J. Field Ornithol., 58(4):464–473

ESTIMATING THE ERROR INVOLVED IN USING EGG DENSITY TO PREDICT LAYING DATES

B. T. Collins and A. J. Gaston
Canadian Wildlife Service
100 Gamelin Boulevard
Hull, Quebec
K1A OE7, Canada

Abstract.—In colonies where it is impractical to revisit nests daily to assess date of laying, egg density can be used as a simple measure of age. This requires a sample of eggs of known age to calibrate the density data. This paper describes how to combine estimates of sampling and prediction errors to compute a confidence interval for the mean date of laying for a colony and to compare the difference in mean laying date between two colonies.

ESTIMADO DEL ERROR ENVUELTO AL UTILIZAR LA DENSIDAD DE HUEVOS PARA PREDECIR LA FECHA DE PUESTA

Sinopsis.—En colonias de aves en donde es impráctico visitar la misma diariamente para determinar la fecha de puesta de huevos, la densidad de estos, puede ser utilizada como una medida de éste estimado. Esto requiere una muestra de huevos de fecha de puesta conocida (edad) para calibrar los datos de densidad. En este trabajo se describe un método para combinar estimados de muestras y predicción de errores para computar un intérvalo de confiabilidad para la fecha promedio de puesta de una colonia y comparar la diferencia del promedio de puesta de huevos entre dos colonias.

The weight of an avian egg declines during incubation as a result of water loss (Drent 1970, 1975; Rahn and Ar 1974; Rahn et al. 1976). Since the volume of the egg remains constant, the density of the egg decreases in proportion to the loss of weight. The rate at which weight is lost remains fairly constant through the incubation period up to the stage at which cracks begin to appear in the shell (Drent 1975, Morgan et al. 1978, Rahn et al. 1976). The progressive and constant change in density has been used to measure the length of time that an egg has been incubated, and hence, by extrapolation to estimate its date of laying (e.g., Dunn et al. 1979, Gaston et al. 1983, Hays and LeCroy 1971, Nol and Blokpoel 1983, O'Malley and Evans 1980, Schreiber 1970, Van Paassen et al. 1984, Wooler and Dunlop 1980).

Two techniques have been used to measure egg densities; the flotation method, involving immersing the egg in water and observing whether and how it floats (Hays and LeCroy 1971, Nol and Blokpoel 1983, Schreiber 1970, Van Paassen et al. 1984), and the density index method comparing the weight to some measure of volume (Dunn et al. 1979, Gaston et al. 1983, O'Malley and Evans 1980, Wooler and Dunlop 1980). We prefer the latter method because the measurements involved are simple to obtain and relatively immune from subjective bias. Also, in many situations it is inconvenient to carry a container of liquid in the field (e.g., where the investigator must climb trees or cliffs). Derivation of laying dates for individual eggs by the density index method is described by Gaston et al. (1983) and O'Malley and Evans (1980). We describe here how to estimate confidence limits for mean laying dates thus derived and how to test for the differences between two such means.

Because weight loss accelerates after the egg begins to pip, the method described can only be used on eggs that have not yet started pipping. However, eggs that have begun to pip are usually within a few days of hatching and hence the state of incubation is readily apparent.

ESTIMATING CONFIDENCE LIMITS

The decline in egg density is assumed to be constant and hence can be described by a linear model:

$$y_i = \alpha + \beta X_i + \epsilon_i \tag{1}$$

where y_i is the observed density index, X_i is the number of days that the egg has been incubated, α and β are parameters describing the regression line and ϵ_i is the random error in the observation. The parameters α and β are functions of species-specific parameters such as egg shape and the rate of moisture loss, the latter controlled by eggshell porosity. Both parameters can be estimated from a set of density measurements on eggs for which the number of days elapsing since the start of incubation is known (the calibration data set), using linear regression.

The regression equation is used to put confidence intervals on estimated laying dates for eggs of unknown age (the estimation data set), using the fitted regression line and the estimated variability about the line. We assume that the variability of the observations about the regression line is the same for the calibration and the estimation data sets. Thus, the calibration data set must involve observations on different eggs measured using the same techniques as the estimation data set (i.e., a calibration data set which consisted of repeated observations on one egg would be inappropriate).

Once the regression constants have been estimated, the laying date (U_i) of any egg of known density can be estimated:

$$\hat{X}_i = (Y_i - a)/b \tag{2}$$

$$\hat{u}_i = D_i - \hat{X}_i \tag{3}$$

where a and b are estimates of the parameters α and β, $\hat{X}_i$ is the estimated age of the egg, D_i the date on which the egg is observed and $\hat{u}_i$ is an estimate of U_i, the laying date.

Let μ denote the mean laying date for the population. Then U_i can be written as

$$U_i = \mu + Z_i \tag{4}$$

where Z_i denotes the difference between the laying date for egg i and the population mean. Equation (3) can be rewritten as follows:

$$\begin{aligned} \hat{u}_i &= D_i - \hat{X}_i \\ &= (U_i + X_i) - \hat{X}_i \\ &= U_i + f_i \end{aligned} \tag{5}$$

TABLE 1. Confidence limits on mean laying dates for two data sets (n = 30).

Data set	Mean date of start of incubation (=laying in TBM)	95% confidence limits	Calibration sample size	Proportion of conf. interval due to calibration
Thick-billed Murre (*Uria lomvia*) 1981	30 Jun.	27 Jun.–3 Jul.	168	23%
Ancient Murrelet (*Synthliboramphus antiquus*) 1984	30 Apr.	26 Jun.–4 Jul.	142	17%

where $f_i = X_i - \hat{X}_i$ is the error in predicting the time since laying for egg i. Combining (4) and (5) yields

$$\hat{u}_i = \mu + Z_i + f_i. \tag{6}$$

Formula (6) includes two sources of error: (a) Z_i, the sampling error, and (b) f_i, the error associated with the estimate based on the calibration curve.

The mean laying date for the colony is estimated as $\bar{u}$ (the average of the $\hat{u}_i$). Calculating the standard sample variation about this mean provides an estimate of the variance, which includes both sources of variability. However, the contribution of the regression is underestimated because the same regression is used to predict laying dates for all eggs. Eggs with the same density are assumed to have an identical age and the age variation in eggs of the same density is not reflected in the set of predicted laying dates.

To calculate a confidence interval for the mean laying date, assuming a normal distribution, we calculate two portions for the regression and the sampling, respectively, and then combine them. Details of these calculations are given in Appendix 1.

We used our method to calculate confidence intervals for mean dates of laying derived from two data sets (Thick-billed Murres [*Uria lomvia*] at Digges Sound, N.W.T., in 1981 and Ancient Murrelets [*Synthliboramphus antiquus*] at Reef Island, British Columbia, in 1984). Both species incubate for 32 d (Gaston and Nettleship 1981, Gaston, unpubl.). Sealy (1976) gives 35 d for Ancient Murrelets but his observations were at sites where periodic disturbance may have caused abnormal neglect. For samples of 30 eggs our method yielded 95% confidence intervals of 5.8 and 7.4, respectively (Table 1). In both cases, the size of the confidence interval was mainly due to the sampling error, with the error due to the calibration making up less than 25% of the total error. Not unexpectedly, there was a big difference in the size of the confidence interval for small samples (<20), but relatively little reduction was obtained by increasing sample size beyond 30 eggs (Fig. 1).

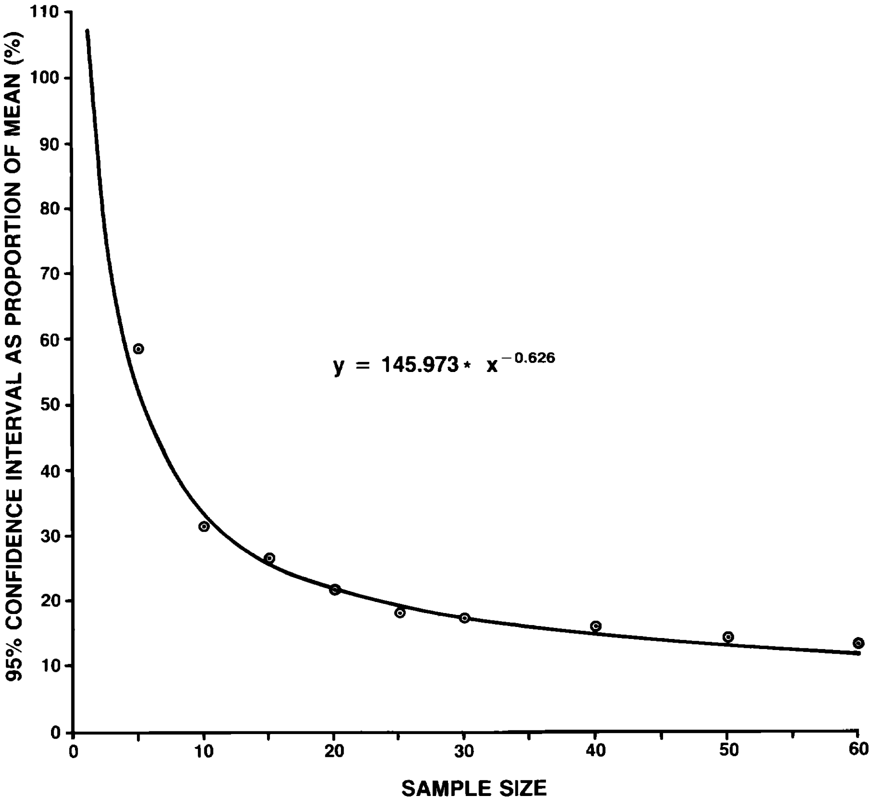

FIGURE 1. Ninety-five percent confidence intervals expressed as a proportion of mean incubation period for Ancient Murrelets, in relation to sample size. Samples were selected randomly from a single data set. The curve was fitted using NWA statpak program "ONEVREG" (Northwest Analytical, Inc. 1984).

As the number of calibration samples increases, the contribution of the regression to the width of the confidence limit declines to a lower limit based on the intrinsic variability in age for eggs with the same density, but this is the portion of the imprecision in the regression that is also included in the sampling portion of the estimate. Thus when the number of calibration samples is large, only the portion of the confidence interval based on the sampling variance needs to be calculated and the confidence interval can be further shortened by setting $\alpha_1 = 0$ and $\alpha_2 = \alpha/2$.

COMPARING MEANS FOR DIFFERENT POPULATIONS

If two populations of the same species are compared for mean laying dates calculated by our method, using the same calibration curve, then a portion of the error associated with the regression is cancelled out. Using the previous notation, the difference in laying date between colonies 1 and 2 would be:

TABLE 2. Comparison of laying dates estimated for Glaucous and Kumlien's gulls at Digges Sound in 1982.

	n	$\bar{x}$[1]	95% confidence limits
Glaucous Gull	19	8 Jun.	3–13 Jun.
Kumlien's Gull	44	23 Jun.	20–26 Jun.

[1] Calibration curve: y = 0.5192 − 0.00283x (n = 39).

$$\begin{aligned}\mu_1 - \mu_2 &= \bar{D}_{1\cdot} - \bar{X}_{1\cdot} - \bar{Z}_{1\cdot} - (\bar{D}_{1\cdot} - \bar{X}_{2\cdot} - \bar{Z}_2) \\ &= \bar{D}_{1\cdot} - \bar{D}_{2\cdot} - (\bar{X}_{1\cdot} - \bar{X}_{2\cdot}) - (\bar{Z}_{1\cdot} - \bar{Z}_{2\cdot})\end{aligned}$$

where μ_h denotes the mean laying date for colony h;
$\bar{D}_{h\cdot}$ denotes the mean date of observation for colony h;
$\bar{X}_{h\cdot}$ denotes the mean time since laying for observations in colony h and
$\bar{Z}_{h\cdot}$ denotes the mean difference between the laying date for the selected eggs for colony h and the mean laying date for the colony.

As for the confidence interval on the single sample, we developed the confidence limits on the difference in mean laying dates by calculating them separately for the sample error and the regression error. The methods used to derive the confidence interval are given in Appendix 2.

An example of the use of our method to compare laying dates of Glaucous Gulls (*Larus hyperboreus*) and Kumlien's Gulls (*Larus glaucoides*) breeding at Digges Sound, N.W.T., in 1982 is given in Table 2. We have assumed that the density index for the eggs of these congeners at a given stage of incubation is the same. We have therefore used the calibration derived from observations of Glaucous Gulls alone (density index = 0.5192 − [0.00283 × days of incubation], n = 39).

DISCUSSION

The method we have described seems most applicable in situations where the timing of laying of the population is well synchronized and the distribution of laying dates approximates a normal curve. Where laying is not normally distributed, the method for estimating date of laying is valid, but the confidence level on the means is not. We have found this method useful on seabird colonies where only one or two visits can be made during the incubation period, and in other situations where nests cannot be revisited regularly to check for dates of hatching. In other situations where daily visits might be possible, this method could be used in preference to direct observations to reduce disturbance at the nest site.

Clearly, 95% confidence intervals of several days constitute unacceptably large errors where incubation periods are short, as is the case for small passerines. As the amount of weight loss as a proportion of the initial weight is more or less independent of incubation period, the daily change in egg density will be higher for birds with short incubation periods. This should result in a more acccurate calibration equation and hence smaller confidence limits, making the method fairly robust with respect to differences in incubation periods.

However, not all eggs are likely to exhibit similar variance in density indices so the power curve shown in Figure 1 cannot be assumed to be typical of all birds.

A more serious limitation for our method in the case of small birds is the accuracy with which eggs can be weighed in the field. The most practical method is by means of a spring-balance (Pesola or similar), but the accuracy of such balances is seldom better than ±0.5 g. For eggs weighing less than 20 g this probably represents an unacceptably large measurement error (≈15% of total weight loss). Battery operated digital electronic balances accurate to ±0.1 g are available, but are less convenient than spring balances and need to be levelled. Probably a method based on flotation would be better than ours for eggs weighing less than 20 g, unless laboratory weighing is possible.

Note that improving the estimate of egg volume by using the formulae of Hoyt (1979), Romanoff and Romanoff (1949), or Stonehouse (1966) will not improve the accuracy of the method, because all depend on multiplying the volume index (LB^2) by a constant. Hence, the resulting regression is no more accurate in predicting laying date than the one that we describe.

The calculations presented in both appendices have been incorporated in a FORTRAN computer program running on an IBM-PC or IBM-AT computer. A copy of the program has been deposited in the Van Tyne Library, University of Michigan (Collins 1987) or may be obtained from the authors upon receipt of a standard 5¼ in. floppy diskette.

ACKNOWLEDGMENTS

We thank Hugh Boyd and Steve Wendt for commenting on earlier drafts of this manuscript.

LITERATURE CITED

COLLINS, B. T. 1987. CIIR: a program to calculate confidence intervals on mean date of laying using egg density, deposited in Van Tyne Library, Wilson Ornithological Society, Museum of Zoology, University of Michigan, Ann Arbor.

DRENT, R. M. 1970. Functional aspects of incubation in the Herring Gull. Behaviour Suppl. 17:1–132.

———. 1975. Incubation. Pp. 333–420, *in* D. S. Farner and J. R. King, eds. Avian biology, Vol. 5. Academic Press, New York.

DUNN, E. H., D. J. T. HUSSELL, AND R. E. RICKLEFS. 1979. The determination of incubation stage in starling eggs. Bird-Banding 50:114–120.

GASTON, A. J., AND D. N. NETTLESHIP. 1981. The Thick-billed Murres of Prince Leopold Island. Canadian Wildlife Service Monograph Series No. 6.

———, D. G. NOBLE, AND M. A. PURDY. 1983. Monitoring breeding biology parameters for murres *Uria* spp.: levels of accuracy and sources of bias. J. Field Ornithol. 54:275–282.

HAYS, H., AND LECROY, M. 1971. Field criteria for determining incubation stage in eggs of the Common Tern. Wilson Bull. 83:425–429.

HOYT, D. F. 1979. Practical methods for estimating volume and fresh weight of bird eggs. Auk 96:73–77.

MILLER, R. G. 1981. Simultaneous statistical inference, 2nd ed. Springer-Verlag, New York.

MORGAN, K. R., C. V. PAGANELLI, AND H. RAHN. 1978. Egg weight loss and nest humidity during incubation in two Alaskan gulls. Condor 80:272–275.

NORTHWEST ANALYTICAL, INC. 1984. Statpak multi-function statistics library, version 3.1. Northwest Analytical, Inc., Portland, Oregon.

NOL, E., AND H. BLOKPOEL. 1983. Incubation period of Ring-billed Gulls and the egg-immersion technique. Wilson Bull. 95:283–286.

O'MALLEY, J. B. E., AND R. M. EVANS. 1980. Variations in measurements among White Pelican eggs and their use as a hatch date predictor. Can. J. Zool. 58:603–608.

RAHN, H., AND A. AR. 1974. The avian egg: incubation time and water loss. Condor 76: 147–152.

———, C. V. PAGANELLI, I. C. T. NISBET, AND G. C. WHITTON. 1976. Regulation of incubation water loss in eggs of seven species of terns. Physiol. Zool. 49:245–259.

ROMANOFF, A. L., AND A. J. ROMANOFF. 1949. The avian egg. J. Wiley, New York.

SCHREIBER, R. W. 1970. Breeding biology of Western Gulls (*Larus occidentalis*) on San Nicolas Island, California, 1968. Condor 72:133–140.

SEALY, S. G. 1976. Biology of Nesting Ancient Murrelets. Condor 78:294–306.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman and Company, San Francisco, California.

STONEHOUSE, B. 1966. Egg volumes from linear dimensions. Emu 65:227–228.

VAN PAASSEN, A. G., D. H. VELDMAN, AND A. J. BEINTEMA. 1984. A simple device for determination of incubation stages in eggs. Wildfowl 35:173–178.

WOOLER, R. D., AND J. N. DUNLOP. 1980. The use of simple measurements to determine the age of Silver Gull eggs. Aust. Wildl. Res. 7:113–115.

Received 14 Oct. 1986; accepted 19 May 1987.

APPENDIX 1: CONFIDENCE LIMITS ON MEAN COLONY LAYING DATE

The confidence interval for the mean laying date is calculated in two portions which are later joined together. These two portions consist of putting a confidence interval about the error in the regression portion of the estimate and the sampling portion of the estimate.

First, from the regression equation, given the selected samples, $\bar{Y}. - a - b\bar{X}.$ is distributed normally with mean 0 and variance

$$\sigma^2\left[\frac{1}{k} + \frac{1}{n} + \frac{(\bar{X}. - \bar{x}.)^2}{\Sigma\,(x_j - \bar{x}.)^2}\right]$$

where $\bar{Y}.$ denotes the average egg density for the k selected eggs,
$\bar{X}.$ the average days since laying for the k selected eggs,
n the number of observations used in the regression,
x_j the j^{th} observed days since laying used in the regression ($j = 1, \ldots n$) and
$\bar{x}.$ the average of the x_j.

In the above variance term it can be seen that only a portion of the variance is reduced through increasing the sample of k values used to estimate the colony mean. This is because errors in determining the regression line cannot be reduced by predicting values using the equation.

We can now create a confidence interval for the predicted mean laying date for the k selected eggs. This is almost identical to the standard procedure for inverse regression (Sokal and Rohlf 1981:496) except that the term $(1 + 1/n)$ is replaced with $(1/k + 1/n)$. (This procedure can fail to produce a useful confidence interval in some circumstances [Miller 1981:117].) The confidence intervals are given by the probability statement

$$P\{L_1 < \bar{X}. < H_1\} \geq 1 - 2\alpha_1 \tag{A1}$$

where

$$L_1 = \bar{x}. - \frac{\bar{Y}. - \bar{y}. - B}{A}$$

$$H_1 = \bar{x}. - \frac{\bar{Y}. - \bar{y}. + B}{A}$$

$$A = \frac{t^2_{n-2}(\alpha_1)s^2}{b\Sigma(x_j - \bar{x}.)^2} - b$$

$$B = \sqrt{(\bar{Y}. - \bar{y}.)^2 - \frac{A}{b}\left[t^2_{n-2}(\alpha_1)s^2\left(\frac{1}{k} + \frac{1}{n}\right) - (\bar{Y}. - \bar{y}.)^2\right]}$$

$\bar{y}.$ is the mean of the n observations used in the regression,
s^2 is the estimate of variance from the regression, and
$t_{n-2}(\alpha_1)$ is the upper α_1 percentile of the t distribution.

If it is assumed the Z_i are normally distributed, then an α_2 percent confidence interval on the mean of the Z_i is given by

$$P\{L_2 < \bar{Z}. < H_2\} \geq 1 - 2\alpha_2 \tag{A2}$$

where

$$H_2 = \frac{t_{k-1}(\alpha_2)s_z}{k}$$

$$L_2 = -H_2$$

$t_{k-1}(\alpha_2)$ is the upper α_2 percentile of the t distribution and $s^2_z = \Sigma(\hat{u}_i - \bar{u}.)^2/(k - 1)$ is an estimate of Σ^2_z. (This will be an overestimate since the $\hat{u}_i$ also include some imprecision due to the regression.)

The two equations (A1) and (A2) can now be combined to provide a confidence interval on $\mu = \bar{D}. - \bar{X}. - \bar{Z}.$ since $\bar{D}.$ is a constant

$$P\{L_1 + L_2 < \bar{X}. + \bar{Z}. < H_1 + H_2\} \geq 1 - 2\alpha_1 - 2\alpha_2$$

thus,

$$P\{\bar{D}. - H_1 - H_2 < \bar{D}. - \bar{X}. - \bar{Z}. < \bar{D}. - L_1 - L_2\} \geq 1 - 2\alpha_1 - 2\alpha_2$$

or

$$P\{\bar{D}. - H_1 - H_2 < \mu < D. - L_1 - L_2\} \geq 1 - 2\alpha_1 - 2\alpha_2 \quad \text{(A3)}$$

setting $\alpha_1 = \alpha_2 = 0.0125$ gives a 95% confidence interval for the mean laying date.

APPENDIX 2: CONFIDENCE INTERVALS ON THE DIFFERENCE BETWEEN TWO COLONIES IN MEAN LAYING DATE

Assume a sample of k_h are taken from colony h and that density determinations y_{hi} are made on each egg. If a subscript h is added to all previous notations to denote colony h, then the difference in laying date between colonies 1 and 2 would be

$$\begin{aligned}\mu_1 - \mu_2 &= \bar{D}_1. - \bar{X}_1. - \bar{Z}_1. - (\bar{D}_2. - \bar{X}_2. - \bar{Z}_2.) \quad \text{(B1)}\\ &= \bar{D}_1. - \bar{D}_2. - (\bar{X}_1. - \bar{X}_2.) - (\bar{Z}_1. - \bar{Z}_2.)\end{aligned}$$

and an estimate of this difference is

$$\bar{u}_1. - \bar{u}_2. = \bar{D}_1. - \bar{x}_1. - (\bar{D}_2. - \bar{x}_2.)$$

As was done previously, confidence limits on the difference in mean laying date are developed by placing confidence intervals separately on the error in regression and error in sampling portions of (B1).

First, from the regression equation, given the selected samples, $\bar{Y}_1. - \bar{Y}_2. - b(\bar{X}_1. - \bar{X}_2.)$ is normally distributed with mean 0 and variance

$$\sigma^2\left[\frac{1}{k_1} + \frac{1}{k_2} + \frac{(\bar{X}_1. - \bar{X}_2.)^2}{\Sigma(x_j - \bar{x}.)^2}\right]$$

Thus, a 100 α_1 confidence interval for $(\bar{X}_1. - \bar{X}_2.)$ is given by

$$P\{L_1 < \bar{X}_1. - \bar{X}_2. < H_1\} \geq 1 - 2\alpha_1 \quad \text{(B2)}$$

where

$$L_1 = \frac{b(\bar{Y}_1. - \bar{Y}_2.) - B}{A}$$

$$H_1 = \frac{b(\bar{Y}_1. - \bar{Y}_2.) + B}{A}$$

$$A = b^2 - \frac{t^2_{n-2}(\alpha_1)S^2}{\Sigma(x_j - \bar{x}.)^2}$$

$$B = \sqrt{b^2(\bar{Y}_1. - \bar{Y}_2.)^2 - A\left[(\bar{Y}_1. - \bar{Y}_2.) - t^2_{n-2}(\alpha_2)s^2\left(\frac{1}{k_1} + \frac{1}{k_2}\right)\right]}.$$

Again if it is assumed that the Z_{hi} are normally distributed with mean 0 and variance σ^2_z than a 100 α_2 percent confidence interval on $(\bar{Z}_1. - \bar{Z}_2.)$ is given by

$$P\{L_2 < \bar{Z}_{1\cdot} - \bar{Z}_{2\cdot} < H_2\} \geq 1 - 2\alpha_2 \qquad \text{(B3)}$$

where

$$H_2 = t_{(k_1+k_2-2)}(\alpha_2)s_z \sqrt{\frac{1}{k_1} + \frac{1}{k_2}}$$

$$L_2 = -H_2$$

and

$$s^2_z = \Sigma\Sigma(\hat{u}_{hi} - \bar{u}_{h\cdot})/(k_1 + k_2 - 2).$$

Combining (B2) and (B3) gives

$$P\{L_1 + L_2 < (\bar{X}_{1\cdot} - \bar{X}_{2\cdot}) + (\bar{Z}_{1\cdot} - \bar{Z}_{2\cdot}) < H_1 + H_2\} \geq 1 - 2\alpha_1 - 2\alpha_2$$

or

$$P\{\bar{D}_{1\cdot} - \bar{D}_{2\cdot} - H_1 - H_2 < \bar{D}_{1\cdot} - \bar{D}_{2\cdot} - (\bar{X}_{1\cdot} - \bar{X}_{2\cdot}) - (\bar{Z}_{1\cdot} - \bar{Z}_{2\cdot}) < \bar{D}_{1\cdot} - \bar{D}_{2\cdot} - L_1 - L_2\} \geq 1 - 2\alpha_1 - 2\alpha_2$$

which, when compared to (B1), provides a confidence interval for

$$P\{\bar{D}_{1\cdot} - \bar{D}_{2\cdot} - H_1 - H_2 < \mu_1 - \mu_2 < \bar{D}_{1\cdot} - \bar{D}_{2\cdot} - L_1 - L_2\} \geq 1 - 2\alpha_1 - 2\alpha_2.$$

Setting $\alpha_1 = \alpha_2 = 0.0125$ provides a 95% confidence interval for the difference in mean laying dates. If the confidence interval does not include 0 then the colonies are significantly different in respect to mean laying date.