Comparison of two regimes for artificially incubating kiwi eggs

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Published by
Department of Conservation
Head Office, PO Box 10-420
Wellington, New Zealand

This report was commissioned by Tongariro/Taupo Conservancy.

ISSN 1171-9834

Reference to material in this report should be cited thus:

Bassett, S.M.; Potter, M.A., 2001.
Comparison of two regimes for artificially incubating kiwi eggs. *Conservation Advisory Science Notes No. 342,* Department of Conservation, Wellington.

Keywords: egg handling, incubation regimes, artificial incubation, kiwi eggs, ratites.

Abstract

Kiwi hatching success and chick survival were compared under two incubation protocols and two chick-rearing procedures, to fine-tune the procedure and determine best practice in terms of hatching rate, chick survival and growth.

Analysis of the combined 1998/1999 and 1999/2000 data show 10 live chicks from 12 eggs incubated under Protocol 1, compared with 7 live chicks from 13 eggs under Protocol 2, suggesting that the old incubation protocol (Protocol 1) is more successful than the new one (Protocol 2). This impression is misleading because there were more cracked eggs in Protocol 2 (8 from 13) than in Protocol 1 (4 from 11) that may have contributed to several embryo deaths under Protocol 2. Two embryos placed into Protocol 2 were severely deformed (almost certainly prior to removal from the wild) and would not have survived under any protocol. When these factors are taken into account, there appears to be little difference between the success rates of the two protocols.

There was no significant difference in the average length of artificial incubation between Protocol 1 (old) and Protocol 2 (new). There was also little difference in the duration of hatching between the two, or in chick hatch weight.

Modifications needed to define the best protocol include:

- Improve egg recovery and transportation to reduce the number of cracked eggs arriving at Rainbow.
- Continue to modify and improve one standard artificial incubation protocol to attempt to minimise the time chicks spend hatching, and also assess the benefits of forced air incubation.
- Improve the palatability and nutrient content of the artificial diet to reduce the time taken for chicks to regain hatch weight.
- Transfer chicks to outside pens at 400 g when possible.
- Release chicks to the wild as soon as possible after they reach 1000 g.

1. Objective

To compare two alternative incubation/early chick-rearing procedures to optimise the procedure and determine best practice in terms of hatching rate, and chick survival and growth.

2. Details of artificial incubation protocols

The Tongariro/Taupo Conservancy of the Department of Conservation sought advice on five issues.

2.1 DIFFERENCES BETWEEN PROTOCOLS

The major differences between the old (Protocol 1) and the new (Protocol 2) incubation protocols are: (i) Protocol 2 uses a lower incubation temperature and holds temperature constant throughout incubation; and (ii) Protocol 2 rolls eggs throughout incubation rather than for just the first 55 days. We intentionally kept the number of variables altered to a minimum so as to maximise our chance of identifying which factor contributed most to any change in hatching success. The incubation protocols have remained the same for the two years of the trial. Details of the two protocols are presented inTables 1 and 2. A minor variation between the protocols was that, in Protocol 2, holes in the lid of the incubator were not covered to control humidity except when absolutely necessary.

Protocol 1 (old)

Incubation Regime

See Table 1.

Egg Turning Regime

(i) From day 0 to 55: Four 90° turns per day (alternating between day 1 and day 2 protocol: two anticlockwise followed by two clockwise, and vice versa),

(ii) No egg turning from day 56 to hatching.

Protocol 2 (new)

Incubation Regime

See Table 2.

Egg Turning Regime

(i) From day 0 to 55: Four 90° turns per day (alternating between day 1 and day 2 protocol as under protocol 1).

(ii) Two 180° turns per day from day 51 to hatching. This was one continuous movement, with the egg being placed back in the incubator with the same side up as when it was removed for turning.

(iii) No turning from internal pip until hatching.

Additional modifications to the 1999/2000 incubation protocol

Fourteen of the 22 eggs received from Tongariro, Waimarino, and Whirinaki Forests, Tongariro /Taupo Conservancy, this season were flown to Rotorua by fixed-wing aircraft in order to reduce the amount of time eggs spent in transit.

Two kiwi eggs from the 1998/1999 incubation trial arrived cracked, and both died (Potter & Bassett 1999). More eggs arrived cracked this season and they were dealt with in one of three ways upon arrival at Rainbow, depending on the magnitude of the crack: hairline cracks (identified by candling) were incubated as normal without special treatment; eggs with more severe cracking (visible by eye) had the cracked area sealed with nail varnish; badly smashed eggs were patched up with another piece of eggshell taped over the damaged area. If identified as cracked on first candling, eggs were splashed with Incusan© solution, avoiding the cracks, rather than being fully immersed in the solution.

Chick rearing

The chick-rearing regime was changed between the 1997/1998 and 1998/1999 seasons. In 1997/1998, the incubator temperature was dropped to 34°C on the day of hatch, 31'C on day 2, and 27°C on day 4. Chicks were not provided with food until they were moved to the brooder (when they were at least 7 days old). Water was not provided until day 14. Under this regime chicks lost considerable weight (up to 25% of hatch weight), took a month or more to regain hatch weight, and usually required forced feeding.

The revised chick-rearing regime used during 1998/1999 (Potter & Bassett 1999) and 1999/2000, outlined below, was identical for chicks hatched under both incubation protocols.

Days 1, 2: Upon hatching, the incubator temperature was reduced progressively to 30°C within 24 h. Depending on the vigour of the chick, it was kept at this temperature for another 24-48 h, potentially delaying the following by one day.

Day 3: When 48 h old, the chick was placed within an enclosed section of the brooder set at 28°C (end of day 2 or early in day 3). The brooder was filled withYates Peat Moss and kept damp. No worms were put in the brooder, but water was supplied ad libitum.

Day 4: The temperature in the brooder box was reduced to 26°C (provided that the chick was alert and active). Worms were placed in the brooder. Most chicks started feeding.

Day 5: The heater in the small section of the brooder box was turned off.

Day 6: Earthworms continued to be supplied.

Day 7: Chicks were fed 50% artificial diet and 50% earthworms.

Day 8-12: The proportion of earthworms was progressively reduced. Chicks were force-fed where necessary to maintain their weight until they were feeding properly on their own. At approximately 400-500 g chicks were moved into an outdoor pen and remained there until release into the wild..

2.2 DIFFERENCES BETWEEN NEW PROTOCOL AND CURRENT NATIONAL PROTOCOL

Both protocols were a continuation of those run during the 1998/1999 breeding season. Our response to question 2 has not altered from that expressed in depth in last year's report (Potter & Bassett 1999) and is reiterated here.

The most biologically important difference between the new protocol and the old protocol is that the average incubation temperature has been reduced from 36.5 and 35.5°C to 34.5°C and held constant throughout incubation. Too high an incubation temperature can cause a range of pre- and post-hatch problems. The close relationship between artificial incubation parameters and hatching success is well documented (Webb 1987, Deeming 1995a, Ar 1996, Bassett 1997). In most extensively studied species, hatching rates under artificial incubation have been improved dramatically in recent years and now often exceed 80%. Lower hatching rates usually indicate sub-optimal incubation conditions. Sub-optimal conditions contribute to embryo death, difficulties in hatching, yolk sac abnormalities, and reduced chick vigour. Also, chicks that experience hatching difficulties have higher post-hatch mortality than chicks that hatch easily (Deeming 1995b, Bassett 1997). For ratites, human assistance during hatching rarely results in healthy live chicks (Potter and Bassett 1998). The four most important factors in hatching success and chick vigour are: incubation temperature; oxygen supply; egg turning; and relative humidity via its influence on the rate of water loss by the egg. Temperature, egg turning and humidity are important throughout incubation (Ar 1996), while oxygen supply is most critical late in incubation and during hatching, when the physiological demands of the embryo are greatest.

For kiwi, incubation and chick rearing protocols vary greatly around New Zealand (Bassett & Potter 1998). Our analyses suggest that too high an incubation temperature might be a major contributing factor to low hatching success. High temperatures are more deleterious to avian embryos than low temperatures, and normal embryonic growth and survivorship diminishes with increased duration of exposure (Webb 1987, Bassett 1997). This supports our observation that hatching success is greater for eggs removed from nests late in incubation than it is for newly laid eggs or young eggs. The period of rapid embryo growth during the second half of incubation is associated with an increase in heat generated by the embryo and an increase in the embryo's ability to thermoregulate (Hoyt 1987,Vleck & Vleck 1987). This may allow an embryo to maintain a body temperature different from that in the incubator, and also to accommodate a degree of heat stress for a period before dying.

Egg turning is important particularly during the first trimester of incubation. Without it the embryo may suffer local dehydration damage, adhesion to the shell, abnormal development of the sub-embryonic fluid and the chorio-allantoic membrane, slowing of embryo development and of albumen absorption, and poor bone calcification - all leading to reduced hatchability (Ar 1996). All ratite eggs, except kiwi, are turned throughout incubation. Unpublished data (Pete and Judy Morrin) obtained from continuously videoed wild kiwi nests suggest that kiwi, too, turn their eggs throughout incubation.

For these reasons we selected temperature and egg turning to be the parameters tried in our modified incubation protocol.

2.3 DIFFERENCES IN KIWI DEVELOPMENT

Experimental design

The allocation of kiwi eggs removed in 1998/1999 from Tongariro Forest is given in Potter & Bassett (1999). During the 1999/2000 breeding season, eggs were removed from Tongariro,Waimarino and Whirinaki Forests. Eggs removed from nests in each of these sites were allocated to each protocol as outlined in Table 3.

Results

Table 4 outlines the main egg outcomes for both the 1998/1999 and the 1999/2000 kiwi breeding seasons. Nine eggs were removed from the Tongariro Forest during the 1998/1999 season, and 22 eggs were removed fromTongariro, Waimarino and Whirinaki Forests in the 1999/2000 season. Four of the 22 eggs from 1999/2000 were not artificially incubated because they were rotten. Of these, two (H5 and Ta2) were infertile, and the fertility of the other two (B1 and B2) could not be determined due to advanced decomposition. Of the 18 fertile eggs (82% of the total number removed) that were artificially incubated, one (D11, in Protocol 2) suffered mid-embryo death and two (D10 and Ml, both in Protocol 2) suffered late embryo death. Fifteen chicks (nine in Protocol 1, and six in Protocol 2) hatched successfully, giving a hatching success of 83% for 1999/2000 compared with two chicks out of seven (29%) fertile eggs from 1998/1999. Ten (56%) of the fertile eggs from 1999/2000 were cracked on arrival at Rainbow (four in Protocol 1, and six in Protocol 2). An additional chick was recovered from the wild when about seven days old, and was included in the chick rearing protocol.

Two of the fertile eggs were removed late in incubation (60+ days old), seven were removed when about 45 to 59 days old, another seven were removed midway through incubation (at about 25-44 days old), and two were younger than the recommended minimum age of 25 days when removed from the wild.

One of these young eggs (D1 1) was removed soon after laying (1-2 days old) following accidental disturbance at the nest.

Ten (56%) of the fertile eggs were cracked on arrival at Rainbow (four in Protocol 1, and six in Protocol 2). Cracks can greatly increase the risk of bacterial contamination, during incubation and may have been a significant factor in the deaths of M1 and D10, which had high levels of bacterial contamination present throughout the yolk sac when autopsied.

Two chicks (both under Protocol 2) died at point of hatch and both had extensively cracked eggs. One (D10) pipped prematurely, and had an unformed umbilicus, and bacterial contamination was evident. The second chick (M1) also had an unformed umbilicus, and died about one week before it should have hatched. The chick was small, poorly developed, and had a twisted bill and a deformed head.

Five chicks (33%) hatched with external yolk sacs. This was related in part to high weight loss during incubation (L1 and L2), and problems associated with maintaining correct weight loss in cracked eggs. These chicks were treated with a course of antibiotics to prevent a secondary yolk sac infection, and in one instance (T8) the external yolk sac was tied off and removed. All five chicks survived despite hatching with some external yolk.

Both of Lucky's eggs (L1 and L2, one in each of Protocol 1 and Protocol 2) had difficulties hatching and high weight loss during incubation. Plop (L1) hatched with an external yolk sac and a badly splayed right leg. The leg was extensively strapped and the chick made a complete recovery. Hola (L2) had a slightly longer hatch time with similar a external yolk sac problem and slightly splayed legs (strapped for a short period of time). Both chicks were treated with Clavulox and did not suffer any secondary yolk sac infections or complications.

Chick survival was very high (93%). The only death was a result of black nightshade poisoning (Hola, at 21 weeks of age).

Analysis of the combined 1998/1999 and 1999/2000 data (Table 4) shows 10 live chicks from 12 eggs incubated under Protocol 1 compared with 7 live chicks from 13 eggs under Protocol 2. This suggests that the old incubation protocol (Protocol 1) is more successful than the new one (Protocol 2). This impression is misleading because there were more cracked eggs in Protocol 2 (8 from 13) than in Protocol 1 (4 from 11) and these cracks may have contributed to several embryo deaths under Protocol 2. Also, two embryos in Protocol 2 (H2 and M1) were severely deformed (almost certainly prior to removal from the wild) and would not have survived under any protocol. One egg allocated to Protocol 2 was removed within just two days of having been laid. It failed to hatch, but that is currently normal for such young eggs incubated artificially. Countering this, in part, is H4. This embryo, allocated to Protocol 1, actually died in the wild before the egg was brought in for artificial incubation. When these factors are taken into account, there appears to be little difference between the success rates of the two protocols.

There was no significant difference in the average length of artificial incubation between Protocol 1 (old) and Protocol 2 (new). There was also little

difference in the duration of hatching between the two, or in chick hatch weight.There was, however, a significant reduction in the age the chicks were moved to the brooder based on the new chick rearing protocol used in the 1998/1999 and 1999/2000 seasons compared with that under the old (1997/1998) chick-rearing protocol (Table 5).

2.4 FORCED AIR INCUBATION

Forced air incubation was not tried in the 1999/2000 season for two reasons. First, a suitable incubator was not available at a price affordable within the constraints of this contract. Second, during discussions with Department of Conservation and Rainbow staff (Whakapapa annual research meeting, March 1999) it became clear that it was more important that we placed all available kiwi eggs into the main incubation trial to improve statistical rigour. Comparing still air and forced air incubation remains worthwhile, but only if the costs of hiring a fully automated ratite incubator are covered.

2.5 OTHER DIFFERENCES

We do not believe that any of the differences was likely to be due to genetic, habitat, or other differences between kiwi populations. We have been unable to find any literature on other species that would support these possibilities.

3. Progress on previous recommendations

(i) Continue the experiment for a second season to increase sample size.

A far greater number of kiwi eggs was obtained for the trial during the 1999/2000 season than during the 1998/1999 season, but no statistically significant difference can be detected between the two incubation protocols.

(ii) Assess whether embryo jarring during transportation is causing embryo death (using emu eggs as a model).

This was achieved. The results are presented in Potter & Bassett (2001).

(iii) Investigate ways to reduce jarring and shaking of eggs in transit.

This was achieved. Egg removal and transportation methods were improved by using a modified padded transportation box. Interestingly, flying eggs to Rotorua rather than driving them resulted in an increase in jarring. See Potter & Bassett (2001) for more details.

(iv) Incubate some eggs in a modern 'forced air' incubator.

This recommendation is still be addressed.

(v) Monitor nests more closely so that eggs are not removed too young.

Achieved.

(vi) Adhere strictly to the experimental incubation and chick rearing protocols.

Achieved.

4. New recommendations

Improve egg recovery and transportation to reduce the number of cracked eggs arriving at Rainbow. This should include removing some eggs from nests at night-time.

Continue to modify and improve one standard artificial incubation protocol to attempt to minimise the time chicks spend hatching, and also assess the benefits of forced air incubation.

Improve the palatability and nutrient content of the artificial diet to reduce the time taken for chicks to regain hatch weight.

Transfer chicks to outside pens at 400 g when possible.

Release chicks to the wild as soon as possible after they reach 1000 g.

Carefully monitor weight loss of Lucky's eggs to improve their hatchability.

5. Acknowledgements

We thank Helen McCormick, Deidre Vercoe, Carole Harvey and Claire Travis (Rainbow) for their enormous and invaluable assistance in running the experiment and for tending to the eggs and chicks; Paul Boyce (Rainbow Park Manager) for continuing to support Massey University research; Tongariro/ Taupo Department of Conservation field staff for monitoring kiwi nests, removing eggs, and transporting them to Rainbow; and Dr Harry Keys and

Mr Cam Speedy (Tongariro/Taupo Department of Conservation) for their continued and enthusiastic support of this research; and the 15 wonderful kiwi chicks that successfully hatched and provided us with invaluable data.

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Table 1: *Incubation temperatures, cooling periods and egg turning in Protocol 1.*

Egg age (days)	Temperature (°C) at top of egg	Cooling period	Turning
1 -55	36.5°C	1 hour	4 x 90° turns/day
56 - hatching	35.5°C	1 hour	no turning
Hatching	35.5°C		

Table 2: *Incubation temperatures, cooling periods and egg turning in Protocol 2.*

Egg age (days)	Temperature (°C) at top of egg	Cooling period	Turning
1 -55	34.5°C	1 hour	4 x 90° turns/day
56 - hatching	34.5°C	1 hour	2 rolling rotations/ day (see below)
Hatching	34.5°C		no turning

Table 3: *Egg allocation to two artificial incubation regimes. Oldest and youngest eggs were determined by candling upon arrival at Rainbow Springs.*

Nest number	Egg age*	Incubation regime
Nest 1	Oldest egg	Protocol 1
	Youngest egg	Protocol 2
Nest 2	Youngest egg	Protocol 1
	Oldest egg	Protocol 2
Nest 3	Oldest egg	Protocol 1
	Youngest egg	Protocol 2

* Oldest egg (55-65 days) and youngest egg (25+ days)

Table 4: *Date and time eggs spent in the incubator to hatch or the estimated age of embryos at time of death under the two artificial incubation protocols for eggs removed from Tongariro/Taupo Conservancy from August 1998 to February 2000. In 1999/2000 the first four eggs were driven to Rotorua, as were Ta 1 , Ta2, B 1 and B2. All others were flown.*

Egg ID	Chick ID	Date removed from nest	Time in incubator to hatch (estimated age of dead embryos) (days)	Incubation regime	Egg outcome
1998/1999 Season					
H1	-	14/8/98	-	Nil	Infertile
H2	-	14/8/98	32 (~45)	Protocol 2	Mid Embryo Death
H4 λ	-	19/11/98	- (2-4)	Protocol 1	Early Embryo Death
D5	-	19/11/98	38 (75+)	Protocol 1	Late Embryo Death
D6 *	-	19/11/98	22 (75+)	Protocol 2	Late Embryo Death
T5	Moenui	8/12/98	57	Protocol 2	Chick
D7	Mahi	8/12/98	43	Protocol 1	Chick
D8 *		15/2/99	14 (~36)	Protocol 2	Mid Embryo Death
H3	-	19/11/98	-	-	Infertile
1999/2000 Season					
D9 * ϕ	Iwa	9/9/98	22	Protocol 1	Chick
D10 *	-	9/9/99	38 (75+)	Protocol 2	Late Embryo Death
T6 *	Koru	9/9/99	42	Protocol 2	Chick
T7 Ψ	Jake	9/9/99	47	Protocol 1	Chick
L1 ϕ	Plop	28/9/99	26	Protocol 1	Chick
L2 ϕ	Hola	28/9/99	46	Protocol 2	Chick
IH1 Ψ	Mawhitiwhiti	30/9/99	21	Protocol 2	Chick
IH2	Pango	30/9/99	24	Protocol 1	Chick
BM1*	Taniwha	30/9/99	48	Protocol 1	Chick
D11	-	18/10/99	43 (~30)	Protocol 2	Mid Embryo Death
Ta1	Taz	26/10/99	19	Protocol 1	Chick
M1 *	-	1/12/99	48 (~70)	Protocol 2	Late Embryo Death
M2 *	Ataahua	1/12/99	62	Protocol 1	Chick
T8 ** ϕ	Te Humua	5/1/00	24	Protocol 2	Chick
D12	Komutu	26/1/00	12	Protocol 2	Chick
D13	Taniko	26/1/00	32	Protocol 1	Chick
IH3	Tuatea	1/2/00	10	Protocol 1	Chick
IH4 * ϕ	Aniwaniwa	1/2/00	39	Protocol 2	Chick
H5		28/9/99	rotten	unincubated	INF
Ta2		26/10/99	rotten	unincubated	INF
B1		26/10/99	rotten	unincubated	Unknown
B2		26/10/99	rotten	unincubated	Unknown

* eggs that were cracked on arrival at Rainbow
Ψ eggs with tiny star cracks on arrival at Rainbow
λ died in wild before artificial incubation.

** severe egg shell damage
ϕ chicks with external yolk sacs post-hatch

Table 5: *Summary of differences in age of chicks when moved into the brooder, moved into outside runs, and age at release under the two different chick-rearing regimes. Note: not all 1999/2000 chicks have yet been released. Data to July 2000.*

	Old chick-rearing protocol 1997/1998 breeding season x±SE(n=6) (range)	**New chick-rearing protocol** 1998/2000 breeding season x±SE (range)
Hatch weight (g)	337.8 ± 10.0 (304.5 - 366.0)	341.8 ± 8.1 (n = 17) (267.0 - 405.0)
Age when moved into the brooder (d)	7.2 ± 0.3 (6-8)	3.0 ± 0.3 (n = 17) (2-5)
Age when moved outside (d)	77.8 ± 4.2 (67-94)	47.1 ± 3.8 (n = 15) (20-77)
Age at release (d)	372.5 ± 35.9 (286-526)	185.1 ± 8.0 (n = 11) (140-228)
Release weight (g)	1428 ± 52.4 (1260 -1570)	1302.6 ± 54.0 (n = 11) (1085 - 1628)