figure 5.4). By 18 months of age females were still slightly heavier on average than males (49.8 kg and 47.6 kg, respectively).

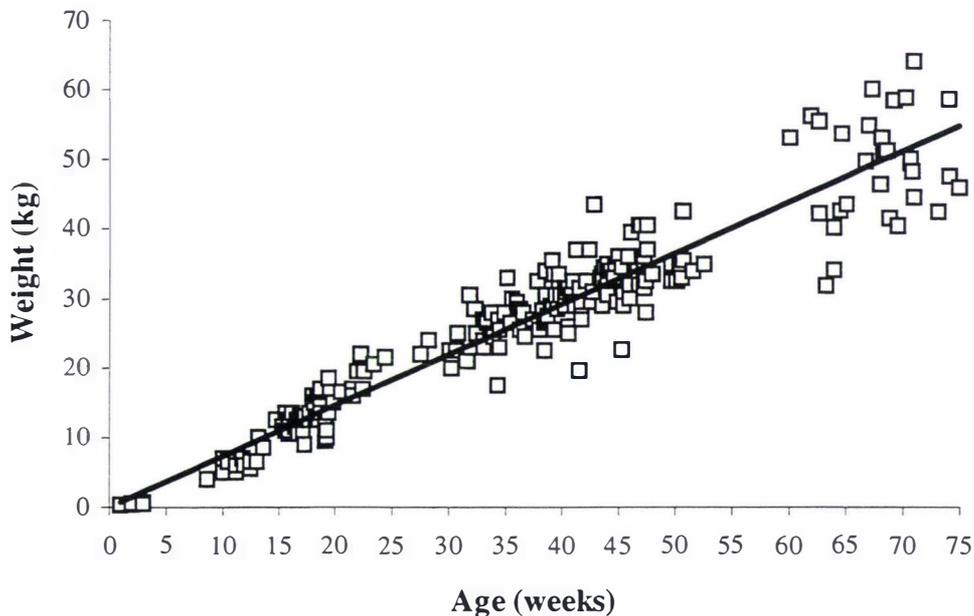


Fig 5.4 Growth rates of emu chicks hatched during 1994 at Colyton, New Zealand ($y = 0.729x + 0.038$, $r^2 = 0.923$, $p < 0.05$).

Discussion

Chick survival

The average survival of 87.6% for emu chicks to three months of age reported here is higher than that reported elsewhere. Bowthorpe and Voss (1968) reported 65% while Potter (1994) found the survivorship of emu chicks to three months was variable, ranging between 70 - 85%. Chick survivorship at a national level in New Zealand in 1995 was also high. Of 578 chicks with known life histories that hatched during 1995, 513 survived to three months of age, representing a survival rate of 88.8% (Appendix ii). In this study, mortality was greatest during the first week of age as a result of post-hatch complications, and again during weeks 8 - 12, when structural bone deformities become evident and the affected birds were euthanased. Potter (1994) reported a mortality rate of 6% in chicks up to 10 days of age, and two large commercial emu farms in Australia recorded post hatch mortalities of 5 - 7% (K. Venn, *pers. comm.*).

Of the New Zealand chicks that died in 1994 and 1995, 8.5% did so from hatching-related and yolk sac problems within one week of emerging from the shell. Nearly a third of the remaining deaths were due to bone rotations of the tibiotarsus, while a small proportion (5%) were due to other causes such as uncommon illness in individual birds (i.e. liver problem, prolapse) and paddock accidents. These high levels of post-hatch survival may reflect the large numbers of farms providing intensive rearing treatment to small numbers of chicks. Such individual chick care would not normally be provided when large numbers of chicks are reared under commercial conditions.

Minnaar and Minnaar (1993) reported a decline in chick viability towards the end of the breeding season. This decline was later confirmed by Potter (1994) who recorded a decrease in chick survival during hatching, and an increase in structural bone deformities as the breeding season progressed, suggesting that body reserves of the hen became depleted as the egg laying season progressed. Similar trends were not apparent during this study and this may possibly relate to dietary differences in the two groups of birds.

The survival of emu chicks in the wild is completely unknown, but limited comparative measures of chick survival in both captivity and in the wild are available for other ratites. For instance Hastings (1994) documented the egg outcomes of eight pairs of captive breeding ostriches (*Struthio camelus*), and post-hatch mortality as a proportion of all eggs set was: 4% mal-absorbed yolk sacs; 5% mortality at hatching; 2% post hatch deformities; 3% mortality under three months; and 28% viable chicks. Of all chicks that successfully hatched, 14% died by three months of age, which is slightly higher than the 8.7% post-hatch mortality recorded in this study from all eggs artificially incubated during 1995. Deeming *et al* (1993) recorded survival rates of 66.7 - 78.3% in domestic ostrich chicks up to three months of age. For these birds mortality was restricted to the first four weeks. Two seasons of observations by Hurxthal (1979) on wild ostriches showed variable but poor survival up to 16 weeks of age, averaging 36.9 and 43.4% per year respectively.

In captive rhea (*Rhea americana*) heavy rain resulted in the death of up to 50% of chicks reared by one male (Bruning 1973). In the wild predation, disease, heavy rain and trampling of young rhea by herds of cattle were the most common causes of mortality, with only 5-10% of chicks surviving (Bruning 1974). Little information is available on the survival of captive-reared kiwi (*Apteryx* spp.) but juvenile mortality in the wild exceeds 92% (McLennan and Potter 1993) due to high levels of predation by introduced mammalian predators (McLennan *et al* 1996).

Most emu chicks that died at hatching were weak, but post mortems failed to determine the causes of death (yolk sacs were normal in appearance). Different problems are caused by a retained yolk sac and an unabsorbed yolk sac. In the first instance, the yolk sac has been completely absorbed into the body but the contents have not been utilized by the chick for normal growth and development. In contrast, in all cases where yolk was exposed or not fully absorbed, hatching assistance had been provided. In four chicks, one of which died on removal from the shell, pipping was accompanied by considerable haemorrhaging. Detailed post mortem examination of two chicks showed evidence of both intestinal and gastric haemorrhage. The heart appeared to be normal in both cases, and though the lungs were poorly inflated this was most likely the result of insufficient time to initiate pulmonary respiration during hatching. In one individual, bone marrow was found to be very pale and examination of blood smears showed very few thrombocytes. Treatment with Vitamin K improves coagulation biochemically (Jensen *et al* 1992), but in this case haemorrhaging appeared to result from lack of blood clotting ability due to low thrombocyte levels.

Death in chicks under three months of age usually occurred suddenly, without obvious prior symptoms, and leg problems accounted for most losses. Emu chicks showed very few outward signs of pain or other symptoms apart from a loss of appetite and weight, lethargy, a hunched posture, and a tendency to stand away from other chicks. Monitoring rates of growth is therefore important in the early identification of chicks with problems. Chick mortality was highest in the first few weeks after hatching and unwell chicks could easily be recognised from their pattern of growth. These chicks generally did not exhibit sustained growth before they died, and in other weak chicks

rate of growth was very slow. It is important to closely monitor chick weights in order to identify any weight losses that may indicate that chicks are not well.

Although impactions (blockage of the proventriculus) have been reported occasionally in emu chicks (Auckland 1995), the problem appears to occur with greater frequency in ostriches (Hastings 1994). Impaction was not a cause of post-hatch mortality during this study and trends show it is most likely related to management. Jarvis *et al* (1985) suggested for ostrich that reducing hatching assistance and correcting incubation problems would allow chicks to hatch on their own, subsequently resulting in healthier chicks with a much lower incidence of yolk sac infection or possible yolk sac retention. This was also found to reduce the amount of post-hatch mortality in emu chicks during the first few weeks of age (Hicks-Allredge 1993, Deeming 1995), and commercial farmers should take heed.

Leg rotation

Leg problems are common in captive emu (O'Malley 1989), as they are in other ratites in captivity, including ostrich (Jensen *et al* 1992), rhea (Bruning 1973), and kiwi (Goudswaard 1996), and there is considerable debate over the cause. Suggested causes include overfeeding and consequent rapid weight gain (Bruning 1973, Stewart 1989, O'Malley 1995), insufficient exercise (Allredge 1993, Angel 1994), nutritional deficiencies (Flieg 1973, Dolensek and Bruning 1978, Fowler 1978, Gandini 1986, and Stewart 1989) and genetic factors (Jensen *et al* 1992). Angel (1994) disputes this, however, and maintains that 90% of leg rotations relate to environment rather than nutrition. Other contributing factors relate to management, as was the case when one pair of breeding emu on a New Zealand farm had a high incidence of leg problems in 1994. The same pair of breeding birds produced chicks in 1995, but the number of leg rotations in chicks was considerably reduced following a change in flooring and management practices.

Ratites have large cartilaginous cones in the distal and proximal tibiotarsal bone at birth. Other precocial birds (such as chickens) also have these structures which persist up to two weeks of age. Ratite chicks, however, retain these embryonic cones for up to six weeks of age (Reece and Butler 1984). This may explain why it is important to avoid

excessive weight gain in chicks, because stress on these cones could result in deviation of the tibiotarsus.

In this study, non-surgical techniques such as hobbling were employed to correct leg rotation, but this was only successful when the rotation was detected early, and the chick was very young. Over the two seasons, three chicks with severe rotations underwent corrective, invasive surgery. This involved an osteotomy of the tibiotarsus, derotation of the leg to the correct conformation (some over-compensation was found to be necessary), and fixation of the bone using pinning or plating techniques to stabilise the fracture site (T. Nickerson, *pers. comm.*). External pinning was performed on two young birds aged 4 - 5 weeks. All five attempts at corrective surgery were unsuccessful and the birds were later put down. Jensen *et al* (1992) and Alldredge (1993) similarly reported surgical derotational success rates as low as 10 - 15% when assessing emu and ostrich survival at 2 to 3 months after surgery. Due to the high cost of surgery and low rate of success surgery is unjustified. If the rotation is severe, it is preferable for birds to be euthanised.

Captive hand-reared rhea chicks show weight gains approximately double that of wild rhea chicks, and while leg problems are common in captive-reared rhea (10 - 30%, Dolensek and Bruning 1978), they have never been recorded in wild rhea (Bruning 1973). Bruning (1973) also determined that parent-reared chicks in captivity had fewer leg problems than hand-reared ones. Splayed legs have also been reported in captive kiwi chicks and may be related to high temperatures and high humidity during incubation (Goudswaard 1996). Again, no occurrence of leg deviations in kiwi have been reported from the wild (J. McLennan, *pers. comm.*).

Oedematous chicks tended to have a high incidence of splayed legs because of their heavy weights at hatching and weak muscles. Prevention is based on proper incubation parameters enabling sufficient egg weight loss. Hobbling is sometimes effective. Over the seasons there were three incidents of curled toes in emu chicks. Curled toes tended to be associated with incubation conditions and possibly chick position in the egg. Small splints were applied for a few days and the problem was self-correcting.

Of the 578 chicks across New Zealand that survived to three months of age, 32 (5.5%) suffered leg rotations. Research is needed to determine the relative influence of nutrition, bird management and genetics. It is currently unclear whether there is a leg bias as in the ostrich (Alldredge 1993), or whether one sex is more prone to leg rotation. These issues should be investigated. Despite the low overall incidence of leg rotations in emu chicks during the study, leg rotations are perceived by the industry as being widespread. Understanding the nutrition requirements of the chicks and their subsequent growth rates is the first step in solving these problems.

Sex ratios

There was no significant sex bias in the chicks hatched during this study. Around the world, very little work has been done on sex ratios in emu so the frequency of biased sex ratios, and of differences between populations in the wild and captivity is unknown. Deeming *et al* (1993) found the sex ratio in 54 ostrich chicks reared artificially, to be skewed 2:1 towards males, and it is unknown if this bias towards males in captive ostrich is widespread, but Bertram (1992) showed that wild adult ostriches in Kenya have a sex ratio of 1:1.4 in favour of females.

Hatch weights

The weights of chicks at hatching and the original fresh weight of the eggs corresponded with those reported by Beutal *et al* (1983). In this study the average chick weight at hatching (380 g) was similar to the 383 g recorded by Curry (1979) but slightly lower than the 393.1 ± 6.9 g reported by Beutal *et al* (1983).

Growth rates

Long (1965) reported average weights of female emu in the wild was higher than of male emu (38.5 and 32.9 kg, respectively) and weights were considerably lower than those recorded in captivity. In this study females tended to grow slightly but not significantly faster. Despite no difference in hatch weights between the two sexes, females were heavier at three months of age and this trend was still evident up to 18 months of age, confirming similar reports by O'Malley (1994b).

All chicks in the study were separated into rearing pens based on body size rather than age. Unfortunately, owing to the small number of chicks available in each batch of eggs (up to 20) it was not possible to separate chicks into groups based on size and retain a control group of mixed sized chicks of the same age. Preliminary observations suggest that separation of the smallest chicks from others in a group is beneficial. This deserves further investigation.

Deeming and Ayres (1995) found that small ostrich chicks lost more weight for a longer period after hatching, and grew more slowly compared to larger chicks. du Preez *et al* (1992) found no significant difference in hatch weights between male and female ostrich, and males grew faster and were heavier at maturity than females but this was not supported in a later study by Deeming *et al* (1993). Furthermore, Deeming and Ayres (1995) found female ostrich chicks lost weight for a shorter period after hatching, and hatch weight and sex had a significant effect on the weight of the chick at a later age. This was not investigated in this study but it would be interesting to compare in emu.

Conclusions

It will be some time before the New Zealand emu industry becomes fully commercial. The problems identified in production such as embryonic mortality, low hatching success and post hatching leg deformities must be dealt with immediately. Space requirements for emu chicks have not been determined. Future research should target the major areas of post hatching leg deformities in chicks and the effects of selective breeding and inbreeding on survival rates. The nutrition of breeding birds and chicks is of fundamental importance to all parameters of reproductive efficiency and growth, and data relating growth and egg production responses to a range of intakes of dietary energy are needed. Annual surveys of New Zealand emu farmers would provide valuable information for tracking production parameters, aid in identifying industry-wide problems, and help in setting research priorities. Chick weights must be monitored in order to identify slow growing birds and weight losses that can indicate ill health, and rapidly growing birds that may be at a greater risk of leg rotation problems.

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CHAPTER SIX

The occurrence and development of twin emu chicks



The occurrence and development of twin emu chicks

Abstract

The occurrence, genetic identity and subsequent development of a twinning event in farmed emu (*Dromaius novaehollandiae*) is described, and comparisons drawn with single chicks hatched in the same season. The twin-bearing egg was significantly larger than any other egg laid in the clutch. It was artificially incubated and hatching assistance was necessary. The hatch weights of the twins, both female, were very low, but their combined weight fell within the range predicted for a single chick from an egg of equivalent weight. DNA analysis of blood collected from the twins showed a complete match with 27 bands/sample larger than 3.8 kb. The estimated probability of this occurring by chance was 1.0×10^{-13} in two unrelated emu and 1.0×10^{-6} in siblings, indicating an extremely high probability that the twins were identical. This appears to be the first established case of identical twinning in birds. Possible causes of twinning are discussed.

Key words: emu, *Dromaius novaehollandiae*, ratite, genetic identity, identical twins, artificial incubation.

Introduction

The phenomena of twin chicks hatching from eggs with double yolks has attracted scientific attention since the time of Aristotle (Romanoff 1949). Twins have been reported for a small but diverse group of birds including the mallard (*Anas platyrhynchos*) (Batt and Cornwell 1972), the Giant Canada Goose (*Branta canadensis maxima*) (Batt *et al.*, 1975), and the American Goldfinch (*Spinus tritis*) and Song Sparrow (*Melospiza melodia*) (Berger 1953). Byerly and Olsen (1934) recorded the incidence of twinning in the domestic chicken (*Gallus gallus*) to be 0.11%, while Olsen and Haynes (1948) discovered three pairs of twins (0.26%) from 1153 eggs in the same species, and Riddle (1923) demonstrated an occurrence of 0.07% in pigeons (*Copidosoma gelechia*), thus underlining the low frequency of twinning in birds.

Most authors (Byerly and Olsen 1934, Cartwright 1939, Sturkie 1946, Batt and Cornwell 1972) have not distinguished between the presence of one versus two yolks when documenting twinning in birds, and Levi (1941), working with pigeons, is the only author we are aware of to state that single yolked eggs have been known to produce twins. The development of twins from single yolked eggs is apparently the result of a single yolk possessing two blastoderms. Newman (1940) distinguished five types of monozygotic chick twins and showed the most common condition of monozygotic twinning in birds is the partial or complete fission of an originally single embryonic axis, resulting in the development of two embryos. Waddington (1941) extended this view and offered an alternative explanation involving the occurrence of two separate endoderm invaginations, rather than the splitting of one embryonic axis, or the elongation of the primitive streak.

Avian twinning is extremely rare. It is even rarer for twin birds, whether monozygotic or polyzygotic, to survive beyond hatching. In cases where twins do hatch, banding and behavioural observations would provide valuable viability data on twins. This study reports on a rare event of twinning in farmed emu, and on the genetic identity of the chicks. It also compares the development and rate of growth of the twins with that of individual chicks hatched in the same (1994) season.

Methods

The observations were made during a study of production and incubation on an emu farm at Colyton (40°14'S, 175°39'E) in the lower North Island of New Zealand. The twin-bearing egg was treated identically to all other eggs during the 1994 breeding season. Egg weights were determined with electronic scales accurate to ± 1 g and linear measurements were taken using Vernier callipers. Before artificial incubation eggs, including the twin-bearing egg, were stored at 15°C for three days and hand-turned 180° three times per day. They were then incubated at $35.6 \pm 0.2^\circ\text{C}$, with a relative humidity (R.H.) of about 32% (*Chapter 3*).

During incubation eggs were weighed at day 5, 10, 20 and 45 and the total weight loss calculated. Embryo development was checked at day 30 - 32 by placing a thin metal rod lengthwise across the shell to detect rocking movements (Minnaar and Minnaar 1993). At day 48 all eggs were transferred to a hatcher which was maintained at a temperature of $34.5 \pm 0.2^\circ\text{C}$ and a R.H. of approximately $40 \pm 5\%$. Chicks, including the twins, were taken from the hatcher when they were able to move around easily, and transferred to a temperature controlled (25°C) brooder room until they were capable of surviving outside during the day (*Chapter 5*).

DNA analysis

Two blood samples (20 μl) were collected from each of the twins on 2 February 1995 when they were 3 months old, and again on 19 August 1995 when they were 10 months old. These second samples were taken to confirm the results from the first analysis. The blood was preserved in 5 ml 70% ethanol. DNA was isolated from blood samples and 5.7 μg DNA was digested to completion by *Hinf* I (Pharmacia) according to the manufacturers' recommendations. Digested DNA was loaded on 0.8% agarose gels (SeaKem Gold Agarose, FMC) and run in Palmers TAE at 23 volts for 5 days. The twins samples were flanked by lanes of DNA from an immortalised chick fibroblast line for standardisation. Gels were capillary blotted using 10XSSC for 16 hours.

Blots were hybridised with the multilocus probe (GGAT)₄ end-labelled with gamma ³²P dATP using Polynucleotide Kinase for 16 hrs at 42°C . Blots were then washed in 1XSSC, 0.1% SDS for one hour at room temperature. The DNA was then bound to the membrane by baking at 80°C until dry, UV crossed-linked for 30 seconds and exposed for autoradiography overnight. Autoradiographs were analysed using Bio-image Whole Band Analyser software.

The similarity between the two minisatellite DNA profiles of the emu twins was determined by comparing the percentage of bands in common between the samples, resulting in the Similarity Coefficient (*D*) for that pair (Wetton *et al* 1987), also known as the "Similarity Index" (Petitte *et al* 1995). The Similarity Coefficient for 251 "unrelated" emu pair combinations was calculated (Appendix iv - equation 1) and the

twin samples were compared to this large reference group. Two samples from the same species very rarely have a D of 0, and we would expect the D of two samples from the same bird to be 1. The mean D in unrelated birds was observed to be 0.45. From this an expected parent-offspring D of 0.69 was calculated using the formula derived by Jeffreys *et al* (1991) (Appendix iv - equation 2; Appendix v).

Statistical Analyses

Data were analysed using Student's t tests, linear regression analysis, analysis of variance, and general linear model statistical analyses (Sokal and Rohlf 1982) where appropriate, on an IBM-compatible personal computer. The software packages Minitab (Version 9.0 for win) and Excel (Version 5.0) were used.

Results and Discussion

Weights and measurements

On 21 October 1994 twin chicks hatched from a single emu egg produced by a two year old female at New Zealand Emu Limited, Colyton, New Zealand. The egg was laid during late afternoon on 25 August 1994 and collected in a dry, clean condition that evening. As little concealing vegetation was present in the pen, the egg was laid in an area surrounded by hay-bales, and partially covered with straw. This was the area in which most of this female's previous eggs had been laid. The twin-bearing egg was the last of a clutch of 18 and was significantly larger ($p < 0.01$) than any other egg produced by the parent bird during the 1994 breeding season (Table 6.1; Figure 6.1), consistent with Riddle's (1923) observation that twin-yielding eggs were much larger than other eggs in the clutch.

Table 6.1 Twin egg measurements in comparison to the other 17 eggs in the clutch.

| Measurement | Twin egg | Average of other eggs in clutch \pm S.E. |
|------------------------|----------|---|
| Initial egg weight (g) | 585.0 | 427.1 \pm 2.9 |
| Egg length (mm) | 140.0 | 117.7 \pm 1.5 |
| Egg width (mm) | 85.0 | 79.4 \pm 0.7 |

Overall, the other eggs in the clutch were fairly small, perhaps because this was the hen's first breeding season. Young birds often lay both smaller eggs and smaller clutches than older birds (Welty and Baptista 1988). Moreover egg size tends to increase over the initial laying period and decrease again near the end of the season (*Chapter 2*). When compared with the total number of eggs laid on the study farm during the 1994 breeding season, the twin-bearing egg was longer and narrower than average (Figure 6.2). Two subsequent eggs laid by the hen were both shell-less, with only a shell membrane enclosing the yolk and albumen. It is not known whether this was influenced by production of the large twin-bearing egg.

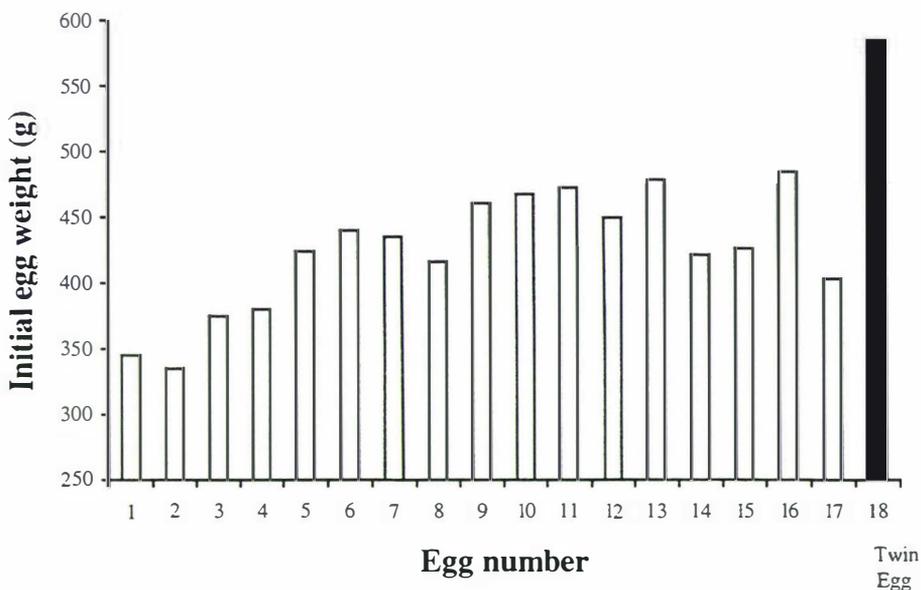


Fig 6.1 Size relationship between the twin-bearing egg and other eggs in the clutch.

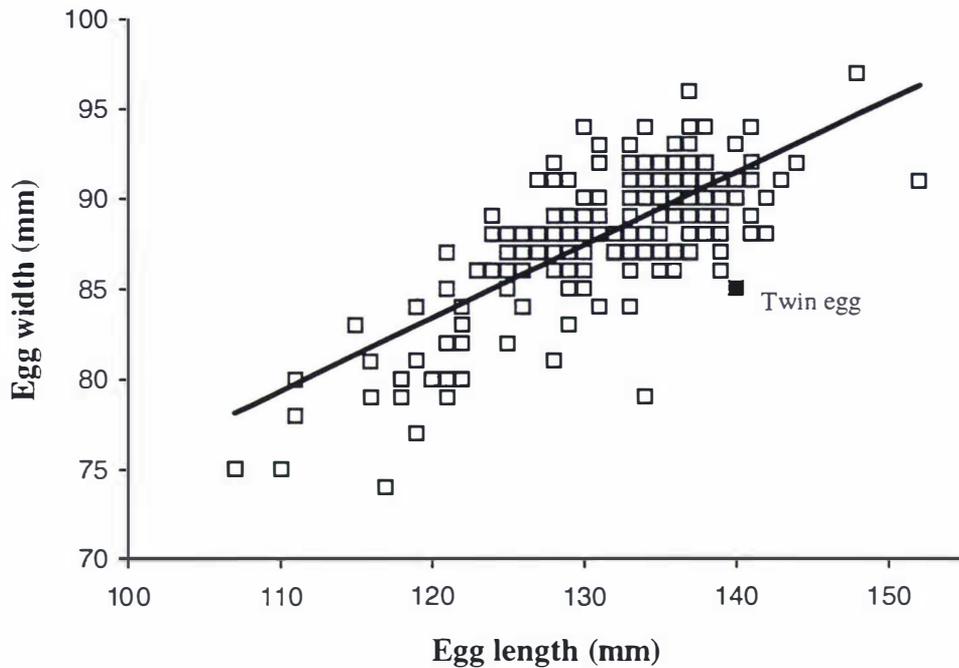


Fig 6.2 Size relationship between the width and length of the twin-bearing egg (■) and all other 1994 eggs (□) ($y = 0.402x + 35.184$, $r^2 = 0.565$, $p < 0.01$).

Embryonic movement was first detected in the egg after 30 days of incubation. Net weight loss for the twin-bearing egg over the entire incubation period (54 days) was 70 g (11.9% of the initial egg weight). This was slightly higher, but not significantly different from the average of 11.3% for the remainder of the clutch. Under artificial incubation the desired weight loss over the entire incubation period is 12-15% according to Ar and Rahn (1980), although Buttemer *et al* (1988) reported that naturally incubated emu eggs lost only 10% of their initial mass (*Chapter 4*).

On day 54 the shell was externally pipped by one of the twins (Twin One), but it appeared unable to emerge from the shell so hatching assistance was given. While enlarging the pipped hole to locate the chick's head and to prevent it suffocating, four legs were discovered. Initially, the visible chick appeared deformed, but when it was removed from the shell a second chick was discovered below it.

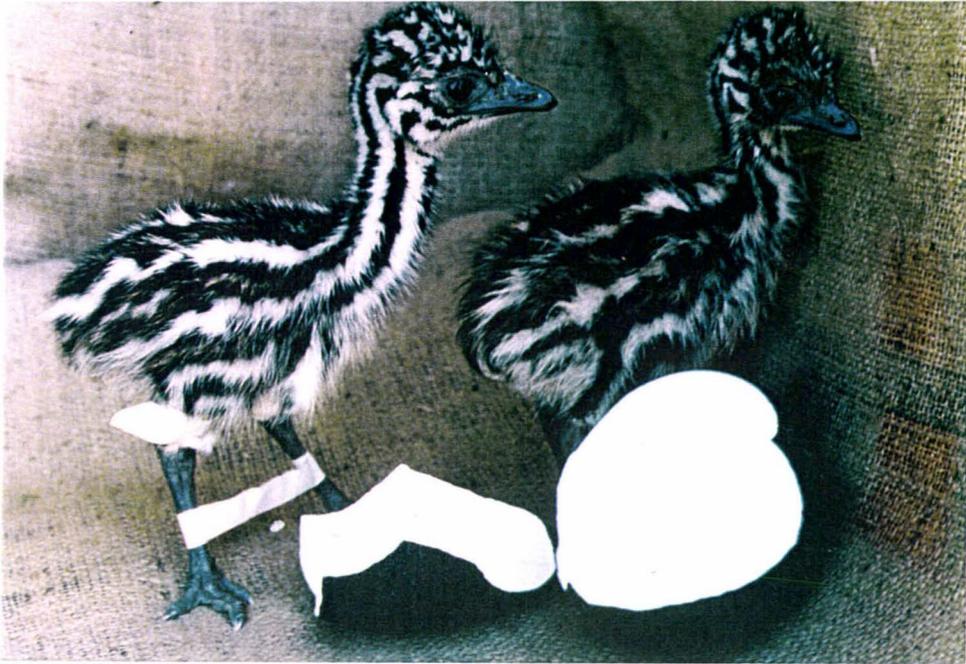


Plate 6.1 Two day old twin emu chicks hatched from a single egg in 1994 at Colyton, New Zealand.

Generally, when hatching assistance is given a hole is made through the shell into the air space and, providing the chick is not malpositioned, it is often left to complete the hatching process unaided. It is highly unlikely the twins would have survived without hatching assistance. The unusual position of the second chick would have prevented it from pipping into the air cell and it would therefore have been at considerable risk of suffocating in the shell. The small size and comparative weakness of the emu twins necessitated hatching assistance. The literature suggests that, even with assistance, extremely few twins survive (Riddle 1923, Cartwright 1939, Newman 1940, Nalbandov 1942, Sturkie 1946, Batt and Cornwell 1972, Batt *et al* 1975, Drent 1975). Interestingly, at least one other pair of emu twins is known to have survived after being assisted from the egg. These hatched on 8 November 1994 in Victoria, Australia, from a 780 g torpedo-shaped egg (Johnston 1995). Their genetic identity is unknown.

The New Zealand twins appeared normal in all respects, except for their relatively small size. DNA profiling showed that both chicks were female (see DNA Analysis below). The yolk sacs were fully absorbed and the navels completely closed.

There was little space in the egg and, probably because of this, the smaller chick (Twin Two) had a rotated right leg. This was corrected over time by hobbling, which involved taping the two legs together above the tarsal (hock) joint, and attaching a fine piece of doweling to keep the legs approximately 5 cm apart - about the width of the chick when it was sitting. This treatment allowed the chick to move fairly normally but prevented further rotation of the tibiotarsus and ancillary damage to tendons. The hobble remained on the chick for approximately three weeks, after which development progressed normally. Nalbandov (1942) reported one chick (*Gallus gallus*) with a deformed right leg and its twin which had crooked toes on the left foot. In this case the chick with the deformed leg was unable to stand and both chicks later died.

The original (fresh) weight of the emu eggs and the hatched weight of the chicks were significantly correlated, consistent with Beutal *et al's* (1983) results. The weights of the twins at hatching were 210 and 189 g respectively, significantly lower than the average chick weight of 392 g (range: 224 - 478 g; Table 6.2). The combined weight of the twins, however, fell within the predicted range for a single chick from an egg of equivalent size (Figure 6.3). In contrast, Nalbandov (1942) reported a case of twin chicks in Austral-white fowls, where both chicks weighed significantly less (11 and 16 g respectively) than the expected combined weight at hatching (35 - 45 g).

Table 6.2 Weight of the emu twins and their egg compared with other chicks and eggs in the same clutch, and with all the other 1994 eggs. Data are reported only for eggs that hatched.

| | Twins | Hatched eggs in twin clutch | Other 1994 hatched eggs & chicks |
|---|-------|-----------------------------|----------------------------------|
| Average weight (\pm S.E.) of eggs that hatched (g) | 585.0 | 443.9 \pm 7.7 | 584.5 \pm 5.2 |
| Average chick weight (\pm S.E.) (g) | 199.5 | 284.5 \pm 8.1 | 392.2 \pm 3.9 |
| Average chick weight as a % of initial egg weight (range) | 34.1 | 64.0 (55.8 - 66.3) | 67.1 (57.8 - 72.0) |
| Sample size | 2 | 8 | 133 |

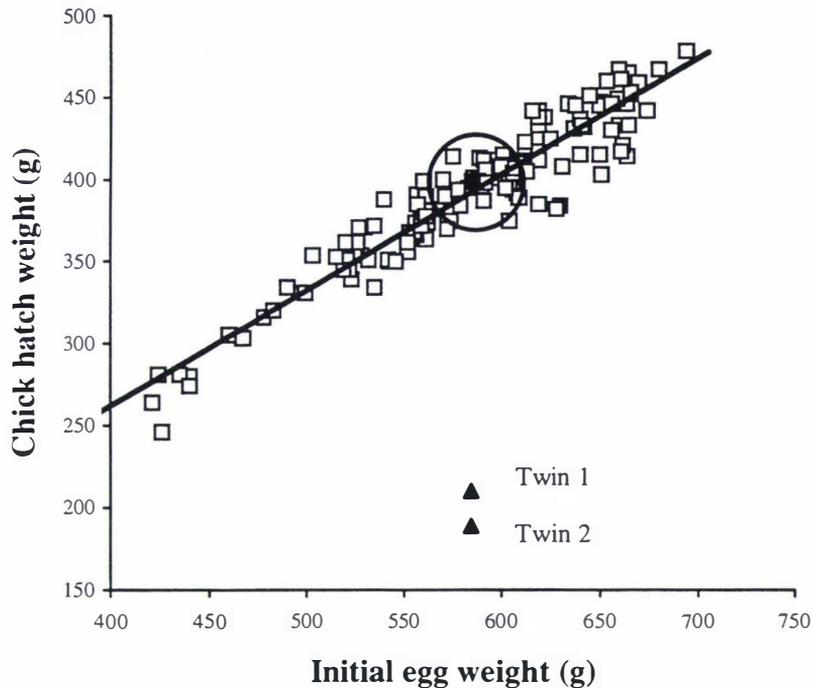


Fig 6.3 Relationship between emu chick weights at hatching and the initial weight of the eggs (□) ($y = 0.706x - 20.301$, $r^2 = 0.896$, $p = 0.01$), individual twin chick weights (▲), and the combined twin weight (■).

Growth Rates

Twin Two, initially the smaller bird, gained weight rapidly after hatching and at three weeks of age weighed 435 g, nearly double the weight of Twin One at 225 g. At this stage Twin One was only 15 g heavier than her hatch weight following two weeks of weight loss. Because of her small size Twin One was held back for an additional three weeks with younger birds on a diet of chick starter crumble, but this was thought not to have greatly affected her rate of growth. Other emu chicks hatching around the same time as the twins averaged 384 g at day zero. By 12 weeks of age the average weight of the other chicks was 7.4 kg, approximately 2 kg heavier than each twin. At this time the weights of the twins were closer to those of 9 - 10 week old chicks. When the twins were 32 weeks old they had both reached a body mass of 21 kg, 7 kg lighter than other birds of the same age. By nearly 14 months of age, they were still slightly lighter than other juveniles hatched in 1994 - 34.6 and 36.1 kg respectively compared to 39.5 kg (Figure 6.4).

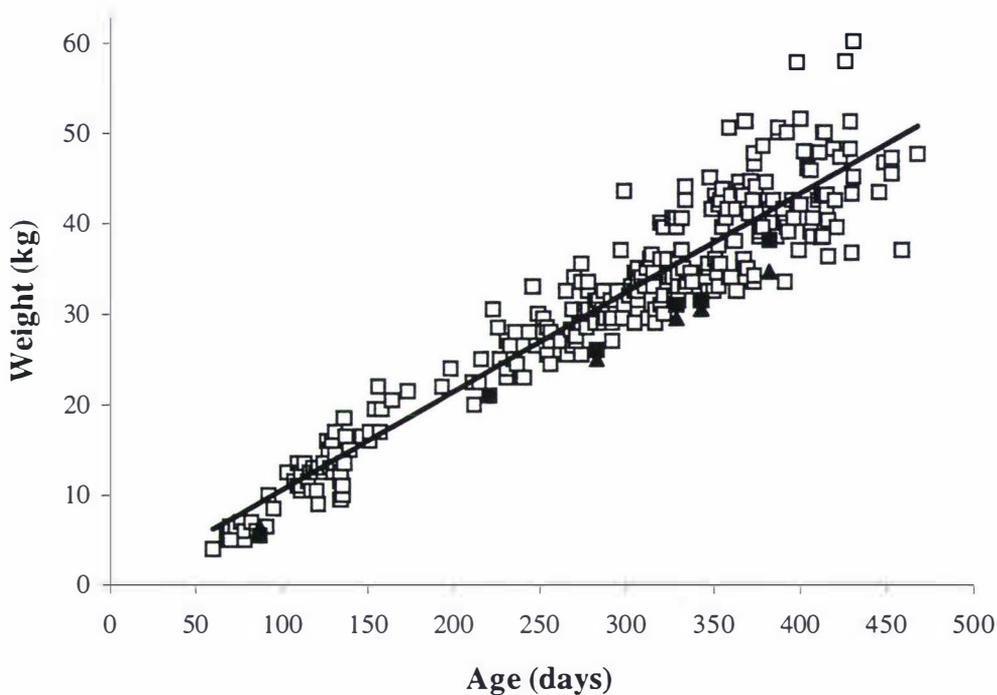


Fig 6.4 The individual weights of Twin One (▲) and Twin Two (■) in comparison to other 1994 chicks (□) ($y = 0.109x - 0.310$, $r^2 = 0.899$, $p < 0.01$).

The difference in initial chick size and subsequent rate of growth may be a result of an uneven division of the yolk sac at point of hatch. Parallel examples occur in human twins where 1 in 4 twins share a mutual blood system (Bryan 1992).

DNA Analysis

The parents of the twin chicks were found to have a Similarity Coefficient (D) of 0.46 which corresponds to a low degree of relatedness, but typical of “unrelated” emu. An increase in D between each parent and the offspring combination is consistent as with any first degree relationship, and a 0.72 D between the sire and each offspring, and a 0.75 D between the hen and each offspring was recorded. The DNA analyses of the emu twins showed a complete match with 27 bands/sample larger than 3.8 kb (Figure 6.5). The estimated probability of identical DNA profiles occurring by chance is 1.0×10^{-13} in two unrelated emu, and 1.0×10^{-6} in siblings (Appendix iv - equation 2 and 3).

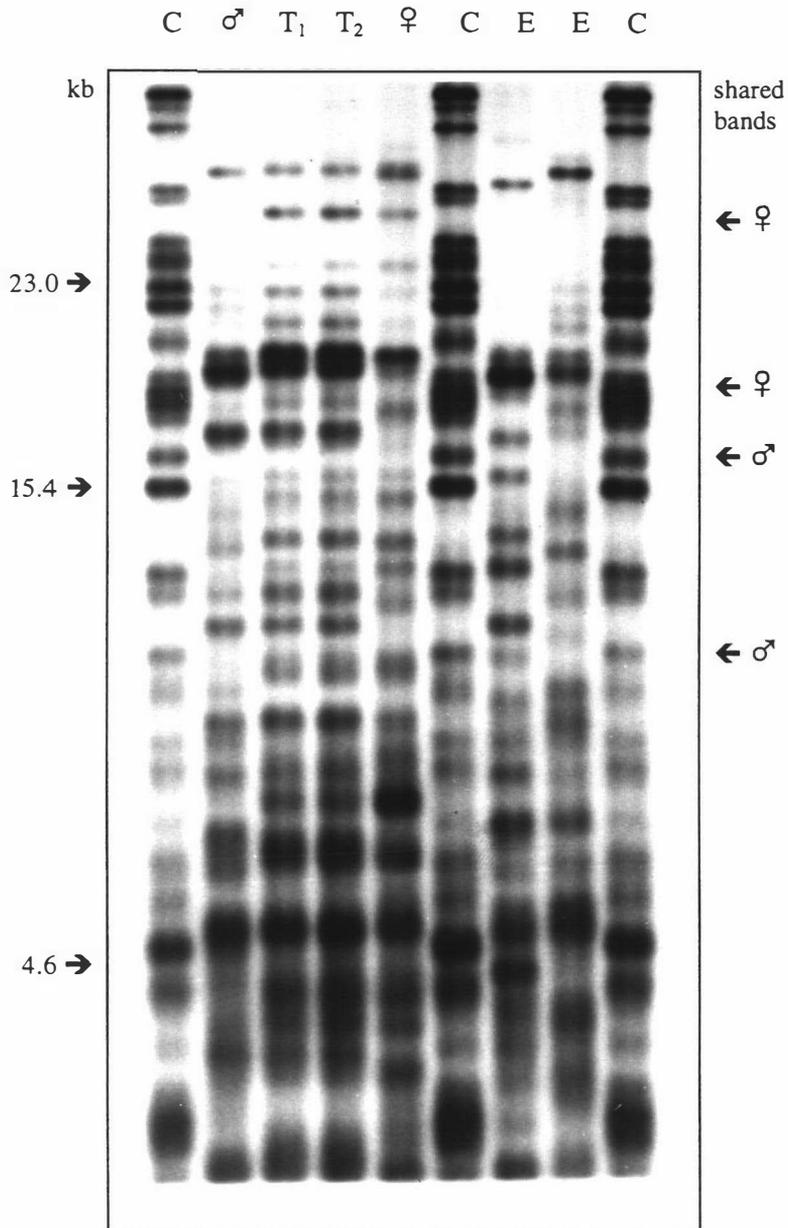


Fig. 6.5 Minisatellite DNA profiles of genetically identical emu twins (T₁ and T₂), the twins' parents (♂, ♀), other emu (E) and chicken DNA (C). Molecular size range (kb) of DNA bands is presented on the right. Arrows indicate bands shared between the parents and the twins on the left. See text for details.

There was a complete match of 1.0 *D* between the two twins which were both female. Blood sharing can occur between avian embryos in the latter stage of incubation (J. Petite, *pers. comm.*), so blood samples taken immediately after hatching could falsely indicate that fraternal twins are identical. This does not account for the results obtained here because DNA analyses were conducted twice, at three and ten months, long after any mixed blood would have been eliminated from the chicks. Accordingly, the identical DNA profiles between the two twins indicate an extremely high probability that the twins arose from a monovular egg. This appears to be the first documented case of identical twins in birds.

Twinning mechanisms

The mechanism of yolk division in monozygotic avian twins is unknown, but apparently the yolk is not always divided equally between the embryos (Nalbandov 1942). An unequal division of the yolk may account for the difference observed in the twins' initial size and subsequent growth rates. Differences in the hatch weight might also reflect unequal distribution of nutrients via the inter-twin haemodynamics of a shared blood circulation - a common phenomenon in monochorionic human twins (Bryan 1977, 1992).

While avian twinning is rare (Riddle 1923, Newman 1940, Waddington 1941), and monovular twinning exceedingly so, the occurrence of two yolks in a single egg appears to be relatively common in emu. One American emu farm reported a hen laying three double-yolked eggs during the 1995 breeding season (L. Baldwin, *pers. comm.*). These twin-bearing eggs were fertile, but in only one case did the chicks develop to the point of hatching. The genetic identity and similarity of these pairs of twins is unknown.

Double embryos also occur relatively frequently in the fowl where gastrulation progresses during laying, whereas in pigeons, where gastrulation is complete before the egg is laid, double embryos are relatively rare (Stockard 1921). Stockard (1921) hypothesised that the occurrence of twinning was associated with premature laying of the blastoderm at or before gastrulation, followed by cooling and subsequent

'developmental arrest'. For emu the timing of gastrulation is unknown and should be investigated.

Riddle (1923), extending Stockard's (1921) view, concluded that twins arose from alterations in the rate of development, but Sturkie (1946) was unable to support Stockard's (1921) hypothesis experimentally. Riddle (1923) hypothesised that twinning is associated with abnormally large yolks. This is interesting, because the emu twins arose from an egg of unusually large size compared to other eggs in the clutch. It is not clear, however, why a monovial egg should be unusually large.

Newman (1940) believed that avian twinning is environmentally, rather than genetically induced, and Batt *et al* (1975) hypothesised that it is induced by exposure to low temperature before incubation. Five pairs of mallard twins occurred in their treatment group of eggs that failed to hatch after being subjected to low temperatures, while none was recorded in their control eggs. They concluded that normal cleavage can be interrupted by low temperature to produce twins, even after oviposition. Four pairs of twins were found to be "normal". In each of these cases the embryos appeared to share the same yolk sac, and therefore were monozygotic (Batt *et al* 1975). There was no adverse cooling of the twin-bearing emu egg prior to incubation nor, so far as we are aware, any other event during incubation that could have resulted in developmental arrest leading to twin-production.

Twin sex

Large yolk size has also been linked to gender. During his analysis of 20,000 pigeon eggs, Riddle (1923) found three pairs of female twins arose from extremely large yolks, and he proposed a correlation between large yolk size and female sex, and small yolk size and male sex. While the emu twins arose from a large egg and were both female, there was no correlation between egg size and gender for other eggs. A gender bias towards females may exist in avian twins. Certainly Nalbandov's (1942) twins were female, and of the five sets of twins investigated by Batt *et al* (1975), three of known sex were female. While the mechanisms for monovular twinning in birds remain elusive, this study demonstrates that it does occur.

Conclusions

We applied the technique of minisatellite DNA profiling to establish the first known occurrence of genetically identical twinning in birds. This raises interesting questions about the mechanism of monozygotic twinning in birds' eggs which have relatively large yolks, and the wider application of DNA analysis in ratite research.

It would be useful to continue to monitor and compare the twins' growth and development, and their future breeding productivity and hatching success. They offer an opportunity to assess the effects of nutrition and environment on egg production, and to provide rare longitudinal bench mark measures of the development of genetically identical twins. Data from other sets of emu twins world-wide may point to the relative frequency of avian twinning and twin viability, and help improve our understanding of the mechanisms involved.

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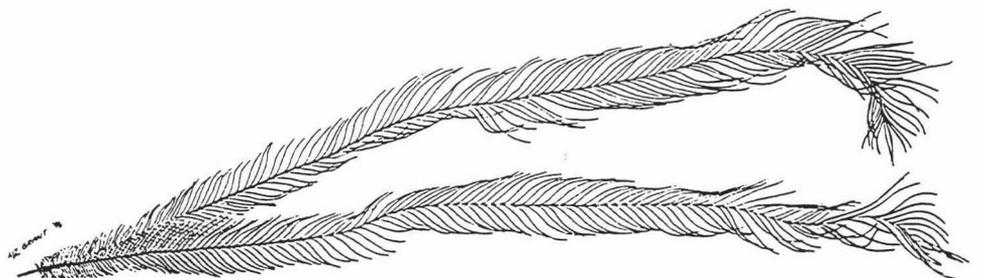
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CHAPTER SEVEN

General discussion and recommendations



Summary and Recommendations

In New Zealand the rarity and value of emu eggs influences incubation practices significantly. At present, hatchability of an individual egg and survival of the chicks takes priority over careful manipulative experimental studies that could determine optimum incubation parameters for maximum hatching success. It will be some time before the New Zealand emu industry becomes fully commercial, but identified problems in production, such as embryonic mortality, low hatching success and post hatch mortality summarised below, should be dealt with immediately.

In the current study, data provided no support for the popular belief that older emu are more productive than first time breeders. Clutch size did not increase over subsequent years, and fertility, hatching success, and chick viability were not found to decline towards the end of the breeding season, as widely perceived in the industry. Once compatible, productive pairs are established, they should be kept together in the same breeding pen in successive years to maximise predictability in egg laying sites. Shrubs should be planted in breeding pens to reduce the number and scatter of laying sites and the time required to gather eggs. Detailed records should be kept for each pair on the time interval between eggs, the time of day eggs are laid, and preferred laying sites. This will reduce bird disturbance and the time spent searching for eggs.

Although captive emu are genetically similar to their wild counterparts, it is clear that their breeding behaviour, especially in respect to egg laying site and duration, is greatly modified by the nature of the surroundings in their enclosure. The effects of pen size, artificial shelter design, and the degree of inter-pair interactions, on productivity are also important considerations that warrant further investigation.

Fertility levels, both on the study farm and at a national level, were found to be greater than hatching success, indicating there is still room for substantial improvement in incubation practices. As infertility does not appear to be a major concern in the majority of captive farmed emu in New Zealand, educating farmers regarding embryo detection, and subsequently improving hatching success of fertile eggs, requires further attention.

It is important that all unhatched eggs are opened and the contents carefully analysed for signs of embryonic development, and that farmers learn to distinguish between early embryonic death and infertility. Subsequent research should further investigate reasons for both early and late embryonic mortality, and monitor stages of incubation to establish normal development and orientation of the emu embryo. It is also essential in order to accurately define what constitutes a malposition in emu chicks.

Although both radiography and ultrasound proved unsuccessful at determining egg fertility at an early stage, a long thin metal rod balanced on each egg provided a very inexpensive, yet accurate, measure of embryo development from 32 days incubation. After field work was completed an infra-red egg candler was purchased and provided by far the most accurate and earliest determination of egg fertility. There are currently no studies reporting on observations of emu eggs during candling, and detailed observations are needed to better understand the hatching process.

Several limitations in the quantitative comparison of hatching success under artificial incubation of ratite species have been highlighted in the present study. Ambiguity of terminology in the literature on avian hatching success restricts the value of comparative studies. In particular, terms used to describe 'hatching success' and 'hatchability' are used inconsistently by both researchers and farmers. It is important to define if only fertile eggs are considered, or all eggs placed in the incubator, as this can result in very different levels of "hatching success".

It is necessary to artificially incubate eggs under controlled conditions to determine the effects of lower temperature and water loss on hatching success. Some eggs should be experimentally incubated at 34°C and 35°C, similar to the temperatures maintained by incubating males. Trialing eggs at different temperatures will determine optimal conditions for the artificial incubation of emu eggs in New Zealand. The percentage weight loss target for eggs should be reduced to a maximum of 12% over the full incubation period. The frequency of turning should be maintained at once every two hours, but the effects of reducing the angle of turn should be investigated further.

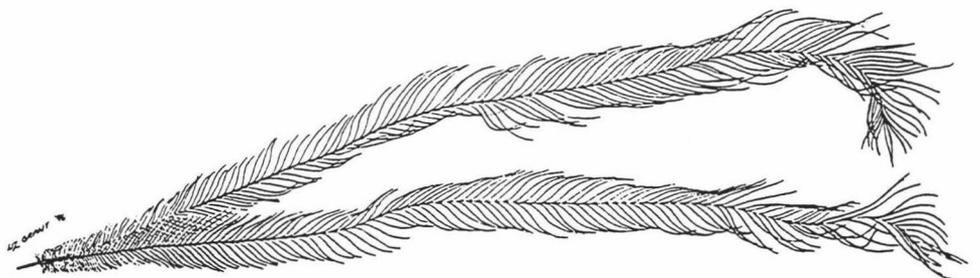
Reducing the temperature in the hatcher should also reduce heat stress to the chicks during hatching. Hatching assistance was usually given to chicks regardless of whether it was required. Because the justification of this practice is far from clear, and evidence suggests it is actually detrimental to chicks, a proper assessment of the need for such a practice is required. The low incidence of malpositioned chicks suggests intervention is seldom necessary. Chicks need adequate time to hatch with minimal interference and post hatching mortality in the first week due to non-absorption of the yolk sac or its retention, should decline with a reduction in hatching assistance.

The lack of standard methodology in determining central egg temperatures during natural incubation in quantitative studies have also hindered the interpretation of comparative studies. It is necessary to consider the effects and accuracy of different telemetric egg designs on the internal egg temperatures recorded. Also of importance, but often overlooked, is the need to monitor nest microclimate and parental attendance during incubation. Although conditions under natural incubation are very different to those used in an artificial incubator, determining parameters used in the wild should help ascertain optimal incubation parameters to be used in artificial incubators.

It is important to continually review and improve farm management practices. Regular monitoring of chick weight gains are necessary in order to identify slow growing birds and weight losses that may indicate ill health. It will also help detect rapidly growing birds that may be at greater risk of leg rotation problems. Reasons for leg problems which are strenuously debated, should be identified and detected as early as possible. As corrective surgery has proved to be expensive and unsuccessful it is recommended affected birds be euthanased.

Finally, this is one of the first studies in New Zealand to highlight emu productivity in a New Zealand environment. In spite of obvious limitations, behavioural studies of captive emu are relevant to the behaviour of animals in their wild state and should not be discounted. Comparisons will allow a better understanding of the birds' needs in a farming environment, and subsequent research addressing the problems outlined in this study should further increase production and hatching success of emu in captivity.

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APPENDICES



Appendix i

1995 New Zealand Emu Breeding Survey

Ratite Research Centre
Massey University

Why a Survey?

This survey is a part of on-going research at Massey University into breeding and production of emu farmed in New Zealand. The research is directed at improving management of farmed emu and ultimately the profitability of the emu industry. The survey will link nation-wide information with an intensive local study.

Aim of the Survey

The aim of the survey is to establish accurately the current (1995) level of productivity and hatching success of farmed emu in New Zealand. This information is vital to:

1. establishing baseline information,
2. identifying problem areas,
3. establishing priority research needs,
4. quantifying future improvements,

and will subsequently provide valuable information on the status of emu breeding at a national level. This will be of great benefit to the New Zealand emu industry. While this survey focuses on the 1995 breeding season, any previous years' emu breeding records you can provide would also be greatly appreciated.

Confidentiality

ALL information you provide will remain strictly confidential at Massey University. General trend data only will be published in a report summarising the survey results.

Further Information

For further information regarding this survey please contact Suzanne Bassett, Department of Ecology, Massey University - telephone (06) 356 9099 - extn 7035, or extn 5486 (Departmental Secretary), fax (06) 350 5623. If Suzanne is temporarily unavailable please contact Dr Murray Potter on extn 5484.

QUESTIONNAIRE

Name (optional)

Location of Emu Farm

Upper North Island

Central North Island

Lower North Island

Upper South Island

Lower South Island

Number of years farming emu?

Number of years farming other livestock?

Numbers of emu farmed

Total number of breeding female emus

Total number of breeding male emu

Total number of non-breeding female emu

Total number of non-breeding male emu

Total number of yearling emu

Incubator

What type of incubator do you use?

Average incubator temperature

Average incubator humidity

Average length of incubation (days)

Frequency of egg turning (e.g. 1 turn/2 hours)

Degree of egg turning (e.g. 180°)

Chicks

Do you use a hatcher? yes/no

Average hatcher temperature

Average hatcher humidity

At what stage of incubation are the eggs transferred to the hatcher (days)?

Is hatching assistance give to chicks yes/no

if yes - please outline

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DNA Sexing

Are you currently getting your chicks DNA sexed? yes/no

if no... would you be interested in this at a later stage? yes/no

Production information

| | | | | | | Additional Pairs |
|--|---|---|---|---|---|------------------|
| PAIR NUMBER | 1 | 2 | 3 | 4 | 5 | |
| Age of breeding female | | | | | | |
| Age of breeding male | | | | | | |
| Start of egg laying/pair | | | | | | |
| Finish of egg laying/pair | | | | | | |
| Number of chicks produced/pair | | | | | | |
| Number of embryos found* | | | | | | |
| Number of infertile eggs found* | | | | | | |
| Number of chicks that died at point of hatch | | | | | | |
| Average weight of chicks at point of hatch | | | | | | |
| Number of chicks with yolk sac problems | | | | | | |
| Number of chicks with leg problems | | | | | | |

* if fertility was not determined please state unknown

Weights

Do you weigh your adult birds yes/no
 if yes - how regularly?

Do you weigh your chicks? yes/no
 if yes - how regularly?

Mortality/Survivorship

- Number of chicks alive at week one
- Number of chicks alive at week four
- Number of chicks alive at week eight
- Number of chicks alive at week twelve

General Comments

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Thank you for your participation - if you have any queries please don't hesitate to contact me. Please place the completed questionnaire in the self-addressed pre-paid envelope and return it to me as soon as possible.

Suzanne Bassett
Ratite Research Centre
Department of Ecology
Massey University

November 1995

Appendix ii

1995 New Zealand Emu Breeding Survey Results

Suzanne Bassett
Ratite Research Centre
Department of Ecology
Massey University

Background

In November 1995, New Zealand emu farmers were surveyed to determine emu production and hatching success during the 1995 breeding season. The data obtained are summarised here. These data establish a baseline against which future improvements can be measured. They identify several problem areas that would benefit from an investment in research.

The questionnaire was developed in consultation with the technical committee and the executive of the New Zealand Emu Farmers Association (NZEFA). The questionnaire (Appendix i) was circulated at the end of the 1995 egg laying season to NZEFA registered emu farmers with breeding birds¹, and was accompanied with a letter of support from the President of the NZEFA, a brief personalised covering letter outlining the importance of the requested information, and a self-addressed, pre-paid envelope. To help maximise return rates, the survey was printed on yellow paper so it would stand out from other printed material received by the farmers.

A reminder notice was placed in the December issue of the NZEFA Journal requesting prompt responses. A guarantee was given that information about specific farms would remain confidential and self identification by respondents was optional. The questionnaire stipulated that only general trend data would be publicised. This report follows the format of the questionnaire.

¹ Breeding birds were defined as birds two or more years of age and producing eggs; non-breeding birds were birds two years or older which had not yet produced eggs; and yearlings were juveniles (or black-heads) around one year of age.

Results and discussion

A total of 34 questionnaires were sent out in November 1995. By February 1996, 28 had been returned - a response rate of 82%.

In 1995 17 farmers bred emu on their properties, and 14 farmers artificially incubated eggs on their properties. Data presented below are for productive birds only. A true estimate of production should include mature, non-breeding birds and although the survey targeted this group, it was not possible to distinguish birds that paired up but failed to breed, or birds that lacked a suitable mate and consequently were unable to breed. Future surveys should request data on total numbers of birds and pairs of birds, including breeding and non-breeding individuals.

Location of Emu Farms

For the purpose of the questionnaire, New Zealand was divided into four geographic regions:

Upper North Island - all farms north of Hamilton.

Central North Island - all farms between Wanganui and Hamilton

Lower North Island - all farms between Wellington and Wanganui

South Island - all farms located in the South Island.

The distribution of emu farmers with breeding birds within each of these regions is shown in Table 1.

Table 1 Distribution of productive emu farms throughout New Zealand in 1995 (n = 17).

| Farm location | % of emu farms |
|-------------------------------|----------------|
| Upper North Island | 12 |
| Central North Island | 35 |
| Lower North Island | 6 |
| South Island (mainly Central) | 37 |

Sixteen farmers answered the questions of how long they had been farming emu. Half (50%) of the emu farms were established in 1994; 38% (n = 6) had been breeding emu for 3-5 years; and 12% (n = 2) had farmed emu for more than 5 years. Twelve (75%) of the emu farmers had previously been employed in agriculture.

Bird numbers

National bird numbers are summarised in Table 2.

Table 2 National bird numbers as recorded in January 1996 from 17 emu breeders in New Zealand.

| | Breeding Females | Breeding Males | Non-breeding Females | Non-breeding Males | Yearlings |
|--------------|------------------|----------------|----------------------|--------------------|-----------|
| No. of birds | 61 | 62 | 22 | 24 | 144 |

A number of respondents did not specify the number of non-breeding birds they had, mainly because the birds' sexes were undetermined at the time of the survey. Non-breeding birds have therefore been underestimated. The proportion of birds in each region is summarised in Table 3.

Table 3 Percentage of birds in each region of New Zealand by breeding status at the end of 1995.

| Emu Breeding Status and Numbers | | | | | | |
|---------------------------------|---------------------|-------------------|-----------------------|---------------------|--------------|------------------------|
| Farm Location | Breeding Female Emu | Breeding Male Emu | Non-breed. Female Emu | Non-breed. Male Emu | Yearling Emu | Total Emu Numbers/area |
| Upper North I | 4 | 4 | 1 | 1 | 20 | 28 |
| Central North I | 22 | 24 | 12 | 13 | 24 | 95 |
| Lower North I | 19 | 18 | 7 | 8 | 24 | 95 |
| South I | 16 | 16 | 2 | 2 | 33 | 69 |
| Total | 61 | 62 | 22 | 24 | 144 | 313 |

The average number of breeding emu per farm was just 3 pairs. Currently 50% (n = 8) of New Zealand emu farmers have only 1 pair of breeding birds, 38% (n = 6) have 5 or fewer pairs, and only 12% (n = 2) have 6 pairs or more.

Farm Management

All New Zealand emu farmers currently use labour-intensive farming techniques and breed birds in pairs within individual breeder pens. There was one trio (2 females : 1 male), and one foursome (2 females : 2 males) reported in the survey. Breeding pen sizes varied from 300 m² (12 x 25 m) up to 3000 m², and 88% of pens were long, narrow and rectangular in shape.

On all farms all the birds had access to pasture, but trees and shrubs (for shelter or nesting sites) were present in only 18% (n = 3) of pens. Artificial shelters were provided in 94% (n = 16) of breeder pens and, where artificial shelter was not provided, trees and shrubs were present. Only three farms provided both trees, shrubs and artificial shelters for breeding birds.

Artificial shelter type or size was not investigated and questions pertaining to this would be useful in subsequent surveys. The most common vegetation in emu pens was flax (*Phormium tenax*), *Eucalyptus* spp, toetoe (*Cortaderia* sp.) and unspecified shelter-belt tree species. Trees and other tall plants provide important shade, shelter and varied surrounding for breeding emu.

Egg handling and storage

Eggs were stored for one to 14 days before artificial incubation, with the majority of eggs been held for 4 - 6 days. Ten breeders (71%) stored eggs at 12 - 16°C, while four (29%) stored them in a cool area at room temperature.

Table 4 outlines egg sanitation practices currently used in New Zealand. Five (36%) out of 14 farmers fumigated eggs with formaldehyde gas either soon after collection or immediately before setting the eggs, and another five farmers used no fumigation procedures at all.

Table 4 Methods of egg fumigation used by farmers in New Zealand in 1995 (n = 14).

| Fumigation Method | Percentage |
|---|------------|
| Egg washing - Virkon | 7% |
| Egg washing - Chickguard | 21% |
| Potassium Permanganate and Formaldehyde | 36% |
| No fumigation procedures | 36% |

Incubation

Fourteen (82 %) emu farmers incubated their own eggs. Six (43%) also incubated eggs from other farms. Incubator models used included Dominion (n = 3), Domex (n = 3), Midex (n = 2), Multiquip (n = 2), Aussie-set (n = 2), Hatchrite (n = 1) and BellSouth (n = 1). Incubator settings varied from 35.4 - 37.0°C, with 69% (n = 9) of farmers incubating eggs at 35.6 - 35.8°C. Relative humidity settings were variable, ranging between 28 - 65%, with 10 (71%) of the 14 farmers choosing a humidity of 35 - 55%. The positioning of eggs within incubators was not investigated in the questionnaire and it was assumed that all eggs were incubated horizontally, as is the common practice for emu eggs. Turning regimes were variable, with the most common frequency and degree of turning being 180° at 2 hourly intervals (Table 5).

Table 5 The frequency and degree of emu egg turning during artificial incubation in New Zealand in 1995 (n = 14).

| Frequency of egg turning | Degree of egg turning | Percentage of farmers |
|--------------------------|-----------------------|-----------------------|
| 1 turn/1 hr | 90° | 7% |
| 1 turn/1 hr | 180° | 22% |
| 1 turn/2 hr | 180° | 50% |
| 1 turn/4 hr | 180° | 14% |
| 1 turn/12 hr | 180° | 7% |

Most emu breeders removed eggs from the incubator between day 45 - 51 of incubation. Two farmers transferred eggs to the hatcher only after they had pipped internally. Incubation usually lasted 51 days, but variations of 2 - 3 days either side of this were reported.

In general, incubation length did not seem to reflect the various regimes of incubation temperature and relative humidity. Farmers tended to open eggs prematurely, making it difficult to determine the effect of various incubation temperatures and humidity on the length of incubation.

Chicks

Several types of hatchers were used by farmers, and hatcher temperatures varied from 34.5 - 36.0°C, with humidity ranging from 28 - 75%. Seventy seven percent (n = 10) of farmers maintained the hatcher at a lower temperature than that used in the incubators. Two farmers kept similar temperatures in the incubator and the hatcher, but humidity was higher in the hatchers.

Hatching assistance was given by 86% (n = 12) of farmers regardless of whether it was required. Assistance varied from cracking a small hole in the shell through into the air sac to locate the beak of the chick, to breaking multiple holes if the chick was malpositioned. In some instances the chick was completely removed from the shell. The justification for providing hatching assistance to a chick is far from clear. Proper assessment of the need for such a practice requires (a) carefully controlled experimentation in artificial incubators and (b) detailed comparison of the hatching process and hatching success under natural as well as artificial incubation. In the mean time it appears that intervention is seldom necessary. Chicks that are allowed to hatch in their own time tend to be stronger than those receiving assistance. Chicks need adequate time to hatch with minimal interference. Assisted chicks show high levels of mortality within 12 - 48 hours after hatching, probably reflecting damage sustained during over-vigorous hatching assistance.

DNA Sexing

Eleven (73%) of the 15 respondent farmers currently sex their emu chicks by means of DNA techniques (blood samples). Of those not yet using this service, 20% (n = 3) stated they would like further information regarding the procedure, and only 7% (n = 1) did not intend to use it.

National productivity

The age of first breeding in captive New Zealand emu is generally two years, but the percentage of birds laying eggs as two year olds was not determined. In 1995, the oldest bird producing eggs in New Zealand was 25+ years old and the youngest was 16 months. The most productive bird laid 45 eggs.

Responses to the survey indicate that a total of 1351 eggs were laid in the 1995 breeding season from 58 breeding female emu, and from this 716 live chicks were produced. This resulted in a national hatching success of 52.9%. Age had no significant effect on production in birds from 2 years old to 5 years old, but females aged 6 years or older were more productive (Table 6). Regardless of breeder age, average productivity was 23 eggs, and 12 chicks, per female per year. From 43 known breeding pairs, the average chick weight at point of hatch was 375 g (range: 280 - 490 g).

National fertility levels could not be determined because a large proportion of unhatched eggs were not analysed for embryo development, or were assessed by untrained observers. Data from an intensive study on emu indicate, however, that fertility levels are considerably greater (average: 90.3% over 1994 and 1995) than hatching success (average: 70.0%). There is thus room for substantial improvement in incubation practices. Many farmers who responded to the questionnaire did not differentiate between unhatched “fertile” and unhatched “infertile” eggs. Hatching success was, therefore, calculated from the total number of eggs set in the incubators and the resulting number of live chicks hatched.

Clutch size varied from 2 to 45 eggs (Table 6). The interval between eggs is fairly variable both within and between emu, but once a regular laying pattern is established it is extremely consistent, especially in experienced breeders. The first egg of the 1995

breeding season was laid on 13 April in the Auckland region, and the last egg was laid on 9 December in Christchurch. The last female to start egg laying began on 8 August, and the first breeding female ceased laying on the 4 July. The average egg laying period was 85 days (± 5 days) with a range of 12 - 188 days. The main egg-laying season extended from mid Autumn (April) until mid Spring (September) with peak egg laying from June to August.

Table 6 Average number (\pm S.E.) of eggs and chicks produced in relation to age of breeding female emu in New Zealand in 1995.

| Age breeding female | 2 years | 3 years | 4 years | 5 years | 6+ years |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| n | 20 | 14 | 12 | 7 | 6 |
| Total eggs | 408 | 348 | 269 | 124 | 202 |
| Average no. eggs/female | 20.4 \pm 2.2 | 24.7 \pm 3.1 | 22.4 \pm 4.2 | 17.7 \pm 3.4 | 33.7 \pm 3.3 |
| Total chicks | 202 | 145 | 168 | 74 | 127 |
| Average no. chicks/female | 10.1 \pm 2.2 | 10.4 \pm 2.7 | 14.0 \pm 3.6 | 10.6 \pm 2.9 | 21.2 \pm 4.3 |
| Hatching success | 49.5% | 41.7% | 62.5% | 59.7% | 62.9% |

Chick survival

From a total of 578 chicks with known life histories, 513 survived to 3 months of age, representing a survival rate of 88.8% of all chicks that hatched in 1995. A further 51 chicks died at point of hatch, equivalent to a 3.8% rate of late embryonic death from all eggs set. The highest frequencies of chick deaths occurred in the first week after hatching, and between weeks 8 - 12, where any birds developing leg rotations were subsequently euthanased (refer Table 7). Yolk sac problems occurred in 5.2% of all chicks hatched in New Zealand (37 out of 716 chicks), and most of these died within one week of emerging from the shell.

Table 7 National chick survival in New Zealand in 1995.

| Week | Number of chicks alive | Percentage surviving |
|------|------------------------|----------------------|
| 0 | 578 | 100% |
| 1 | 545 | 94% |
| 4 | 539 | 93% |
| 8 | 528 | 91% |
| 12 | 513 | 89% |

Incidence of leg problems

From the 578 chicks surviving up to 3 months of age, 32 (5.5%) were affected with leg rotations. Research is needed to determine whether this problem is related to nutrition, bird management, or genetics. It is currently undetermined whether the left or right leg is more commonly affected, or whether there is a sex bias in leg problems. It would be useful to investigate this in future surveys.

Bird weights

Only 26.7% (n = 4) of farmers weigh their adult birds once a year, although one farm took weights monthly. Eleven (73%) out of 15 farmers weighed their chicks at hatching but only 6 (55%) out of 11 continued to weigh their chicks after this. Additional weights were generally taken at weekly intervals until the chicks reached 6 - 8 weeks of age. It is important to closely monitor chick weight gains in order to identify slow growing birds, weight losses that may indicate ill health, and rapidly growing birds that may be at greater risk of leg rotation problems.

Summary and Recommendations

Surveys such as this are extremely important in evaluating breeding and management conditions on New Zealand emu farms, and similar surveys should be conducted annually. That would allow yearly comparisons to be made of the national flock size, and national productivity. It would also indicate areas in which future research should focus. Currently in New Zealand, farm management practices, hatching success, and

chick survival vary widely. With data from only one season it is too early to state which practices are more effective but this would become increasingly apparent if repeat surveys were conducted annually.

Nationally, chick survivorship is high but the hatching success obtained from artificial incubation is low. Recent studies on farmed emu have shown fertility levels are extremely high, and embryo mortality is greatest during the first two, and the last week, of artificial incubation. Late embryonic deaths were recorded in this survey, but most farmers do not bother, or lack the necessary skills, to accurately assess early embryonic deaths. There is an urgent need for farmer training to correctly distinguish between early embryonic death and infertility. Subsequent research should aim at further assessing reasons for early and late embryonic death, and determine optimal conditions for the artificial incubation of emu eggs in New Zealand.

It is important to continually review and improve farm management practices. Leg problems and chick growth rates should be closely monitored to identify sick chicks as early as possible. Subsequent surveys should assess the proportion of young birds producing eggs in their first breeding season.

No comparable surveys from overseas are known for emu, but ostrich farmer surveys have been conducted. These have targeted incubation and health problems. The majority of the problems identified were due to poor management practices, a situation that probably applies equally to emu. Assessment of farming conditions in regard to emu behaviour and breeding productivity requires further research to maximise emu egg production, hatching success and chick survival in New Zealand.

Appendix iii

Measurements (in millimeters) of emu embryos

| Egg number | Embryo number | Body width | Body length (<i>in situ</i>) | Body length (<i>straight</i>) | Head width | Head length | Leg length | Wing length | Eye (diameter) | Age estimate (day) |
|-------------|---------------|------------|-----------------------------------|------------------------------------|------------|-------------|------------|-------------|-------------------|--------------------------|
| 0725-293 | 95-32 | | | 3.95 | | | | | 0.50 | 4 |
| 0725-372 | 95-28 | | | 4.00 | | | | | | 4 |
| 0725-307 | 95-31 | | | 4.95 | | | | | 1.25 | 4 |
| 0725-372 | 95-1 | | | 5.20 | | | | | | 5 |
| 47-38 (1) | C-4 | 2.10 | 3.70 | 5.90 | 2.30 | 3.80 | | | | 6 |
| S27A-267 | 95-33 | | | 6.80 | | | | | | 6 |
| C42-70 (20) | C-5 | 2.70 | 4.00 | 7.00 | 3.10 | 3.25 | | | 1.20 | 6 |
| 19-94 | 94-4 | | | 7.40 | | | | | 1.45 | 8 |
| P22-108 | 94-10 | 3.40 | 7.40 | 8.60 | 3.30 | 4.00 | | | 1.60 | 7 |
| 0725-325 | 95-34 | 2.60 | 7.00 | 7.50 | | | | | 0.70 | 5 |
| AC-25 | AC-1 | 3.65 | 8.20 | 9.95 | 4.35 | 4.90 | | | 1.80 | 8 |
| S27A-91 | 94-9 | 5.90 | 7.75 | 12.70 | 4.70 | 5.50 | | | 2.05 | 7 |
| 013-127 | 94-11 | 5.00 | 9.20 | 12.40 | | | | | | 7 |
| 001-60 | 95-14 | 5.60 | 10.70 | 13.30 | 5.50 | 6.40 | 5.80 | 2.20 | 3.60 | 13 |
| S27A-35 | 95-6 | 7.40 | 11.00 | 14.80 | 6.30 | 7.20 | 5.50 | 2.60 | 3.50 | 12 |
| S27A-47 | 94-2 | 8.00 | 10.40 | 14.85 | 5.90 | 6.20 | | 1.65 | 3.70 | 13 |
| S27A-234 | 95-36 | 6.10 | 9.35 | 15.00 | 4.95 | 5.20 | 4.70 | 1.95 | 2.60 | 10 |
| 5236-317 | 95-20 | 8.00 | 11.40 | 15.25 | 6.50 | 8.40 | 7.00 | 2.70 | 2.50 | 13 |
| 013-58 | 94-3 | 7.50 | 12.20 | 16.70 | 7.20 | 9.10 | 10.00 | 1.60 | 5.00 | 13 |
| P21-253 | 95-5 | 5.20 | 10.85 | 17.10 | 7.00 | 7.85 | 4.95 | 1.80 | 2.45 | 12 |
| 0725-45 | 95-35 | 6.65 | 11.00 | 17.40 | 6.00 | 6.80 | 6.30 | 2.55 | 3.20 | 10 |
| S27A-3 | 95-29 | 8.40 | 10.60 | 17.45 | 8.00 | 8.00 | 7.00 | 3.15 | 4.50 | 14 |
| C17-83 (19) | C-2 | 6.60 | 11.20 | 17.50 | 5.40 | 8.10 | 3.60 | | | 14 |
| V08-21 | 94-8 | 8.20 | 12.00 | 18.00 | | | 9.10 | 2.70 | 5.10 | 14 |
| V08-30 | 95-13 | 8.20 | 12.20 | 18.95 | 8.80 | 8.60 | 7.40 | 3.30 | 4.00 | 16 |
| 2536-389 | 95-3 | 8.55 | 12.45 | 19.60 | 7.00 | 7.60 | 7.05 | 2.20 | | 14 |
| 013-65 | 94-1 | 9.85 | 12.70 | 19.85 | 7.95 | 9.00 | 8.30 | | 6.20 | 14 |
| 0725-269 | 95-30 | 8.65 | 13.95 | 20.00 | 8.20 | 8.60 | 6.60 | 2.70 | 4.80 | 14 |
| 013-84 | 93-2 | 8.30 | 14.10 | 20.50 | 9.20 | 10.00 | 8.80 | 2.20 | 5.80 | 14 |
| 0725-57 | 95-12 | 7.00 | 11.60 | 21.00 | 6.50 | 9.05 | 7.95 | 4.10 | 5.00 | 12 |
| C56-46 (5) | C-3 | 9.10 | 12.40 | 22.00 | 8.30 | 9.50 | 10.00 | 3.70 | | 16 |
| P22-54 | 95-27 | 8.40 | 11.70 | 22.00 | 10.50 | 10.30 | 10.20 | 2.80 | 3.90 | 18 |
| 9940-300 | 95-25 | 8.55 | 13.10 | 22.05 | 9.30 | 12.00 | 7.00 | 2.00 | 5.50 | 14 |

| Egg number | Embryo number | Body width | Body length (<i>in situ</i>) | Body length (<i>straight</i>) | Head width | Head length | Leg length | Wing length | Eye (diameter) | Age estimate (day) |
|------------|---------------|------------|-----------------------------------|------------------------------------|------------|-------------|------------|-------------|-------------------|--------------------------|
| C63-48 (7) | C-1 | 10.10 | 14.00 | 23.00 | 6.75 | 10.80 | 8.40 | 3.80 | | 14 |
| V08-22 | 95-8 | 10.20 | 15.00 | 23.50 | 9.40 | 12.30 | 15.00 | 5.00 | 7.70 | 24 |
| V08-55 | 94-10 | 10.50 | 17.25 | 23.60 | 11.70 | 15.05 | 17.90 | 4.90 | 7.20 | 24 |
| 0725-31 | 95-7 | 12.00 | 16.70 | 24.10 | 11.10 | 17.00 | 20.40 | | 8.80 | 22 |
| 0725-19 | 95-15 | 13.20 | 19.00 | 25.00 | 12.20 | 16.20 | 19.50 | 3.95 | 8.00 | 22 |
| CWW15-1 | C-6 | 11.50 | 14.00 | 25.20 | | | 8.55 | 2.50 | | 18 |
| 0725-64 | 95-26 | 10.20 | 18.00 | 25.50 | 9.35 | 12.00 | 14.75 | 5.55 | 7.10 | 20 |
| 0725-220 | 95-22 | 7.60 | 14.50 | 25.60 | 8.40 | 10.40 | 8.15 | 3.00 | 4.90 | 22 |
| 004-178 | 95-19 | 10.30 | 14.20 | 26.10 | 9.20 | | 1.07 | | | 21 |
| 0725-151 | 95-17 | 8.95 | 18.50 | 26.20 | 11.10 | 14.00 | 15.80 | 4.65 | | 20 |
| S27A-79 | 95-10 | 9.70 | 15.30 | 26.20 | 10.50 | 13.70 | 12.40 | 4.60 | 6.00 | 20 |
| S27A-402 | 95-37 | 12.20 | 19.20 | 28.10 | | 15.70 | 20.80 | 4.05 | | 20 |
| A26-207 | 94-5 | 12.70 | 18.00 | 29.70 | 11.30 | 15.50 | 17.30 | 4.40 | | 24 |
| 5232-258 | 95-18 | 13.70 | 19.50 | 30.00 | 11.00 | 18.40 | 25.20 | 7.45 | | 24 |
| 0725-51 | 95-24 | 14.00 | 20.00 | 30.60 | 14.00 | 18.00 | 20.50 | 7.70 | 7.45 | 24 |
| 001-74 | 94-6 | 11.60 | 19.90 | 32.10 | 14.20 | 17.00 | 16.10 | 6.00 | 9.85 | 25 |
| 2534-375 | 95-2 | 12.75 | 20.80 | 32.12 | 13.80 | 18.65 | 21.65 | 4.35 | | 24 |
| 013-72 | 93-1 | 12.00 | 18.50 | 32.15 | 12.45 | 18.30 | 20.50 | 7.00 | 7.30 | 24 |
| V08-50 | 95-16 | 11.95 | 19.70 | 32.40 | 12.00 | 17.10 | 18.00 | 4.20 | | 24 |
| P22-63 | 95-11 | 14.20 | 23.65 | 33.00 | 12.00 | 20.30 | 39.40 | 9.40 | | 24 |
| 0725-39 | 95-4 | 12.95 | 21.00 | 36.00 | 14.20 | 18.30 | 18.95 | 7.45 | 9.00 | 22 |
| 0725-315 | 95-23 | 16.60 | 28.70 | 40.00 | 15.70 | 20.50 | 36.60 | 8.50 | | 27 |
| S27A-25 | 95-9 | 17.60 | 31.00 | 41.30 | 17.90 | 28.80 | 39.50 | 10.10 | | 30 |
| 5233-237 | 95-21 | 17.30 | 35.80 | 45.30 | 13.75 | 27.50 | 42.50 | 11.20 | | 30 |

All emu embryo measurements in millimeters.

Appendix iv

Similarity Coefficient Statistics

PE Zoogens's emu population research statistics based on 40 assumed unrelated emu in the U.S.A, with an average number of 22 bands per bird.

1. Similarity Coefficient = 0.45 ± 0.10

$$D = 2N_{AB} / (N_A + N_B)$$

where N_{AB} = number of bands shared by both A and B

N_A = number of bands in individual A

N_B = number of bands in individual B

D values vary from zero when there are no bands in common, to one when the two samples are identical.

2. Probability of match in unrelated emu = 1×10^{-13}

$$P = 1/t \sum (1 - 2x + 2x^2)^{ni/x}$$

where P = probability of match

n = number of bands in an individual

x = mean D in unrelated birds

t = number of individuals

3. Probability of a match in related emu = 1×10^{-6}

$$P = 1/t \sum [1 - 1/2q (1-q)^2 (4-q)]^{ni/x}$$

where P = probability of match

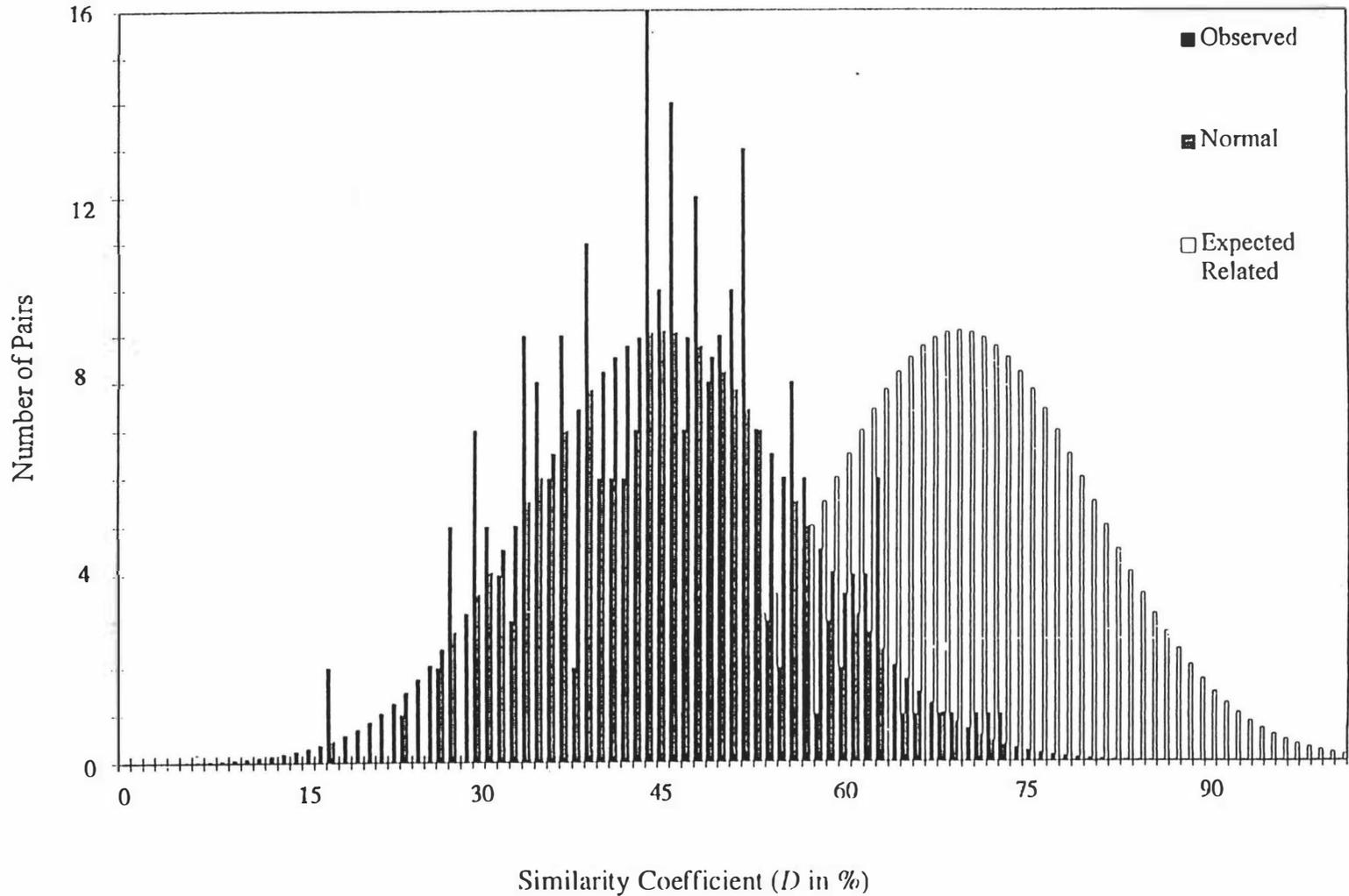
n = number of bands in an individual

x = mean D in unrelated birds

t = number of individuals

$q = 1 - (1-x)^{1/2}$

PE Zoogen's Similarity Coefficient of related and unrelated emu.



Similarity Coefficient distribution chart