

# Effects of transportation-induced jarring on ratite embryo development and hatching success

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# Abstract

Advice is provided on five questions that were addressed by subjecting emu eggs, as a model for kiwi eggs, to transport-induced jarring during three stages of embryo development. The jarring associated with two kiwi egg recovery operations was also measured.

Jarring during transportation had no demonstrable effect on embryo development, the need for hatching assistance, or the incidence of embryo malpositions.

Jarring did not increase physical abnormalities or defects in developing or hatched chicks within the level of jarring applied in this experiment.

There was no evidence that any stage of embryo development was more sensitive to jarring than any other stage.

Maximum jarring occurred when transported on a quad, and when eggs were moved on to or into vehicles, including aircraft. Extreme care is needed at these times to avoid knocking the egg container against hard structures. Transportation by fixed-wing plane had higher average jarring than transport by car over either sealed or unsealed roads. The practice of holding egg transport boxes in vehicles should continue to minimise jarring. The reasons for continuing to transport eggs by air should be re-evaluated.

Other factors during transport (e.g. temperature fluctuations, shock, torsional forces) were important to manage, and eggs should not be left sitting for prolonged periods during transportation as was the case with the Waimarino eggs.

## 1. Introduction

This report quantifies the magnitude of jarring (acceleration) experienced by ratite eggs during transportation and assesses the effects of this jarring on subsequent embryo development and hatching success. The report addresses five specific questions:

1. Does jarring associated with egg transport reduce hatching success?
2. Does jarring increase physical abnormalities or defects in developing or hatched chicks?
3. If the answer to either 1 or 2 is yes, is the risk of damage at some stages of embryo development more or less than at others? What practical steps can be taken to minimise jarring effects?

4. Are there likely to be any other factors during transport (e.g. temperature fluctuations, shock, torsional forces) that might be important to manage also?

## 1.1 BACKGROUND

Potter & Bassett (1999) identified egg and embryo damage from jarring during kiwi-egg removal from the wild into captivity as a possible contributing factor to the low hatching success (2 chicks from 9 eggs) during the 1998/99 breeding season. Kiwi eggs are precious, and too few are removed from the wild to enable a large-scale direct experimental assessment of the effects of jarring on kiwi embryos. We therefore opted to use emu eggs as a model for kiwi eggs. While adult emu and kiwi are physically very different, their eggs are similar. Freshly laid emu eggs range in weight from 335 to 724 g (Bassett 1996), nearly spanning the 306 to 509 g range (Bassett & Potter unpublished data) of Northern brown kiwi eggs. The similarity in mass of kiwi and emu eggs suggests that the effects of jarring during transportation are likely to be similar. Further, emu and kiwi are both ratites. Emu and cassowary are genetically the closest living relatives of kiwi (Cooper et al. 1992; van Tuinen et al. 1998), and emu eggs are readily available.

To answer the questions on which advice was sought we:

- (i) Ran an experiment in which sets of emu eggs were removed from an incubator during the first, second and third trimesters of incubation and transported by car for 4 h over sealed and unsealed roads, and assessed the effect on embryo development.
- (ii) Quantified the magnitude of the jarring experienced by the experimental emu eggs and compared this to the magnitude of jarring experienced by kiwi eggs during two egg-removal operations.

## 1.2 DEFINITIONS

In this report the following definitions are used:

*Hatchability* is defined as hatch of fertile eggs set.

*Egg weight loss* in the emu eggs is determined from the time of setting the eggs through to day 45 of incubation, both for eggs that hatched and for those that failed to hatch.

*Embryonic mortality* was categorised at the time of death following the definitions given by Minnaar & Minnaar (1993) and Bassett (1996). Embryos estimated to have died within the first 3 weeks of incubation are classified as early embryonic death (EED). Mid-embryonic death (MED) was from 3 to 5 weeks of age. These embryos usually measure between 90 and 150 mm in length and possess feathers, toenails, and the chorioallantoic membrane completely surrounding the inner shell. Late embryonic death (LED) applies to

embryos that die after 5 weeks of age. This category includes fully developed chicks that pip externally but that die before hatching.

*Malpositioned embryos* are those that do not lie in the normal position within the egg. The most common malposition in emu is when the embryo's head lies in the small pointed, end of the egg rather than in the large blunt end.

The unit of jarring used here is the maximum acceleration ( $G$  = gravitational unit) experienced within an egg-container each second.

## 2. Methods

### 2.1 SOURCES OF EGGS

Emu eggs laid in mid to late August 1999 were obtained from five emu breeders in the lower North Island. No eggs were more than two weeks old when received.

### 2.2 EGG STORAGE, HANDLING AND ALLOCATION TO TREATMENTS

The eggs were stored for 2-5 days in boxes at 10°C and hand-turned 180° once per day. Storing eggs in this manner is standard practice in commercial emu production. All eggs were weighed, measured, labelled and washed in Incusan® (20%) before being randomly assigned to treatment groups and randomly placed in the incubator. Eggs were incubated in a Dominion incubator regulated at 35.5°C ± 0.5°C with a relative humidity of 56-62%. Eggs were turned automatically 20° every hour and turned by hand 180° four times per day. Eggs were weighed during incubation on days 5,7,9,15,20,30 and 45, and the total weight loss calculated. Eggs that started to smell rotten during incubation were removed and examined for embryo development. Embryonic development was assessed in all remaining eggs on day 34 by placing a thin metal rod lengthwise across the shell to detect rocking movements of the embryo.

### 2.3 EXPERIMENTAL DESIGN

The 52-day incubation period was divided into three equal trimesters: Trimester 1 (early incubation, day 1-17); Trimester 2 (mid incubation, day 18-34); and Trimester 3 (late incubation, day 35-52).

Eggs were assigned to one of seven groups (Table 1). Group A eggs were left in the incubator throughout the experiment and were not manipulated. They formed a 'grand control' group. Group B, D, and F eggs were jarred (transported) in incubation trimesters 1, 2, and 3 respectively. Eggs in groups C, E, and G were control eggs for those in groups B, D, and F, respectively.

### 2.3 EGG JARRING PROCEDURE

Treatment eggs were removed from the incubator on their designated day of jarring, wrapped in newspaper, and placed in a large chilly-bin. A Tinytag© temperature probe (Gemini Data Loggers (UK) Ltd) was attached to the surface of one of the emu eggs and the egg was then wrapped. A second temperature probe and a Tinytag high-sensitivity shock data logger (Range 0-5 *G*) were placed inside the chilly bin between the wrapped eggs. The chilly-bin was carried to a vehicle and strapped into a back-seat seatbelt.

The appropriate control eggs were removed from the incubator, wrapped in newspaper, placed in a chilly-bin and left on a bench in the incubator room to cool during the period their associated treatment eggs were being transported.

The treatment eggs were driven 237 km on both sealed and unsealed gravel roads. The trip took four hours. A typical jarring profile that treatment emu eggs were subjected to is shown in Figure 1. Average and maximum acceleration (as a measure of jarring) recorded for different stages during egg transportation are presented in Table 2.

The surface temperature of the transported and control eggs did not drop more than 3 °C (i.e. below 33 °C) during any transport period.

On arrival back at the incubation room, the treatment and control eggs were unwrapped, wiped with Incusan to minimise the risk of bacterial contamination, and placed back into the incubator in their original position and orientation.

### 2.4 HATCHING ASSISTANCE AND POST-HATCH CARE

During hatching, temperature was maintained at  $35.5 \pm 0.5$  °C, and relative humidity was maintained at 55-62%. As far as possible, eggs were left to hatch unassisted. On several occasions hatching was protracted so assistance was provided. If chicks had difficulty hatching, 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 opened after day 54 of incubation and the contents examined for evidence of embryonic development.

After hatching, the umbilical area of each chick was disinfected liberally with Betadine (an iodine solution) to minimise bacterial contamination. Once the chicks were dry, they were weighed on an electronic balance and transferred to a brooder pen.

### 2.5 MONITORING OF JARRING DURING KIWI EGG REMOVAL FROM THE WILD

A Tinytag high-sensitivity shock data logger (Range 0-5 *G*) was also used to monitor jarring during the removal of kiwi eggs from the wild. The shock

logger was placed in the padded transport box next to kiwi eggs being removed from both Tongariro Forest (Doug's nest, 26 January 2000) and Waimarino Forest (Kung Fu's nest, 1 February 2000). Event records were kept during egg transportation to Rainbow Essentially New Zealand (Rotorua) enabling analysis of the magnitude of jarring during different types of transportation.

## 3. Results

### 3.1 EMU EGG INCUBATION TRIAL

Of a total of 76 eggs artificially incubated, 64 eggs (77%) were fertile, and 53 chicks hatched (83% of fertile eggs, 70% of eggs set). During incubation there were one EED (1.6% of fertile eggs), two MED (3.1% of fertile eggs) and six LED (9.4% of fertile eggs). Data on hatching success by treatment are presented in Table 3.

Hatching success did not differ significantly between treatments. The initial weight of eggs (allocated randomly to each treatment) did not differ significantly between treatments, and treatment had no significant effect on the amount of weight that eggs lost during incubation. Treatment did not significantly alter the incidence of embryo deformities.

### 3.2 COMPARISON OF JARRING DURING EXPERIMENT AND KIWI EGG REMOVAL OPERATIONS

The maximum jarring (acceleration) experienced by kiwi eggs from Tongariro and Waimarino Forests are presented for a range of types of transportation in Table 4.

Data comparing the magnitude of jarring experienced by the experimental eggs, and kiwi eggs during egg removal operations in Tongariro Forest and in Waimarino Forest, are presented in Figure 2.

The mean ( $\pm$ SE) acceleration recorded during emu egg transportation was  $0.212 \pm 0.001 G$ . This compares with  $0.111 \pm 0.002 G$  during the Tongariro kiwi egg removal, and  $0.098 \pm 0.002 G$  during the Waimarino kiwi egg removal.

There was a significant difference ( $F_{3,3340} = 88.74$ ;  $P < 0.0001$ ) between mean jarring versus transport category for the combined data from Tongariro and Waimarino Forests, with each of the categories differing from each other category ( $P < 0.05$ ) for all combinations except hand-held and in a vehicle over sealed roads, which were not significantly different (Tukey's Studentised Range (HSD) Test). For these eggs, mean jarring was most severe during flights on aircraft. For Tongariro eggs, the second highest mean jarring occurred on unsealed roads. The Waimarino eggs showed little difference in jarring on sealed and unsealed roads.

## 4. Discussion

Jarring, within the limits applied here, had no demonstrable effect on embryo mortality or egg hatchability. On average, the level of jarring applied to the emu eggs was twice as great on both sealed and unsealed roads as that recorded during two kiwi egg removals, so the emu data are a robust test of whether this type of jarring causes problems. One reason for the difference in magnitude of jarring within a vehicle between the emu experiment and the kiwi operations is that the emu eggs were placed directly on to a seat and belted in, whereas the kiwi eggs were held on a person's lap within the vehicles. This practice should continue for kiwi eggs to minimise jarring.

On average, the levels of jarring when carrying eggs was comparatively low, but peaks in jarring also occurred at these times. Severe jarring appeared to be associated with placing the eggs on the ground, or moving them into and out of vehicles. Extreme care is required during these periods to avoid banging the box containing the eggs against hard structures.

While mean jarring was, on average, greater on unsealed than on sealed roads, the higher maximum acceleration at both Tongariro and Waimarino occurred on sealed roads. This is probably due to care being taken to avoid bumps on unsealed roads, while on sealed open roads bumps are often hit at 100 km/h.

The significantly higher mean levels of jarring recorded in aircraft than in vehicles (even when they are driven on unsealed roads) for both kiwi egg removal operations, is a concern. This jarring is due to turbulence, not due to the acceleration of the aircraft during take-off and landing. While transporting eggs by air gets them to their destination quicker and hence minimises cooling, it does not reduce the level of jarring to which the eggs are subjected. The reasons for continuing to transport eggs by air should be re-evaluated, although results from the emu experiment suggest that jarring due to aircraft transportation falls within safe limits for developing embryos. Overall, the quad ride in Tongariro Forest caused the most severe jarring. Extreme care needs to be taken during this stage of egg removal.

## 5. Conclusions

1. *Does jarring associated with egg transport reduce hatching success?*

No, not within the limits of jarring imposed during this trial with emu eggs, nor given the 1999/2000 hatching success of kiwi eggs removed from the Tongariro/ Taupo Conservancy.

2. *Does jarring increase physical abnormalities or defects in developing or hatched chicks?*



No, not for chicks in the emu trial. We caution however, that kiwi eggshells are much thinner than emu egg shells, and hence more prone to cracking. Cracking of kiwi eggs appears to cause problems during hatching. Bassett & Potter (2001) documented that 9 out of 18 (50%) kiwi eggs removed from Tongariro Forest during the 1999/2000 season were cracked.

- 3 *If the answer to either 1 or 2 is yes, is the risk of damage at some stages of embryo development more or less than at others?*

There was no evidence that any stage of emu embryo development was more sensitive to jarring than any other stage, within the jarring parameters applied.

4. *What practical steps can be taken to minimize Jarring effects?*

The greatest accelerations (jarring) recorded during egg transportation occur on the quad in Tongariro Forest, and at all sites when the eggs were moved between, on to or into vehicles and aircraft. While the periods of moving eggs between modes of transport are brief, the jarring associated with these events, and with the quad ride, may contribute to kiwi eggs cracking (notably hairline cracks). Extreme care should be taken when placing eggs on the ground, and when moving them in and out of vehicles, and when transporting them by quad.

Where possible, eggs removed from the wild should be candled before and after transportation to monitor whether cracking is occurring during transportation. Jarring data should be collected routinely to quantify where problems might have occurred if cracks do appear, and to identify where more care is required. Also, when practical, eggs should be removed from nests at night to minimise the occurrence of kiwi-induced egg damage. Data are needed on the incidence rate of cracks in the wild. This should come from eggs examined at night when the male is off, because some eggs appear to be cracked by agitated males during daytime removal of eggs.

The practice of holding egg transport boxes in vehicles should continue to minimise jarring.

5. *Are there likely to be any other factors during transport (e.g. temperature fluctuations, shock, torsional forces) that might be important to manage also?*

The monitored Waimarino eggs were left sitting for two extended periods (23 minutes between putting eggs in the box and starting to walk to the vehicle, and 28 minutes between arriving at the vehicle and starting to drive). Intervals such as these subject the eggs to unnecessary cooling and should be avoided.

## 6. References

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**Table 1:** Assignment of eggs to treatments.

Group	Treatment
A	Grand control - no manipulation
B	Jarred middle of trimester 1 (day 8)
C	Control for trimester 1 eggs
D	Jarred middle of trimester 2 (day 25)
E	Control for trimester 2 eggs
F	Jarred middle of trimester 3 (day 43)
G	Control for trimester 3 eggs

**Table 2:** Average and maximum jarring applied to the emu eggs during transportation.

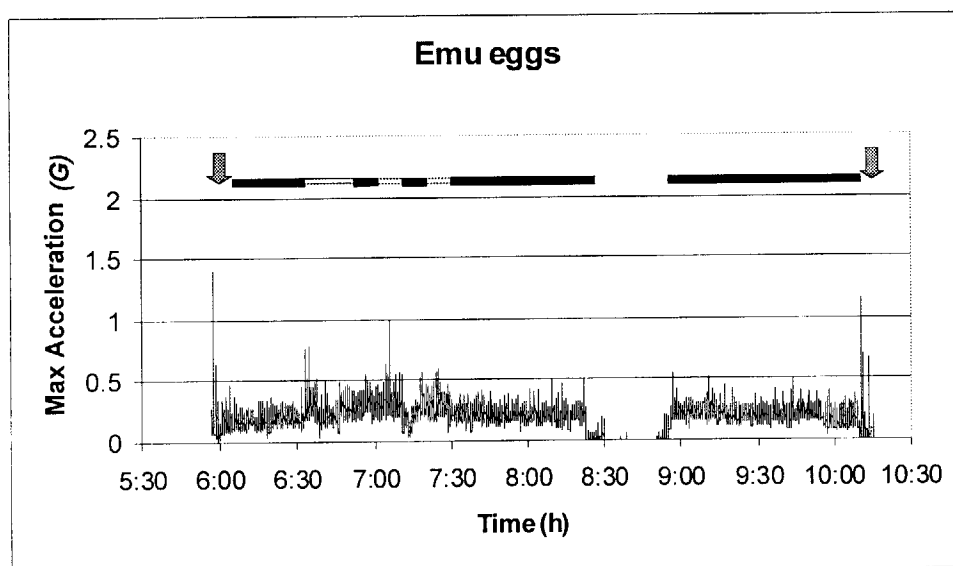
Type of transport	Mean ( $\pm$ SE) acceleration	Maximum acceleration (G)
Carried / hand-held	0.100 $\pm$ 0.009	1.41
In vehicle on unsealed road	0.287 $\pm$ 0.002	1.00
In vehicle on sealed road	0.196 $\pm$ 0.001	1.16

**Table 3:** Fertility rates and hatching success for emu eggs in each treatment.

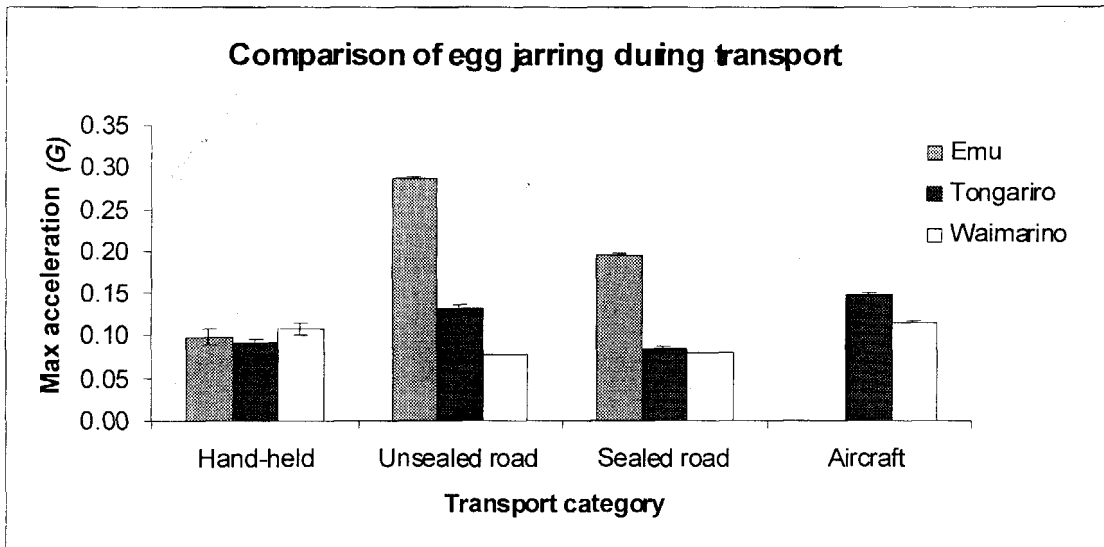
Treatment	Number of fertile eggs / number of eggs set	Number of chicks (hatching success)
Control control	11/11	9 (82%)
Tri 1 treatment	9/11	8 (89%)
Tri 1 control	7/10	5 (71%)
Tri 2 treatment	9/10	9 (100%)
Tri 2 control	8/10	6 (75%)
Tri 3 treatment	8/10	8 (100%)
Tri 3 control	9/10	7 (78%)

**Table 4:** Maximum jarring applied to kiwi eggs during removal operations in Tongariro and Waimarino forests.

Forest	Type of transport	Maximum acceleration (G)
Tongariro	Quad ride on rough tracks	2.31
	Carried / hand-held	0.71
	In vehicle on unsealed road	0.90
	In vehicle on sealed road	0.98
	Aircraft	0.39
Waimarino	Carried / hand-held	1.76
	In vehicle on unsealed road	0.12
	In vehicle on sealed road	0.18
	Aircraft	0.59



**Figure 1:** Maximum acceleration (jarring) versus time during transportation of treatment emu eggs. Legend: — = in vehicle on a sealed road; - - - = in vehicle on unsealed road; ↓ = hand held.



**Figure 2:** Comparison of mean acceleration ( $\pm$  SE) experienced by eggs transported in different ways in the emu-egg experiment, and in two kiwi egg removal operations.