PARENTAL CONTROL OF HATCHING ASYNCHRONY IN RING-BILLED GULLS (*Larus delawarensis*)

by

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PARENTAL CONTROL OF HATCHING ASYNCHRONY IN RING-BILLED GULLS (*Larus delawarensis*)

BY

GEORGE E. SHNIER

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

Eggs in some bird species may hatch up to several days apart, whereas the young of some species tend to hatch synchronously. Since asynchrony has a large effect on the growth and survivorship of the young in the brood, many hypotheses have arisen to explain it. Underlying most adaptive hypotheses is the assumption that parents can control the asynchrony of their brood, yet this has rarely been tested. The goal of my study was to test this assumption for ring-billed gulls (*Larus delawarensis*). I determined when effective parental incubation began and what the early incubation temperatures were using thermocouples affixed to eggs at nests of ring-billed gulls in the field. Data loggers recorded egg temperatures throughout the laying period. Early incubation temperatures correlated strongly with hatch asynchrony (R^2=0.58, P=0.0009). Using data on incubation temperatures in combination with temperature-dependent developmental rate data obtained for laboratory-incubated eggs, it was possible to predict the degree of hatch asynchrony (R^2=0.66, P=0.0007). Additional studies were also undertaken to control for the effects on hatch asynchrony of other plausible variables, including laying intervals, differential within-clutch incubation or intrinsic developmental rates, egg size, and ambient temperatures. None of these variables had a significant effect except ambient temperature when temperatures were unusually low. The results from all studies support the conclusion that parental incubation is the major determinant of hatch asynchrony in this species. The fact that parents can control asynchrony is consistent with at least three current adaptive explanations for hatch asynchrony: the brood reduction hypothesis, the sibling rivalry reduction hypothesis, and the peak load reduction hypothesis. Results were not supportive of the hormonal control hypothesis, the hurry-up hypothesis, or the hypothesis that asynchrony is the result of random variation.

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INTRODUCTION

In some bird species parents do not start incubating until all their eggs are laid, which results in a relatively synchronous hatch. Synchronous hatching is important in many precocial species whose broods leave the nest soon after hatching, as late hatched chicks would be in danger of getting left behind (Magrath 1990). In many other birds, including most semi-precocial and altricial species, the eggs in the nest hatch asynchronously, usually staggered out over the course of more than one day (Amundsen and Slagsvold 1991). Because the chicks grow rapidly at the start, by the time the last hatched chick in these species hatches, it is typically considerably smaller than its siblings, and is at a competitive disadvantage (Mock 1984a). These youngest chicks tend to have relatively high mortality rates, and when food is scarce, they often starve because their siblings prevent them from eating. In extreme cases they may be outright killed by their older and larger siblings (Mock 1984b). The frequent elimination of younger chicks, whether from starvation or siblicide, is termed brood reduction (Ricklefs 1965).

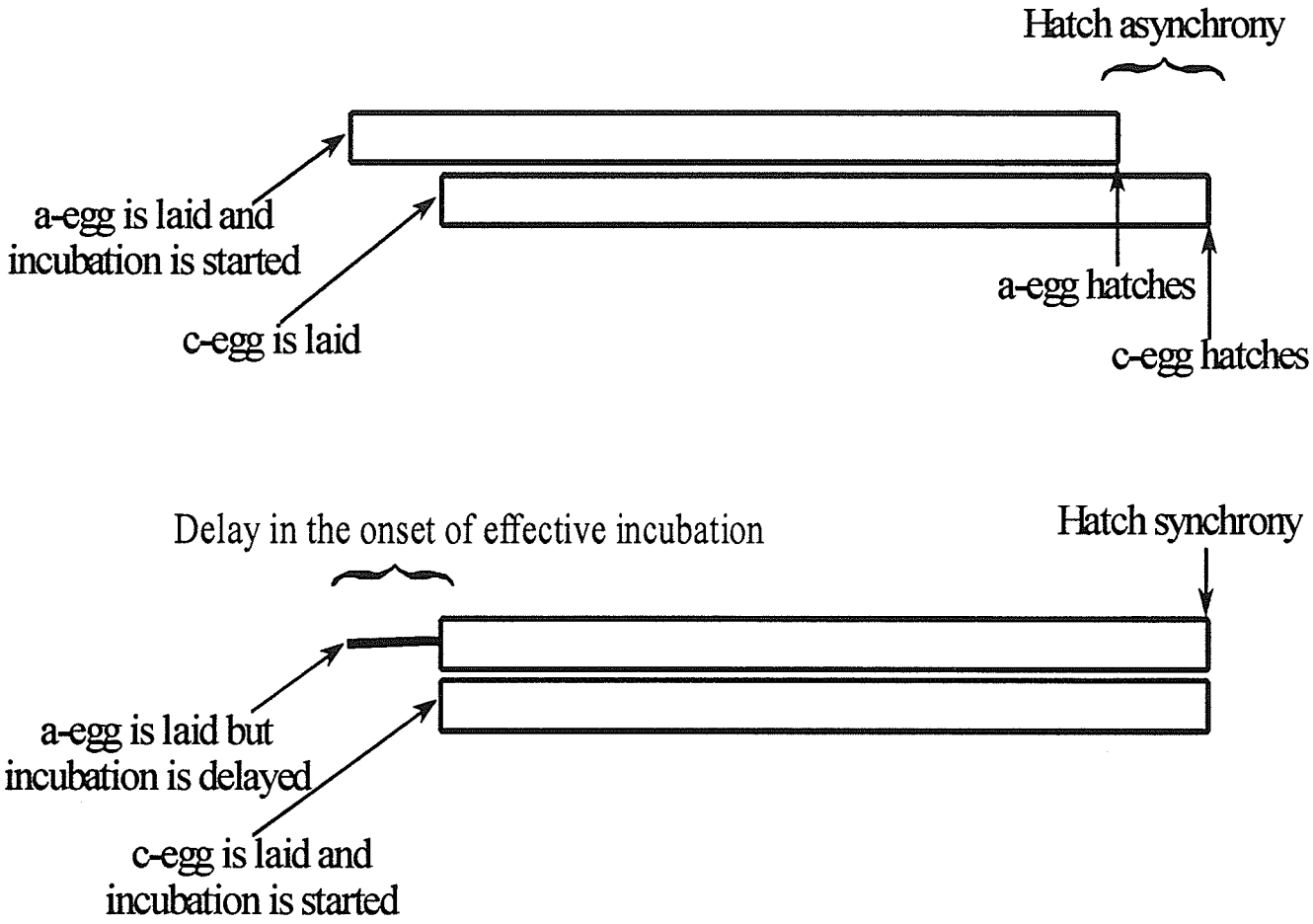
Experimental studies from a wide range of avian species have now shown that brood reduction is facilitated by hatch asynchrony (Slagsvold 1982, Slagsvold 1985, Slagsvold 1986, Mock and Parker 1986, Magrath 1989). David Lack (1954) first suggested that brood reduction and hatch asynchrony are adaptive because they enable the parents to reduce the number of young they have to feed in years when food turns out to be scarce, while permitting them to rear the entire brood in years when food is abundant (Pijanowski 1992). In obligate brood reducing species, the younger chick is thought to represent insurance against the possibility of the first egg failing to hatch or the first chick dying accidentally (Dorward 1962,

Forbes 1990). There are also other less widely accepted adaptive, and some non-adaptive, hypotheses to explain hatch asynchrony and brood reduction (reviewed in Mead and Morton 1985, Magrath 1990).

Most adaptive hypotheses that attempt to explain brood reduction assume, like Lack's hypothesis, that the parents are able to control the hatching asynchrony of their brood by beginning incubation before all the eggs have been laid (Magrath 1990). Because there is generally at least one day between the laying of each of the eggs (Pettingill 1985), the exact timing of the onset of incubation could in theory be used to exert a significant degree of control over asynchrony. For example, if there were 4 days between the laying of the first and third eggs, and the parents initiated incubation immediately after the first egg was laid, one would expect there to be four days between the hatching of the first and third eggs (Fig. 1, top). Conversely, if parents delayed the onset of incubation until the third egg was laid, one would expect significantly more synchrony between the hatching of the first and third eggs (Fig. 1, bottom).

The main objective of my study was to determine whether ring-billed gulls (*Larus delawarensis*), a typical brood reducing species with asynchronous hatching (Woulfe 1989), start incubation before all their eggs are laid and, if they do, to determine whether this behavior allows them to control the magnitude of hatching asynchrony within their brood. A multifaceted approach to this problem was employed. To determine when effective incubation started, egg temperatures were measured automatically, then used to predict, with the aid of regression models, how many days apart the eggs would hatch. To translate incubation temperatures into predicted hatch dates, it was necessary to examine early developmental rates of eggs held in the laboratory at different controlled temperatures. To clarify

Figure 1: Illustration of the idealized way in which the onset of effective incubation is expected to affect the hatching asynchrony.


Hatch asynchrony
a-egg is laid and
incubation is started
c-egg is laid
a-egg hatches
c-egg hatches
Delay in the onset of effective incubation
Hatch synchrony
a-egg is laid but
incubation is delayed
c-egg is laid and
incubation is started

interpretations, additional studies were conducted to control for the possibility that other plausible variables significantly affected hatch asynchrony. These included egg size, laying intervals, differential incubation temperatures within the clutch after the onset of full incubation, ambient temperatures, and intrinsic variation in incubation period depending on the position in the laying order.

Ring-billed gulls typically lay three eggs. The first, second and third-laid eggs will be referred to as the a-, b-, and c- eggs, respectively. Usually there are approximately 2 days between the laying of successive eggs, hence there will usually be a total of 4 days between the laying of the first and third eggs, although it is not uncommon for some clutches to have intervals a day shorter or longer than the modal (Vermeer 1970, Ryder 1993). The c-egg is usually significantly smaller than the a- and b-eggs, and in some studies the b-egg has been found to be significantly smaller than the a-egg (Meathrel and Ryder 1987). These egg size differences are also reflected in the chick weights (Ryder 1993). The natural incubation period, that is, the time between the laying and hatching of a given egg, ranges from 23 to 28 Days (Vermeer 1970). Nocturnal desertion may be one important source of this variation (Chardine and Morris 1983). The b-egg usually hatches on the same day or one day later than the a-egg, whereas the c-egg can hatch 1 to 3 or more days after the a-egg (Vermeer 1970).

The chicks from c-eggs typically experience higher mortality rates in gulls (Parsons 1975, Woulfe 1989), likely due to the competitive effects of their size differences with the other chicks which in turn is due largely to their later date of hatching (Mock 1984b). This selective difference in mortality rates is the primary cause of brood reduction (the 'third chick disadvantage', Pierotti & Bellrose 1985). My study focuses primarily on the overall hatch asynchrony for the brood, between the a- and c-eggs, i.e. the a-c hatch interval.

METHODS

Ring-billed gulls were studied in the springtime of the years 1991-1993 at Kaweenakumik Islands ecological reserve on Kaweenakumik Lake, which lies about 400 km north of Winnipeg, Manitoba (52°48'N, 99°31'W) (Koonz 1985). There are numerous breeding colonies on the lake for several bird species including the American white pelican (*Pelecanus erythrorhynchous*), double-crested cormorant (*Phalacrocorax auritus*), and herring gulls (*Larus argentatus*). There are up to four distinct colonies of ring-billed gulls containing a total breeding population estimated at about 12,000 pairs (Koonz and Rakowski 1985). To minimize disruptions, study colonies were visited only once or twice daily (see Vermeer 1970).

INCUBATION TEMPERATURE

To determine incubation temperatures, fine thermocouple probes (30 gauge) were attached to first-laid eggs (a-eggs) on the day of laying. External probes were attached with adhesive (micro-pore) tape to the outside of the egg at the blunt end with the sensor at the center. The end of the egg at the center was chosen because it stays the same distance away from the brood patch even when the egg is turned. In this way, the effect of egg turning on temperature readings was minimized. Sufficient slack was left in the wire so that the parents could turn the eggs without being hindered. Parents accepted and incubated the eggs normally. Probes were led to one of two eight-channel Grant instruments™ data loggers which were set to record egg and ambient temperatures automatically to the nearest 0.1°C every 10 minutes throughout the day and night. Data from the loggers were downloaded to a

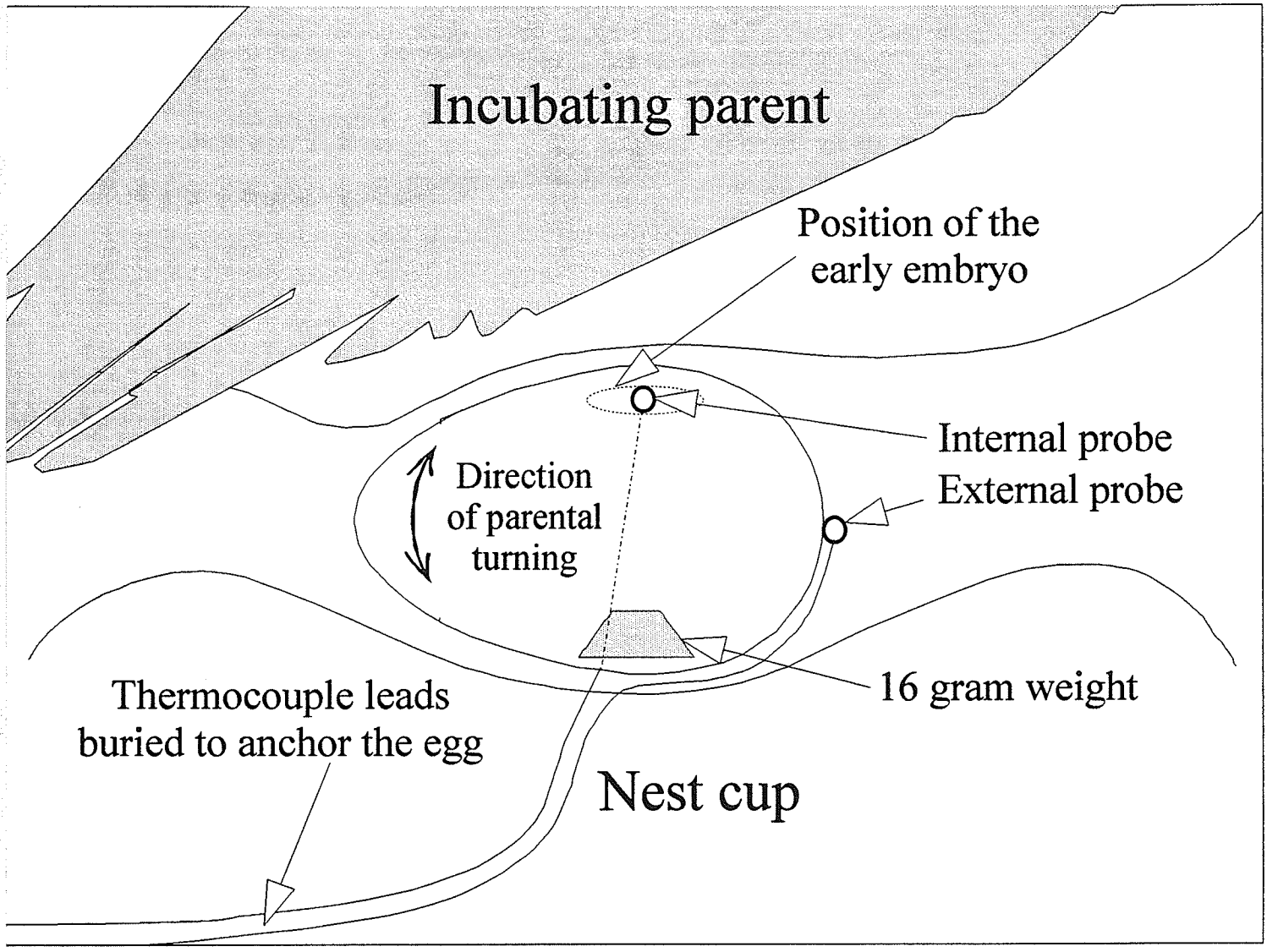
laptop computer and stored on disk every 1-2 days. Readings continued for the first four or five days, encompassing the usual a-c egg laying interval, and therefore the time when incubation behavior is postulated to regulate hatching times and asynchrony. I also recorded laying dates and pipping and hatching dates of the a-, b-, and c-eggs, and any egg losses from nests where temperatures were being recorded. These data were used to determine whether or not the parents began incubation before all their eggs had been laid, to see if incubation temperatures correlate with hatching times, and to make predictions on hatch asynchrony at individual nests.

CORRECTION FOR PROBE LOCATION

During early incubation, gull embryos typically float to a position above the yolk, about 0.5 cm below the upper side of the shell whenever the egg is turned (Drent 1970). Because the thermocouple probes were attached at the blunt end of the eggs in the center, they were necessarily away from the position of the embryos and therefore likely to record a temperature different than the actual temperature of incubation. Positioning the probe in the middle of the egg, closer to the embryo, would mean that when the parents turn the egg, as they do frequently throughout the day, the measured temperature would fluctuate depending on whether the probe was nearest the ground or the parent's warm brood patch (Fig. 2).

To estimate the temperature difference between the blunt end and embryo position, I substituted dummy eggs for real gull eggs at a sample of 17 nests. The dummy eggs were constructed from shells of real ring-billed gull eggs, strengthened inside with Fiberglas and filled with agar, which approximates the density and thermal properties of the natural egg

Figure 2: Probe positions on dummy eggs.


Incubating parent
Position of the
early embryo
Internal probe
External probe
Direction
of parental
turning
16 gram weight
Thermocouple leads
buried to anchor the egg
Nest cup

contents (Evans 1989). An internal probe was placed in position and held in place with masking tape before filling the egg with agar. The internal probe was located at the embryo position, that is, about 0.5 cm below the upper surface of the shell inside the egg, nearer the blunt end where the egg is the widest. The dummy eggs also had a thermocouple probe attached at their blunt ends (where all field egg temperatures were taken) for comparison to the embryo position temperatures. Dummy eggs were kept from being turned by a 16-gram weight opposite the internal probe, and by burying the probe leads to take up any slack, in effect tying them down.

Temperature readings from the probes were recorded every 10 min for one day at each nest during early to mid incubation. Ambient temperature, time of day, and the number of eggs in the nest were also recorded at the time temperatures were being taken. The regression of blunt end temperature on embryo temperature was then used to correct blunt end data from live eggs to obtain a more exact estimate of actual incubation temperatures of the early embryos.

The Relationship Between Incubation Temperature and Development

To assess developmental effects of different incubation temperatures during the laying period, sets of freshly laid eggs were incubated in the laboratory for two days at a range of temperatures, in increments of 2°C, from 24°C to 40°C . Preliminary tests indicated that this range starts below the temperature at which development begins and runs to, and possibly above, the optimal temperature (Drent 1970). Higher temperatures are likely to slow development due to heat stress (Lundy 1969, Webb 1987). Three to five eggs were incubated at each temperature. The eggs were then cracked and development was measured. An

additional five freshly laid eggs were opened and the embryos examined as a control treatment.

To gauge the level of development of the embryos, the blastodisc with the embryo intact was removed from the egg, preserved in Bouin's fixative, and examined under a dissecting microscope. Rugh's (1977) embryology laboratory manual was used as a guide to determine the level of development. Rugh (1977) shows chicken embryos incubated at optimal temperatures for various periods of time over the first four days. Written descriptions and photographic plates are also given. Morphological features used to assess development included the level of development of the primitive streak and the neural folds, the number of somites, the turning of the head and the general appearance of the embryo. Although ring-billed gull and chicken eggs do not necessarily develop at identical rates, the pattern of development is comparable (Drent 1970) so that the gull embryos can be aged relative to each other according to which embryos are more advanced. Other studies have similarly assumed that the sequence of development is comparable in chickens and gulls (Bennet and Dawson 1979, Bennet *et al* 1981). Since all the embryos were incubated for 48 hours, the embryos that were most advanced (presumably those incubated at an optimum or near-optimum temperature), were assumed to show 48 h of development. The developmental ages of less developed embryos were then determined using simple ratios from Rugh's data on chicken eggs as a guide.

MEASUREMENTS OF OTHER FACTORS

To determine the extent of parental control of hatch asynchrony by incubation during the laying period it is necessary to control for other variables that may play a role in hatch asynchrony. The following were examined.

Differential incubation: In some species parents incubate some eggs more than others, which can have a significant effect on hatch asynchrony (Bortolotti and Weibe 1993). If this were so in ring-billed gulls, then it would confound the hypothesis that the parents control hatch asynchrony by starting incubation before all their eggs are laid, even though both theories suggest parental control. To test for this possibility, I attached thermocouple probes to each of the three eggs (a-, b-, and c-) in a sample of 14 nests, all near the middle of the incubation period. Temperatures were recorded every 10 minutes for a 24 hour period at each nest.

Egg size and laying order: It has been postulated that egg size affects hatch asynchrony (hatch intervals) in gulls because smaller eggs take less time to hatch and c-eggs are usually smaller (Parsons 1972). It is also possible that there is a difference in hatching times between eggs within a clutch that is not due to size but some other intrinsic factor associated with position in the laying order. To test for the possible confounding effects of these two variables, eggs of known position in the laying order were collected on the day of laying and incubated until hatching under identical controlled conditions in the laboratory. For this test, eggs were incubated at 36°C, ±0.5°C. Three small bowls were placed in the bottom of the incubator to provide necessary humidity (Lundy 1969) and the eggs were turned three

times a day. A small fan was placed in the incubator to circulate air and keep temperatures uniform throughout. However, different spots in the incubator still may have received slight variations in humidity and temperature. To compensate for this, eggs were shifted in the incubator at the time they were turned in such a way as to ensure that all eggs spent equal time in all locations in the incubator. Length (L) and breadth (B) of eggs were measured to the nearest 0.1 mm with dial calipers and used to calculate Volume (V) according to the formula $V=.489 \times LB^2$ (Ryder 1975). Hatching times were recorded to the nearest 4 hours.

Ambient temperature: Ambient temperature could affect hatching asynchrony indirectly if it was sufficiently cold during the laying period that parents were required to protect the eggs by incubating them as soon as they are laid, thereby causing asynchrony simply to keep the eggs from perishing. Ambient temperature could also affect hatch asynchrony more directly by actually contributing to development, if it was warm enough. These possibilities were tested by holding a total of 30 freshly laid a- and b-eggs at colony ambient temperature for two to seven days in a covered wire-mesh enclosure that kept out rain, direct sun and animals. Eggs were then fostered out to active nests for incubation. Laying date, hatching date, and the amount of time spent at ambient temperatures prior to the onset of incubation were all recorded. As an additional test of the effects of ambient temperature, I also recorded ambient temperatures during the study using thermocouple readings at natural nests. The data on ambient temperatures were regressed against incubation periods and hatch asynchrony.

STATISTICAL TESTS

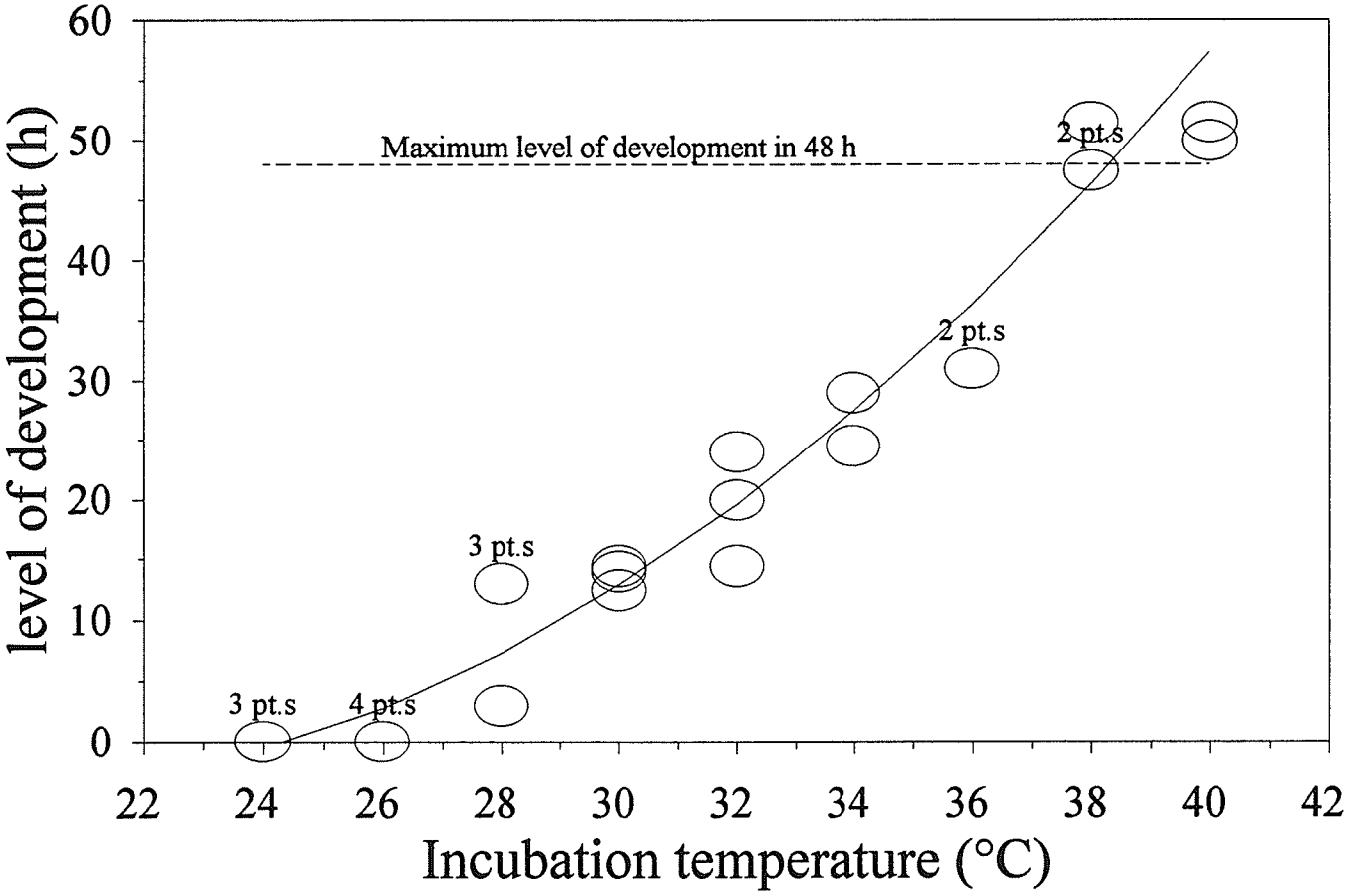
Statistical tests included Ordinary Least Squares regression, Multiple regression, Second Order Polynomial regression, Forward Stepwise regression, One Way ANOVA, Two Way ANOVA, Bartlett's test of equal variances, Durbin-Watson test for autocorrelation, Wilk-Shapiro test for normality, Normal Probability plot and t-tests. Software used for analysis included the Statistix (version 4.1, Analytical Software) and Quattro® Pro for Windows (version 6, Borland International).

RESULTS

TEMPERATURE AND EARLY DEVELOPMENTAL RATE

From 47 eggs dissected, useable data were obtained from 26. In 10 cases the egg turned out not to be fresh when taken and had to be discarded. In 11 others the dissection appeared to have broken the yolk prematurely and damaged the embryo. Development over the range of incubation temperatures for the remaining eggs is illustrated in Fig.3. The relative level of development is expressed as hours required to reach an equivalent stage at optimum temperature. Level of development was then plotted against actual incubation temperature in Fig. 3. For example, the level of development of an egg incubated for 48 hours at 32°C was equivalent to the development shown by eggs incubated for approximately 20 h at an optimum temperature. No development occurred at or below 26°C. Above that temperature, there was a significant correlation between the incubation temperature and the level of development shown by the embryos (R^2=0.95, $F_{1,24}$=409.09, P=0.0000). However, this model tested positive for autocorrelation (Durbin-Watson statistic=1.27, $P_{\text{POSITIVE CORRELATION}}$=0.01, see appendix 1). To correct for autocorrelation I used a second order polynomial regression. Since it is not biologically possible for the level of development to exceed the 48 h of incubation time actually used (Fig. 3, horizontal line), I included data only up to the 48 h point, excluding the 3 data points lying above that level. This model was also highly significant (R^2=0.95, $F_{2,21}$=203.46, P=0.0000). The residuals from this model were normally distributed (Relatively straight Normal Probability plot, Wilk-Shapiro=0.9384), and not significantly auto-correlated (Durbin-Watson statistic=1.44, $P_{\text{POSITIVE CORR}}$=0.17, $P_{\text{NEGATIVE CORR}}$=0.83). To use this model for predictions of

Figure 3: Regression of relative level of development against temperature of incubation during the first two days of development. Best fit regression equation: hours of development = 44.25 + temperature × -5.16 + temperature^2 × 0.14.


level of development (h)
60
50
40
30
20
10
0
Maximum level of development in 48 h
2 pt.s
2 pt.s
3 pt.s
3 pt.s
4 pt.s
22
24
26
28
30
32
34
36
38
40
42
Incubation temperature (°C)

development in naturally incubated eggs, any embryo temperature reading of 38.31°C or more was assumed to show the maximum development rate of 48 h per 48 hours of incubation. The value 38.31 was determined by the temperature at which the regression model predicted 48 h of development (Fig. 3).

CORRECTING FOR PROBE LOCATION

Figure 4 shows typical temperature readings from thermocouple probes mounted on dummy eggs and placed in nests that were then measured for 24 h each during early and mid incubation. Of 17 nests studied, 12 yielded useable data. In two, the egg was abandoned and in three others there were technical difficulties with the probe. In all cases (n=12), once the dummy eggs warmed up, embryo position temperatures were usually higher (89% of the readings overall) than readings taken from the blunt pole of the egg. The empirical relationship between blunt end and embryo position temperature from all relevant dummy egg trials is shown in Fig. 5. Although variance increased at the higher temperatures, there was a highly significant correlation between blunt end and embryo position temperature (R^2=0.84, $F_{1,1246}$=6471.78, P=0.0000). This regression was just one of several models considered. The best fit regression model was:

$$b = C + Ee + Aa + Tt + Nn$$

(Forward stepwise regression, adjusted R^2=0.85, $F_{4,1243}$=1778.03, P=0.0000), where b is blunt end temperature, e is embryo temperature, a is ambient temperature, t the coded time of day, n the number of eggs in the nest, C a constant (Y-intercept), and E, A, T, and N are coefficients for embryo temperature, ambient temperature, time, and the number of eggs in the nest, respectively. The actual coefficients are given in Table 1. For reasons discussed in

Figure 4: Sample temperature readings from dummy eggs placed in three nests. The nest from which the data in the first graph were taken from contained only one egg. The data in the third graph are from a nest that had three eggs. All three graphs are from data obtained during the first week of the incubation period.

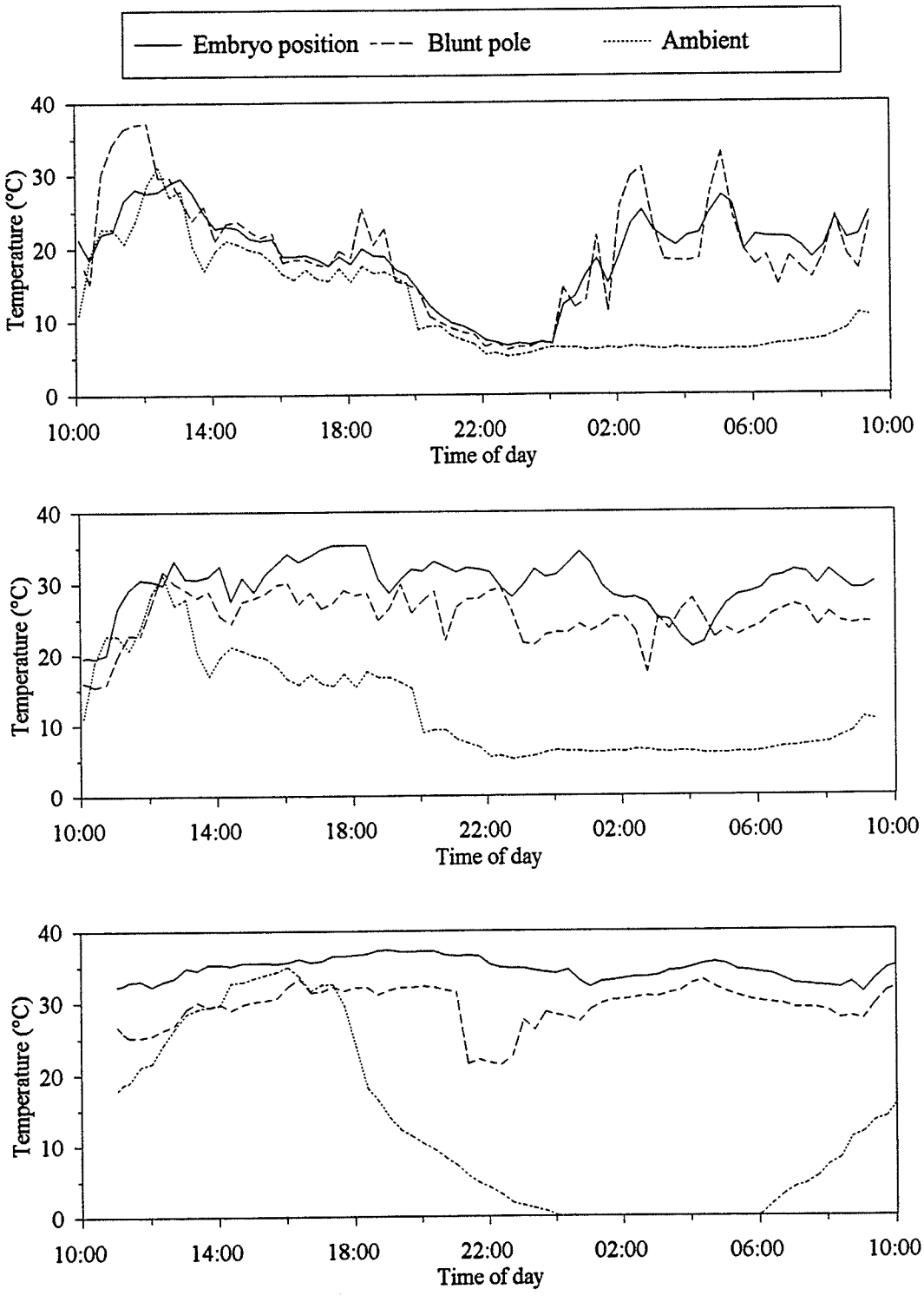

Embryo position
Blunt pole
Ambient
Temperature (°C)
40
30
20
10
0
10:00
14:00
18:00
22:00
02:00
06:00
10:00
Time of day

Figure 5: Embryo position temperature in relation to blunt end temperature.

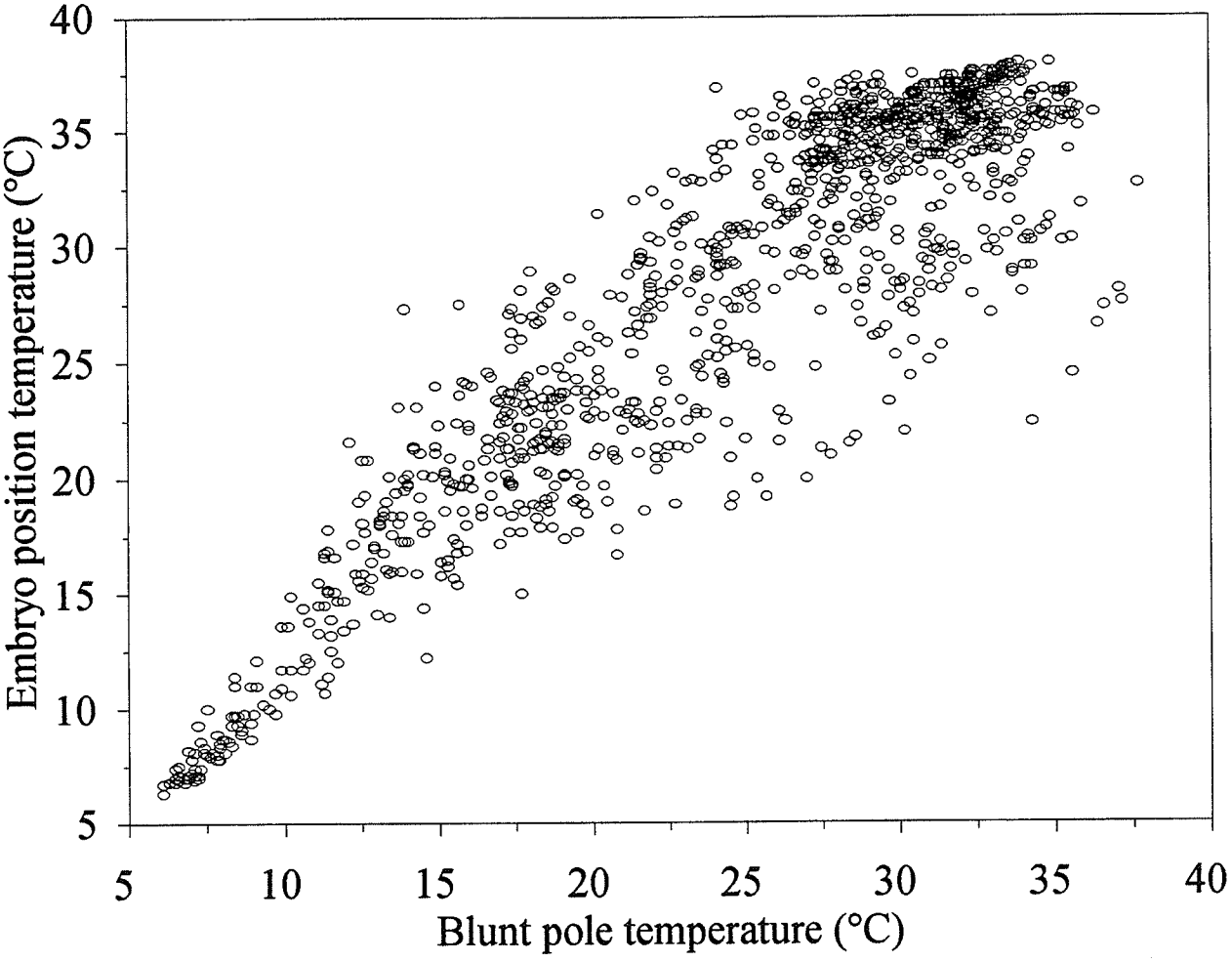

Embryo position temperature (°C)
Blunt pole temperature (°C)
5
10
15
20
25
30
35
40

Table 1: Coefficients for the multiple regression equation used to calculate embryo position temperature.

Variable	Symbol	Coefficient Value
Constant	(C)	-0.438
Embryo temperature	(E)	0.894
Ambient temperature	(A)	0.144
Coded time of day	(T)	-0.502
The number of eggs in the nest	(N)	-0.686

appendix 2, this model uses blunt end temperature as the dependent variable. To use this model descriptively to estimate embryo temperature, the formula can be re-written as:

$$e = \frac{C + Aa + Tt + Nn - b}{-E}$$

Figure 6 is a graphical comparison of measured blunt end temperature readings from a nest and the embryo temperatures calculated with the formula above.

ONSET OF INCUBATION AND INCUBATION PERIOD

Onset of incubation: Embryo temperatures corrected for probe position (see above) from four typical nests are illustrated in Figure 7. Incubation was highly irregular for the first one to three (usually 2) days, but thereafter stayed well above the minimum temperature necessary for development. The usual pattern prior to the laying of the b-egg was for egg temperature to drop precipitously over-night, which suggests a period of desertion or gradually reduced incubation attentiveness. However, even on days when incubation was intermittent, the eggs were significantly warmed for blocks of hours, especially during the day when incubation temperatures tended to climb well into the range where development could occur. After the laying of the b-egg, temperatures usually remained within the developmental range, even at night, for the two days elapsing before the c-eggs were laid. This was the modal pattern among all three-egg nests studied (19 of 30 nests). The period of intermittent incubation at the beginning was sometimes approximately a day longer (3 nests) or shorter (6 nests).

Figure 6: Comparison of temperature readings from the blunt end of a naturally incubated egg and the calculated embryo position temperature.

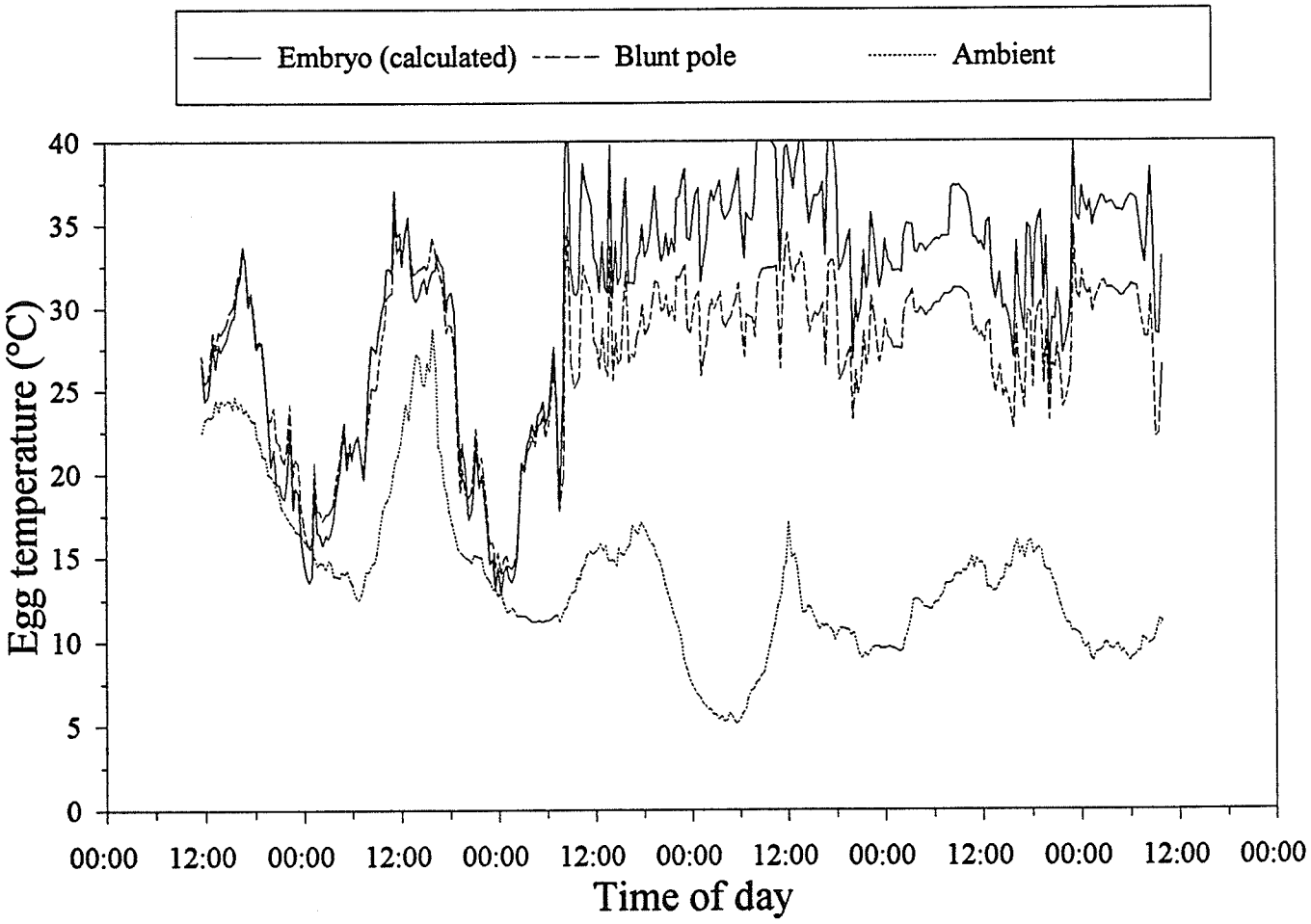
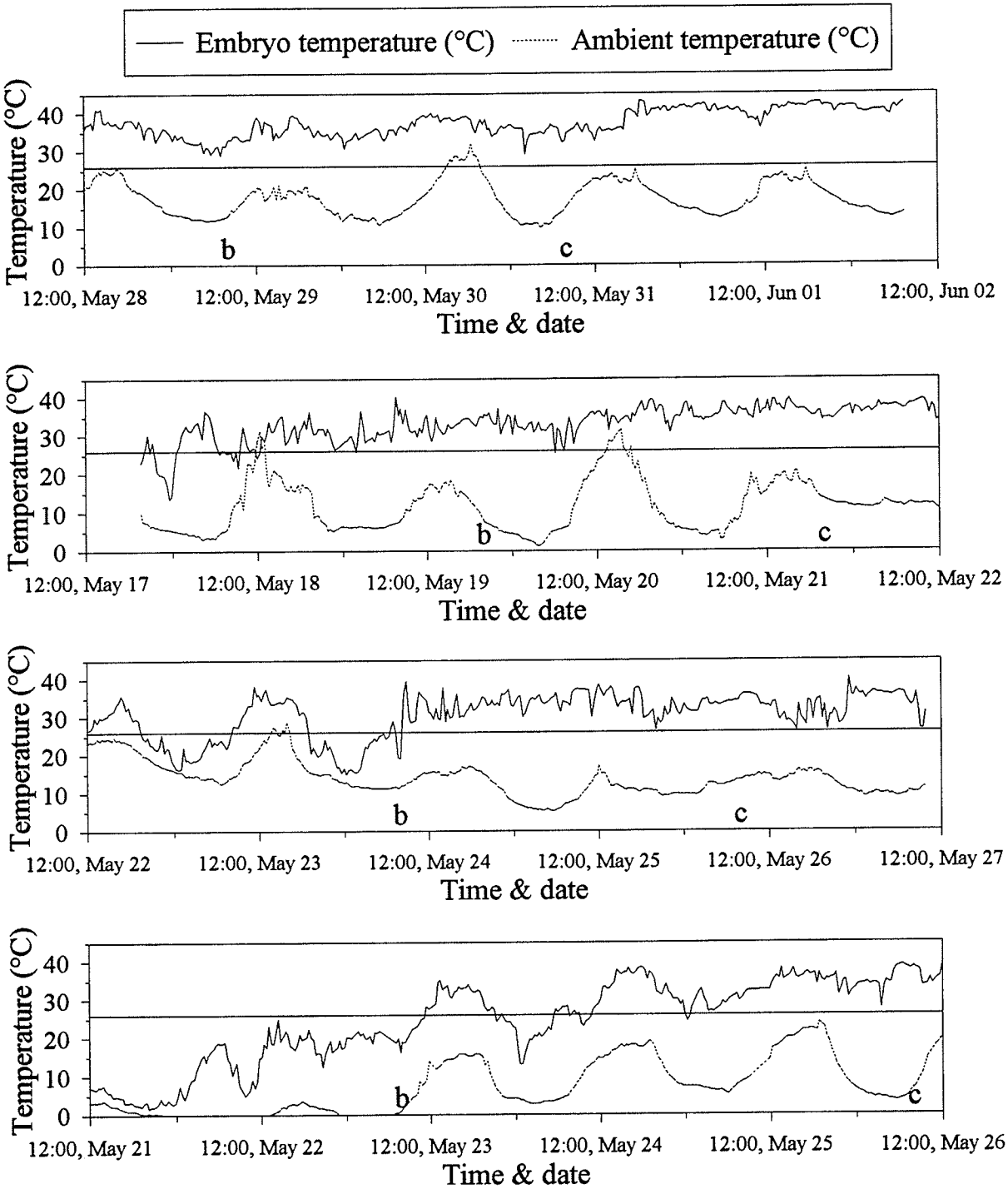

Embryo (calculated)
Blunt pole
Ambient
Egg temperature (°C)
40
35
30
25
20
15
10
5
0
00:00
12:00
00:00
12:00
00:00
12:00
00:00
12:00
00:00
12:00
00:00
12:00
00:00
Time of day

Figure 7: Temperature readings from the a-eggs in four nests during early incubation. Embryo temperatures corrected for probe position are plotted along with ambient temperature and a straight line drawn at 26°C to indicate the lower end of the development range (see above). The time when the b- and c-eggs were laid is indicated by the letters 'b' and 'c' just above the X-axis. Nests were chosen to show the range of periods spent below 26°C. The nest at the top showed the least amount of time below 26°C while the nest at the bottom showed the most.


Embryo temperature (°C)
Ambient temperature (°C)
Temperature (°C)
40
30
20
10
0
b
c
12:00, May 28
12:00, May 29
12:00, May 30
12:00, May 31
12:00, Jun 01
12:00, Jun 02
Time & date
Temperature (°C)
40
30
20
10
0
b
c
12:00, May 17
12:00, May 18
12:00, May 19
12:00, May 20
12:00, May 21
12:00, May 22
Time & date
Temperature (°C)
40
30
20
10
0
b
c
12:00, May 22
12:00, May 23
12:00, May 24
12:00, May 25
12:00, May 26
12:00, May 27
Time & date
Temperature (°C)
40
30
20
10
0
b
c
12:00, May 21
12:00, May 22
12:00, May 23
12:00, May 24
12:00, May 25
12:00, May 26
Time & date

Incubation period: Implicit in the hypothesis that parents control hatch asynchrony, is the assumption that parents can control overall incubation period by manipulating incubation temperature during the laying period. In Figure 8, incubation periods for all eggs studied were regressed against incubation temperature, corrected for probe position, averaged for each egg over the first two days after it was laid. Data points are plotted separately for a-, b-, and c-eggs. For all eggs combined, there was a significant negative correlation between temperature and incubation period (R^2=0.45, $F_{1,85}$=67.94, P=1E-12). Thus, as predicted, eggs that were warmed more by their parents during the first two days of incubation tended to hatch in fewer days after laying.

Incubation temperatures for a-, b-, and c-eggs shown in Figure 8 were in all cases based on thermocouple readings from probes attached to the a-egg in a given nest. For this reason, and also because it is the early a-egg incubation periods that should have the most direct impact on a-c hatch asynchrony, the relationship between a-egg incubation periods and incubation temperatures was examined more closely. In Figure 9, a-egg incubation periods, taken alone, exhibited stronger negative correlation (R^2=0.65, $F_{1,26}$=48.47, P=0.0000). To determine if this relationship carried over to the a-c hatch interval, Figure 10 illustrates the relationship between the average temperatures the a-egg received in the first two days after being laid against the a-c hatch intervals at the same nests. There was a significant correlation between a-egg incubation temperature in the first two days and the length of the a-c hatch interval (R^2=0.63, $F_{1,13}$=22.69, P=0.0004). To further examine this relationship, a-egg incubation period was plotted against the number of temperature readings in the developmental range (between 26 and 40°C, see Fig. 3) during the first two days after laying (Fig. 11). There was a highly significant negative correlation (R^2=0.69, $F_{1,26}$=59.24,

Figure 8: Incubation period versus average incubation temperature at the embryo position averaged over the first two days after each egg was laid. Symbols used represent a, b, and c-eggs. Regression equation: Incubation period = 29.29 + Mean incubation temperature × -0.13.

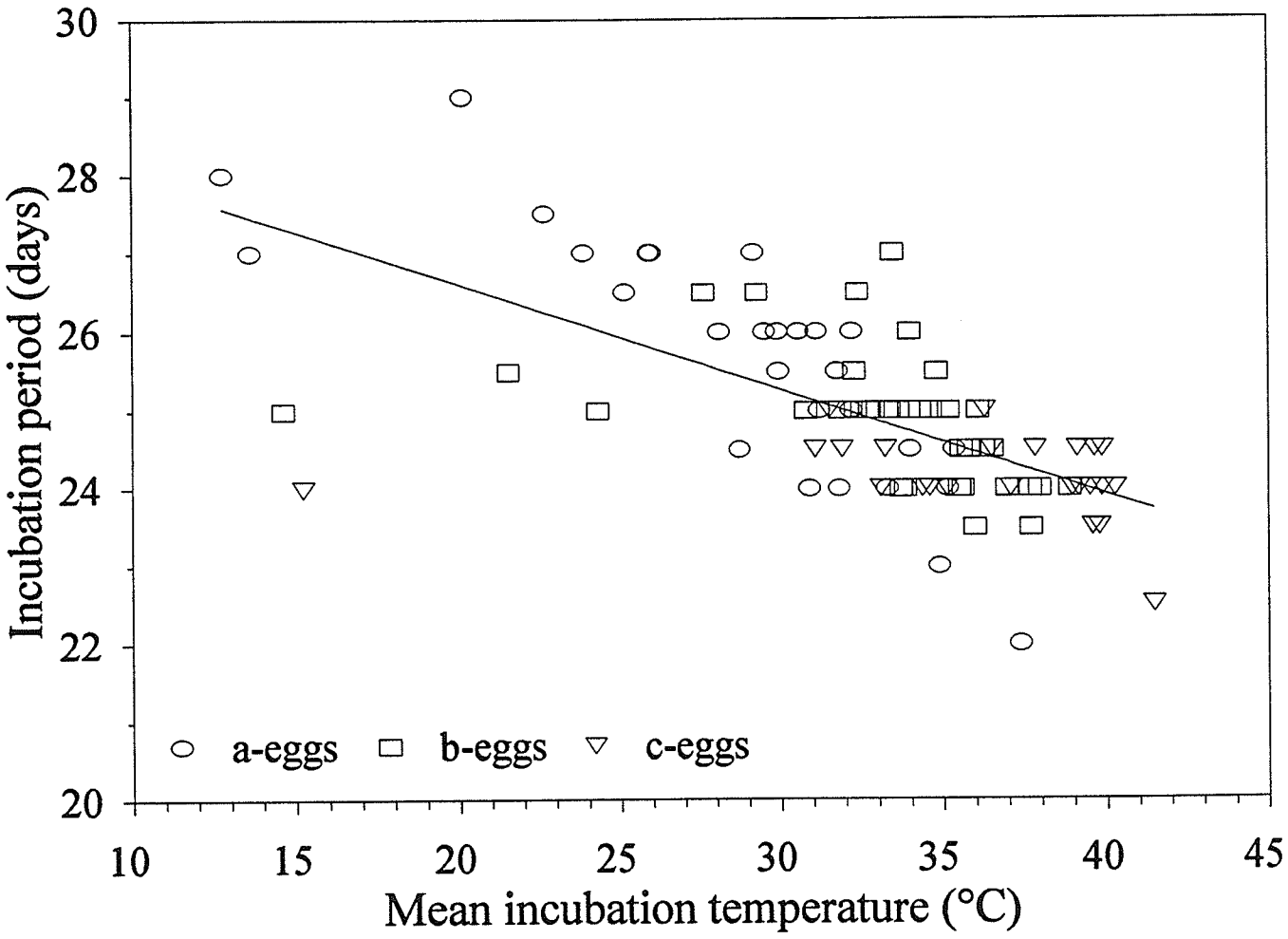

30
28
26
24
22
20
Incubation period (days)
10
15
20
25
30
35
40
45
Mean incubation temperature (°C)
a-eggs
b-eggs
c-eggs

Figure 9: Incubation period in relation to incubation temperatures averaged over the first two days, for a-eggs only. Regression equation: Incubation period = 31.77 + Mean incubation temperature × -0.23.

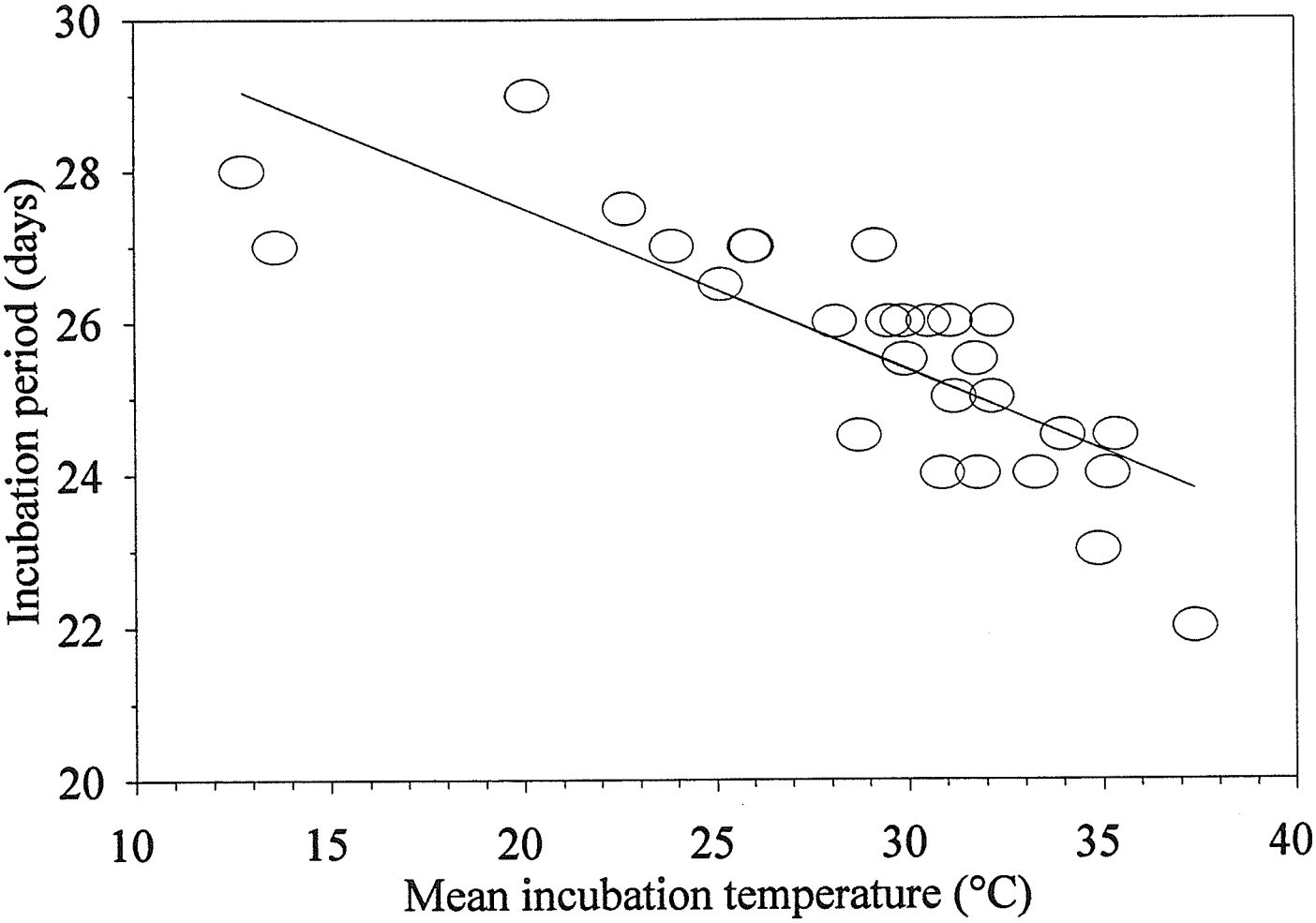

30
28
26
24
22
20
Incubation period (days)
10
15
20
25
30
35
40
Mean incubation temperature (°C)

Figure 10: The a-c hatch interval in relation to incubation temperatures averaged over the first two days. Regression equation: a-c hatch interval = -5.78 + Mean incubation temperature × 0.26.

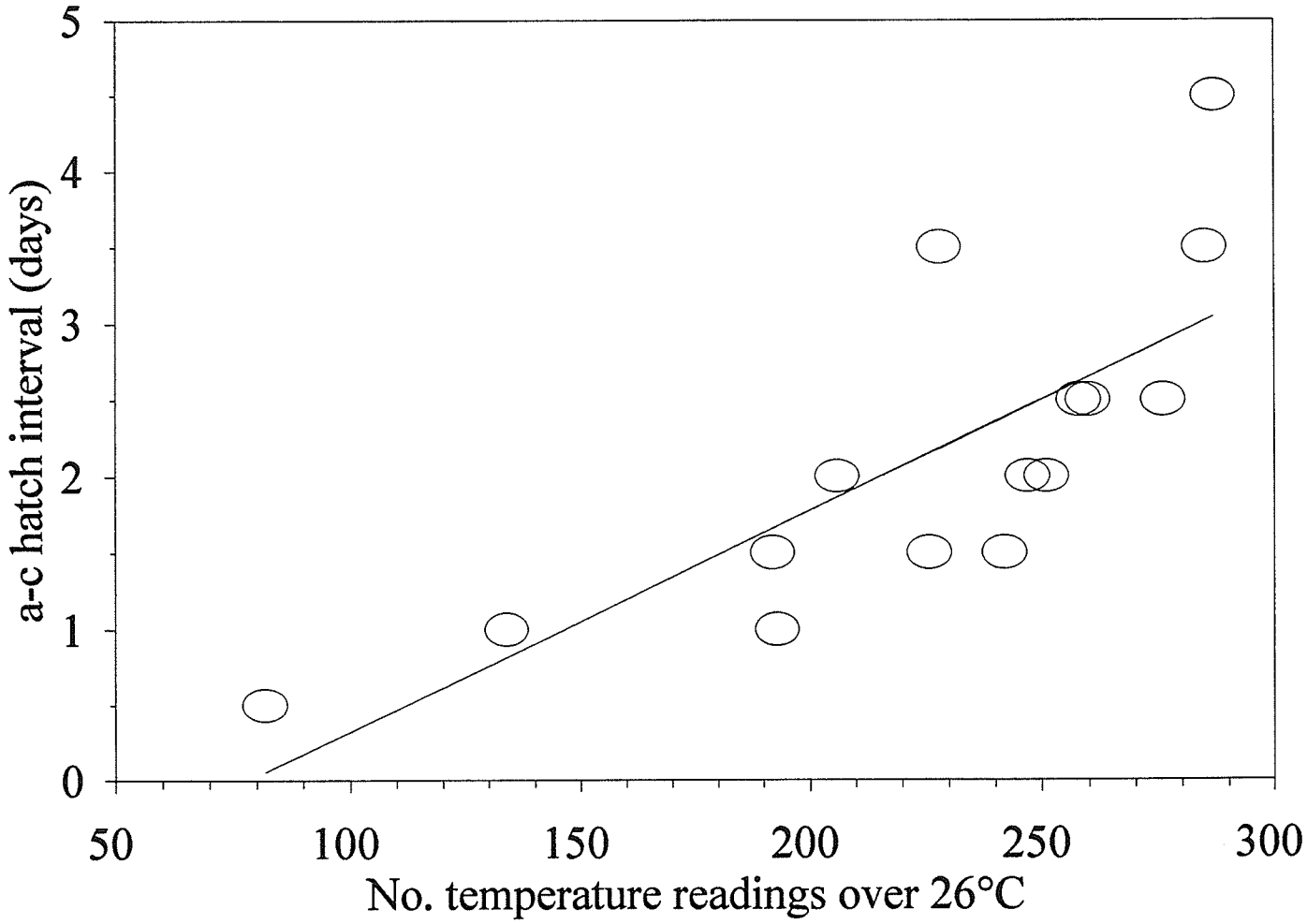

5
4
3
2
1
0
a-c hatch interval (days)
50
100
150
200
250
300
No. temperature readings over 26°C

Figure 11: Incubation period of a-eggs in relation to the number of temperature readings during the first two days that were in the developmental range (above 26°C). Regression equation: a-c hatch interval = 28.94 + No. of readings above 26°C × – 0.16.

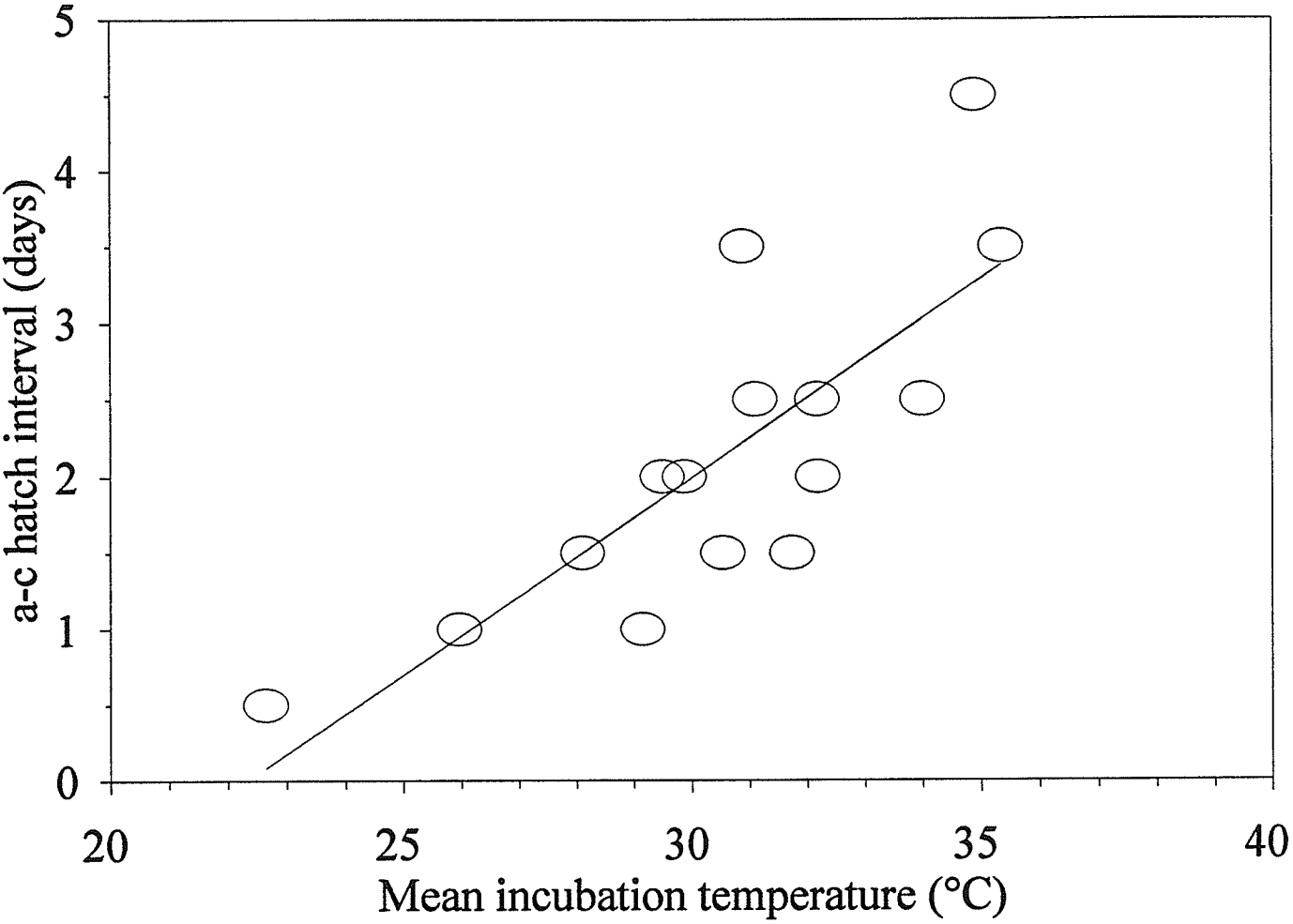
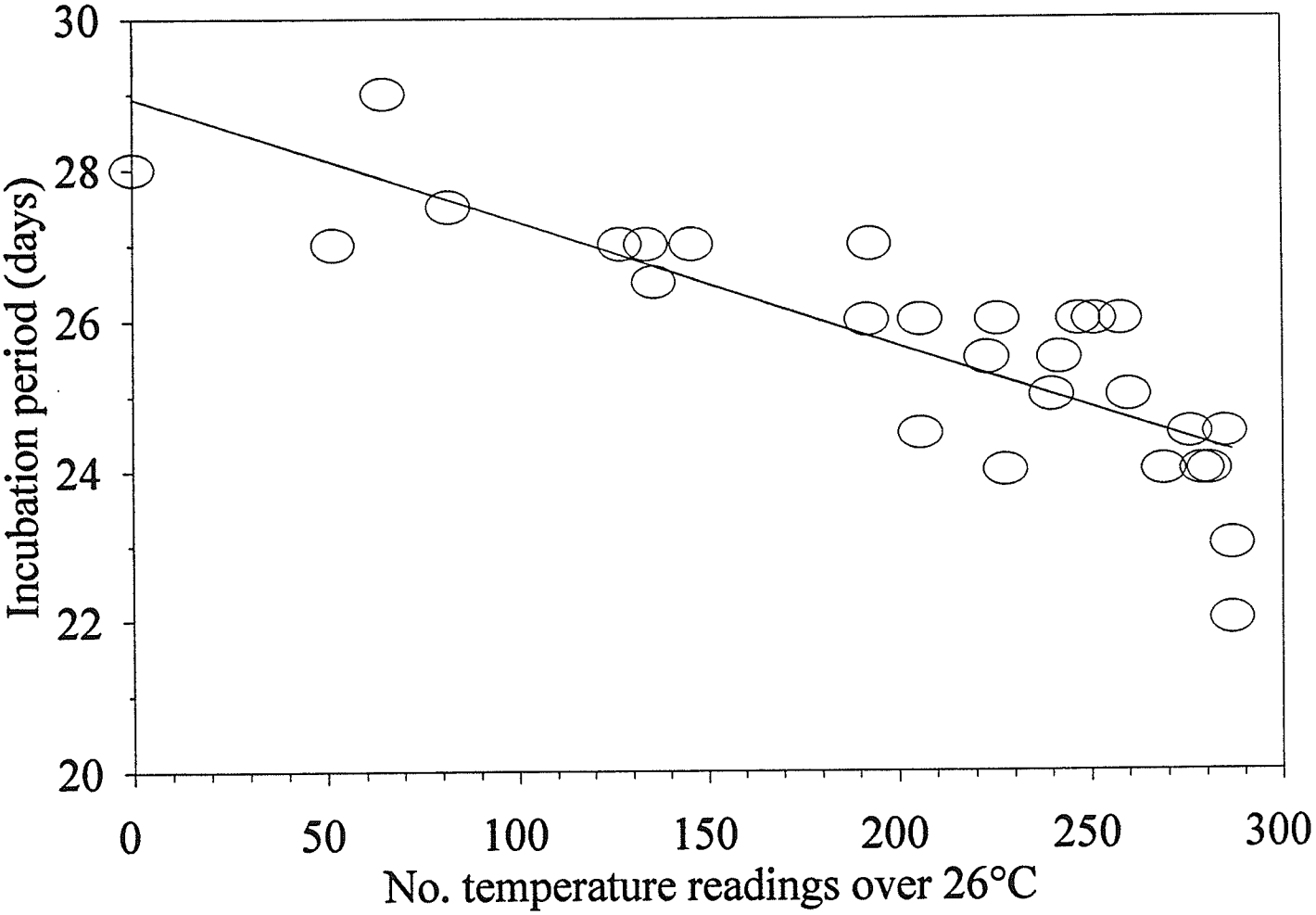

a-c hatch interval (days)
0
1
2
3
4
5
20
25
30
35
40
Mean incubation temperature (°C)

Figure 12: The a-c hatch interval in relation to the number of temperature readings in the development range (above 26°C) in the first two days. Regression equation: a-c hatch interval = -1.13 + No. of readings above 26°C × 0.15.


30
28
26
24
22
20
Incubation period (days)
0
50
100
150
200
250
300
No. temperature readings over 26°C

P=0.0000) and again, this relationship carries over to the a-c hatch interval (Fig. 12; R^2=0.58, $F_{1,13}$=18.49, P=0.0009). The greater the number of developmentally effective temperature readings above 26°C during the first two days, the shorter the incubation period and the longer the a-c hatch interval. The R^2 with readings above 26°C is higher than that for the regression using average temperature (0.69 versus 0.65), suggesting that the number of developmentally effective readings provides a less variable estimate of development than overall averages. This result is not surprising given the large number of readings below 26°C (Fig. 7), all of which should have the same null effect despite their different contribution to overall mean temperature. In the following section, the problem of using mean temperature is avoided by basing calculations on expected amount of development at each temperature reading.

PREDICTING HATCH ASYNCHRONY

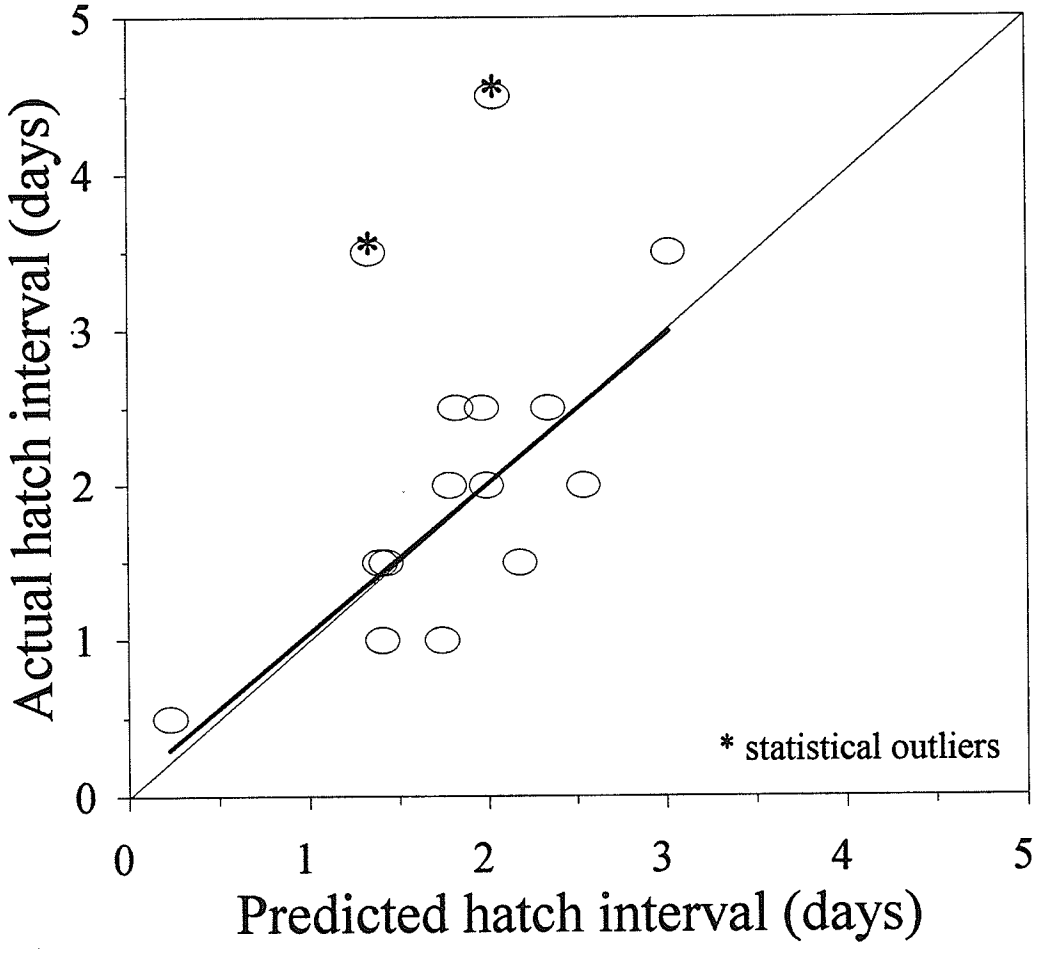
To predict hatch asynchrony, I calculated the number of hours of fully effective incubation equivalent that occurred during the interval between the laying of successive eggs. An example of the calculation can be illustrated using a 48 h period: If, say, an egg spent 48 h of incubation at a temperature where development occurred at a rate equivalent to half the rate of an egg incubated at optimal temperatures, then the egg would have achieved a developmental equivalent to ($\frac{1}{2}$ x 48 h =) 24 h of *effective* incubation. I calculated the equivalent amount of development this way for each 10-min period over all temperatures, after making the above described corrections for probe position. Each 10-min reading was taken as an estimate for that 10-min period and the number of minutes of equivalent development that should have occurred during that 10-min was calculated with the aid of the

regression from the data in Fig. 3. For example, eggs incubated at 32°C for 48 h showed an equivalent of 19.65 h of development. Proportionately, this is equivalent to 4 min. and 6 sec of development during a 10-min period of incubation. Minutes of equivalent development for each 10-min temperature reading were then totaled to determine the overall effective incubation time for periods between the laying of each egg.

To make predictions on the length of a-c hatch intervals, I used the calculated number of hours of effective incubation that the a-egg experienced before the c-egg was laid. This should give an estimate of how far advanced the a-egg was at the time the c-egg was laid, and hence the predicted hatch interval between them. For example, if the a-egg experienced two days of equivalent development in the 4 days before the c-egg was laid, and if both eggs subsequently received the same incubation and had the same inherent incubation periods (see below), the a-egg should hatch out two days before the c-egg.

Observed a-c egg hatch interval is regressed against predicted values for eggs at 15 nests in Figure 13. There was a significant correlation (R^2=0.28, $F_{1,13}$=4.96, P=0.04). The data points from two of the nests were statistical outliers (i.e., greater than two standard deviations from the mean). These are marked with asterisks. When these points were removed and the analysis was run again, there was a stronger correlation (R^2=0.66, $F_{1,11}$=21.55, P=0.0007) between predicted and actual values. With outliers removed, the slope of the regression was not significantly different from 1 (t=0.17, P=0.97) and the intercept was not significantly different from 0 (t=0.18, P=0.86). Except for the outliers, the actual asynchrony was within three quarters of a day from the predicted value. Thus the calculated degree of hatch asynchrony derived from early incubation temperatures was a good direct predictor of actual asynchrony.

Figure 13: Regression of actual against predicted a-c hatch intervals. The extended diagonal line represents a perfect correlation with slope of 1 and intercept of 0. Two outliers are marked with an asterisk. Regression equation: (excluding outliers): a-c hatch interval = 0.07 + No. of readings above 26°C × 0.96.


Actual hatch interval (days)
Predicted hatch interval (days)
0
1
2
3
4
5
* statistical outliers

OTHER EFFECTS ON HATCH INTERVALS

Laying intervals: One might expect that longer intervals between the laying of any two eggs would tend to lead to longer intervals between the hatching of those two eggs, but this did not occur. Table 2 lists laying and hatching intervals for a total of 27 nests in which more than one egg hatched. All a-c laying intervals were either three or four days. There was no significant difference in the variance ($F_{6,7}$=1.83, P=0.22) or the mean (t_{13}=0.99, P=0.34) of the a-c hatch intervals from nests with three day (2.43 ± 0.47) compared with four day (1.88 ± 0.32) a-c lay intervals.

Egg size and laying sequence: There was no correlation between egg volume and incubation period when eggs were incubated under identical conditions in the laboratory (R^2=0.09, $F_{1,17}$=1.74, P=0.21). There was also no difference in the standard deviation of the incubation periods when a-, b-, and c-eggs were compared (Bartlett's test: χ^2=4.40, df=2, P=0.11) nor was there significant difference of the mean incubation period according to position in the laying sequence ($F_{2,48}$=0.00, P=1.00). These variables are therefore unlikely to represent systematic constraints on the parents' ability to regulate hatching asynchrony by varying incubation during the laying periods.

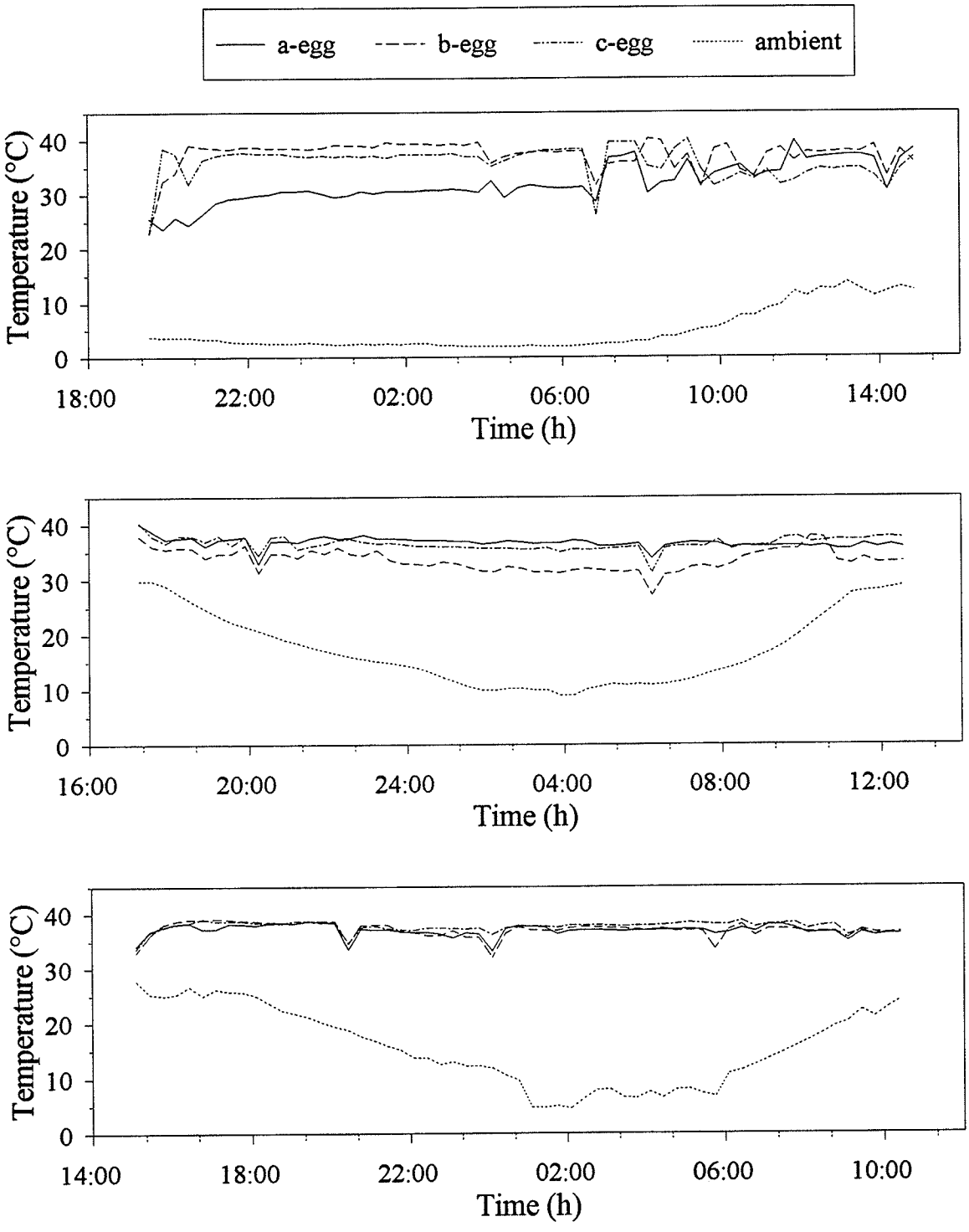
Natural incubation temperature and laying sequence: Graphs of temperatures from three nests, illustrating the range in variance among a-, b-, and c-eggs during mid incubation, are illustrated in Figure 14. There were no significant differences between average a-, b- and c-egg temperatures (ANOVA for non-independent samples: $F_{2,26}$=0.68, P=0.51 (a-, b-, and c-eggs in each nest were not independent as they were all incubated by the same parent)). Evidently, control of hatching intervals is not affected by differential treatment of eggs during normal incubation of the full clutch.

Table 2: Laying intervals and the corresponding hatching intervals (in days) between eggs within clutches (entries in the same row are not necessarily from the same nest)

a-b intervals:		b-c intervals:		a-c intervals:	
laying	hatching	laying	hatching	laying	hatching
1	-0.5	1	0	3	1
1	0	1	1	3	1.5
1	0.5	2	0	3	1.5
1	1.5	2	0	3	2.5
1	2	2	0.5	3	2.5
1	2	2	0.5	3	3.5
2	0	2	1	3	4.5
2	0	2	1	4	0.5
2	0	2	1	4	1
2	0.5	2	1	4	1.5
2	0.5	2	1.5	4	2
2	0.5	2	1.5	4	2
2	0.5	2	1.5	4	2
2	0.5	2	1.5	4	2.5
2	1	2	2	4	3.5
2	1	2	2.5		
2	1.5	3	2.5		
		3	3		

The shaded numbers are the statistical outliers identified in Fig. 13

Figure 14: One day of temperature readings recorded simultaneously from all three eggs from each of three nests


a-egg
b-egg
c-egg
ambient
Temperature (°C)
0
10
20
30
40
18:00
22:00
02:00
06:00
10:00
14:00
Time (h)
Temperature (°C)
0
10
20
30
40
16:00
20:00
24:00
04:00
08:00
12:00
Time (h)
Temperature (°C)
0
10
20
30
40
14:00
18:00
22:00
02:00
06:00
10:00
Time (h)

Ambient temperature: The incubation period of eggs left at ambient temperature for periods of time starting on the day of laying and lasting up to 7 days correlated strongly with the amount of time they were left out (Fig. 15; R^2=0.98, $F_{1,8}$=1.74, P=0.0000). The slope of the line was not significantly different from 1 (t=2.19, P=0.06), indicating that every day an egg was left at ambient temperature added approximately one day to the incubation period, with little effect from ambient temperature on development.

Ambient temperatures at naturally incubated nests sampled early in the laying period with thermocouples (above) had a slight but significant negative effect on a-egg incubation periods (R^2=0.28, $F_{1,26}$=10.30, P=0.004), but only when temperatures were extreme (Fig. 16). The correlation between a-egg incubation periods and ambient temperature was negative, which suggests that cooler ambient temperatures during the laying period may lead to longer incubation periods. However, three points on the graph are from a-eggs that experienced uncommonly low ambient temperatures in the first 3 days after being laid (*see* Figs. 17, 18, and bottom panel of Fig. 7). Only once over the previous 25 years did ambient temperatures drop below 0°C on 21-23 May (Environment Canada official weather record for Grand Rapids Hydro, approximately 40 km from the study site). If the three points that experienced this cold period are removed, the correlation is no longer significant (R^2=0.03, $F_{1,23}$=0.65, P=0.42). Thus ambient temperature appears to have an effect on incubation period mainly when it is extremely low. No a-c hatch asynchrony measurements were possible for nests where a-eggs were laid when temperatures were at or near 0°C because only five out of 12 nests laid c-eggs and none of these hatched. Ambient temperature at nests where all three eggs did hatch had no effect on hatch asynchrony (R^2=0.00, $F_{1,13}$=0.00, P=0.96).

Figure 15: Effect on incubation period of holding freshly laid eggs at ambient temperature for up to 7 days. Regression equation: Incubation period = 24.30 + No. of days left at ambient temperature × 1.11.

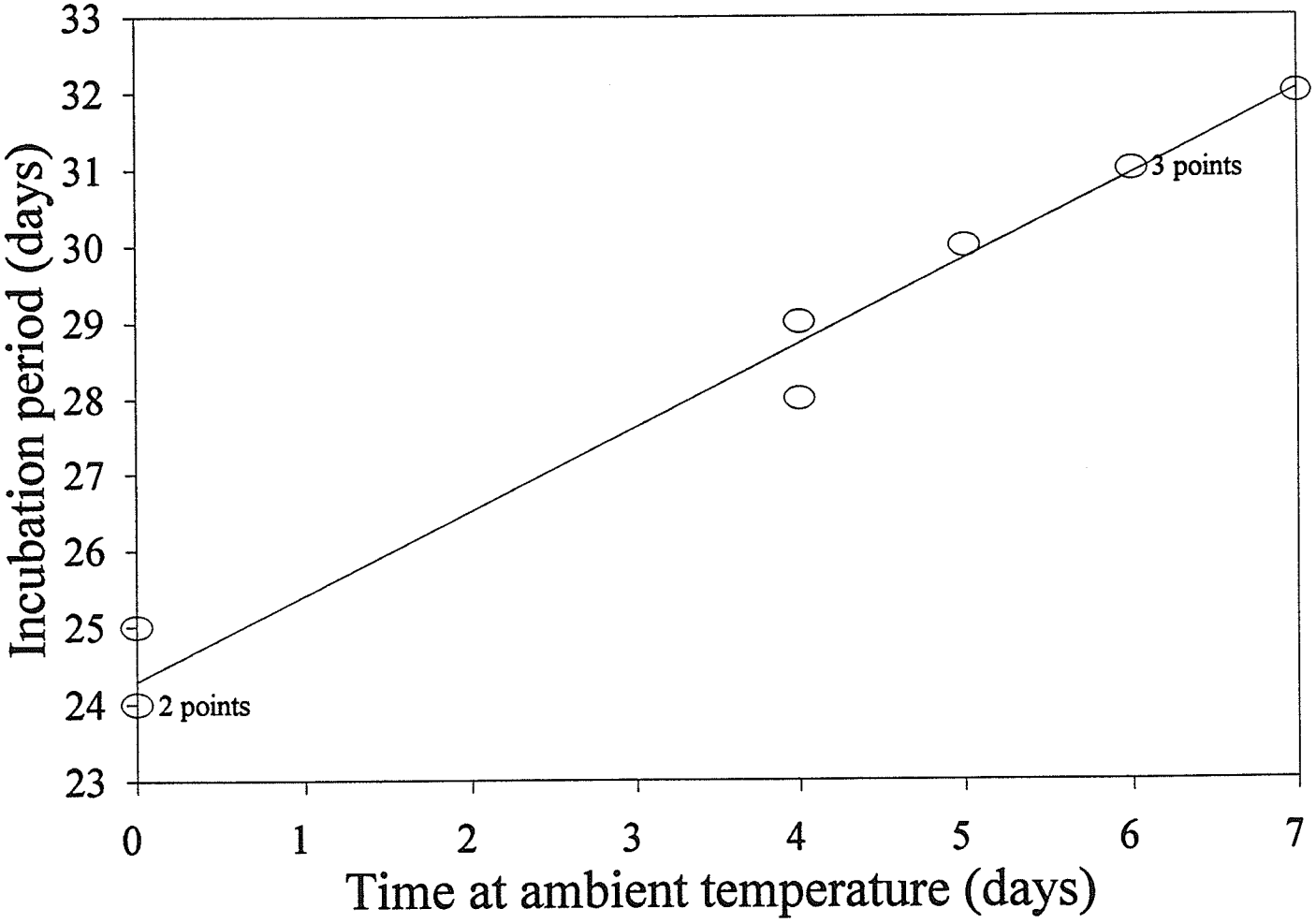

Incubation period (days)
33
32
31
30
29
28
27
26
25
24
23
3 points
2 points
0
1
2
3
4
5
6
7
Time at ambient temperature (days)

Figure 16: Average ambient temperature during the first two days after laying versus incubation period; a-eggs only. Regression equation: Incubation period = 27.86 + Average ambient temperature × -0.17.

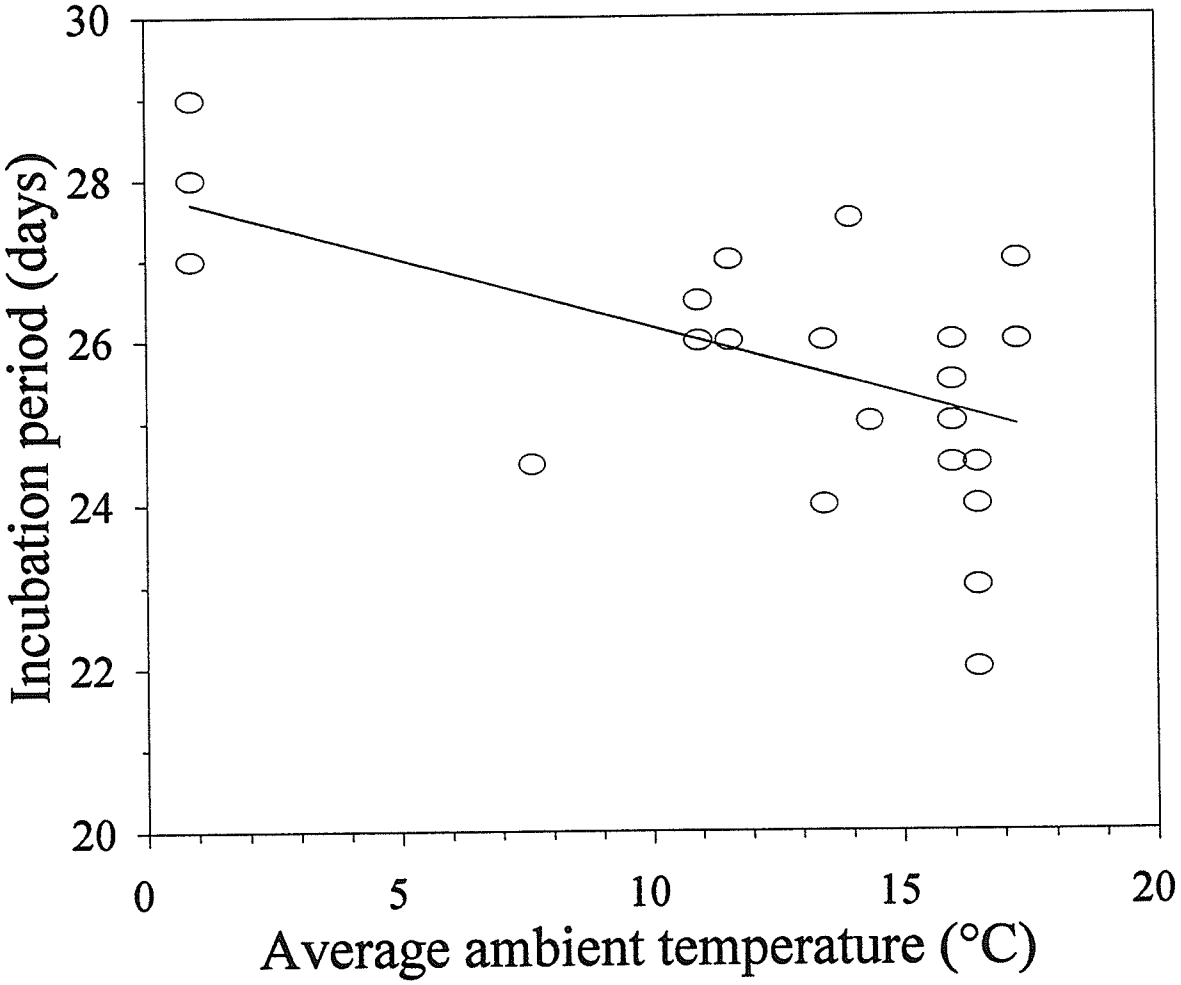

30
28
26
24
22
20
Incubation period (days)
0
5
10
15
20
Average ambient temperature (°C)

Figure 17: A comparison of normal maximum and minimum daily temperatures and the daily maximum and minimum in 1992 for the period from May 21 to May 23 at Grand Rapids, Manitoba (approximately 40 kilometers from the study site)(Environment Canada official weather record).

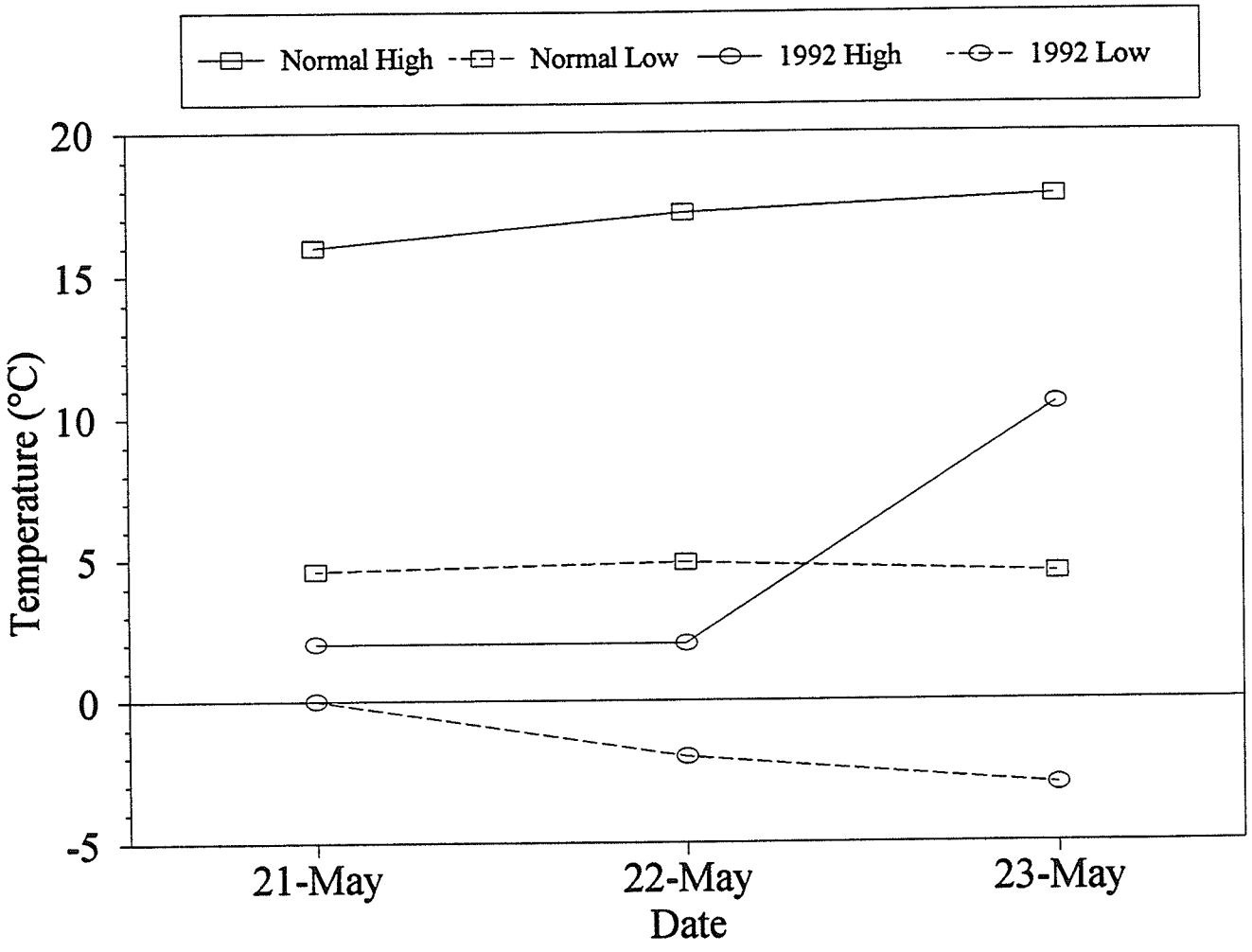
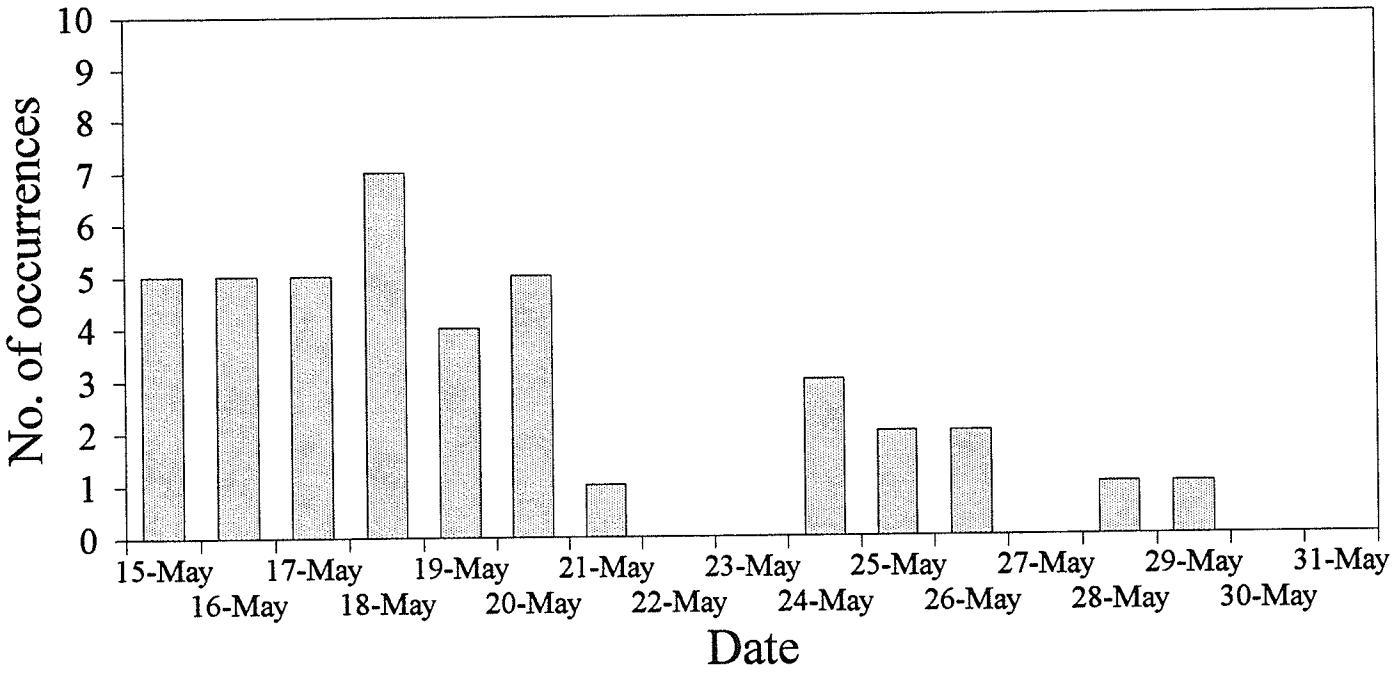

Normal High
Normal Low
1992 High
1992 Low
20
15
10
5
0
-5
Temperature (°C)
21-May
22-May
23-May
Date

Figure18: Number of occurrences of a minimum temperature less than 0°C for the last two weeks in May at Grand Rapids, Manitoba in the 25 years previous to 1992.


No. of occurrences
10
9
8
7
6
5
4
3
2
1
0
15-May
16-May
17-May
18-May
19-May
20-May
21-May
22-May
23-May
24-May
25-May
26-May
27-May
28-May
29-May
30-May
31-May
Date

DISCUSSION

The findings of this study support the hypothesis that parent ring-billed gulls can control hatching asynchrony within their brood. This conclusion was supported by lab evidence that early incubation temperature influences a-egg development rate, and by the subsequent finding that temperature measurements during the first four to five days after laying of the a-egg caused a predictable variation in a-egg incubation period and the resulting a- to c-egg hatch asynchrony. No single factor other than the warming influence of the parents had a significant correlation with hatch asynchrony.

INCUBATION AND EARLY EMBRYONIC DEVELOPMENT:

For incubation to be an effective mechanism to control hatching asynchrony, it is essential that variations in incubation of the a-egg prior to laying of the c-egg result in corresponding differences in the amount of early development of the a-embryo. This can, in theory, be achieved by the parent in at least three ways, by varying (1) the onset of effective incubation, (2) the constancy of incubation once started, and (3) incubation temperature when above the threshold needed for development. The variability in the data shown in Figure 7 suggests ring-billed gulls employ all three mechanisms.

Lab results showed that for development to proceed, eggs in this species must be above about 26°C. This value is the approximate 'physiological zero' (Webb 1987) for ring-billed gulls. This is also the approximate value for physiological zero in the domestic chicken (Drent 1975). In all nests studied here, this temperature was attained during the first two days, and within the first 12 h in 93% of all nests studied. Once started, incubation was consistently

maintained above 26°C in only one nest. A few showed brief periods from 20 minutes to a few hours where temperature was below 26°C. All others showed from one to three major periods of time (from 4 to 24 h) where temperature went below 26°C. Figure 7 shows 4 nests chosen to show the range in the length and number of drops in temperature. These drops in temperature to below 26°C stopped after the b-egg was laid in almost all nests. The significant correlation found between number of sample times when temperature was over 26°C and incubation period (Fig. 11) suggests this constituted an important source of variation in incubation period of the a-egg, hence, in hatching asynchrony. Nocturnal desertions of up to 10 h during laying were also reported in a more southerly colony (Morris and Chardine 1983), but egg temperatures were not reported. If nocturnal desertion occurred in my study, the longest period was likely no more than 2 hours, the longest time egg temperatures remained near ambient before beginning to rise again.

There was also a marked variation in incubation temperature during the time eggs were above the minimum needed for incubation (Fig. 7). In the lab, different incubation temperatures over this range had a significant effect on developmental rate during the first two days, suggesting that incubation temperature could also be a major source of variation in a-egg incubation period and hence asynchrony. The lab study showed that developmental rate is dependent on incubation temperatures (Fig. 6). At this early stage the eggs seem physiologically capable of developing normally at slower rates, depending on the incubation temperatures to which they are subjected.

In addition to controlling how often or for how long they incubate, parents could also control incubation effectiveness by maintaining partial contact or by staying just above the surface of the egg. The rate of brood patch formation could also play a role, as has been

reported in the Goshawk, *Accipiter gentilis* (Holstein 1943, quoted in Lack 1947). This seems unlikely to have been a large factor in my study since egg temperature often climbed well into the developmental range and then fell below again, sometimes several times in the first few days. Repeated variations of this magnitude could not be explained by feather loss or vascularization of brood patches.

PARENTAL CONTROL OF HATCHING ASYNCHRONY:

The variations in the data on early incubation (Fig. 7) also provided support for the hypothesis of parental control over hatching asynchrony. Given the relationship between developmental rates and temperature, parentally controlled embryo temperatures should mean that parents are similarly controlling the incubation periods and subsequent hatching asynchrony. Supporting this conclusion are the correlation studies showing that incubation periods were significantly correlated with incubation temperatures over the first two days, whether based on average temperatures (Figs. 8, 9) or the number of readings within the developmental range (Fig. 11). Direct support was provided by the finding that these correlations also applied to actual hatch asynchrony data (Figs. 10, 12).

By measuring observed incubation temperatures and using lab data to calculate the predicted development occurring in the first egg before the last egg was laid, one can, in theory, predict the early rate of development of the first egg, and therefore the a-c hatch asynchrony. In this study, these predictions were necessarily made after the eggs were hatched, but were calculated from temperature data independently of actual hatch asynchrony. For a-c hatch asynchrony these predictions were normally within three-quarters of a day (18 h) and strongly correlated with actual results. This lends further credence to the hypothesis

that parents are able to manipulate hatch asynchrony by controlling early incubation temperatures, incubation periods of individual a-eggs, and hence a-c hatch asynchrony.

The deviation between actual and predicted hatch asynchrony was surprisingly small, given that measuring methodologies by necessity included sizable sampling error. Data could only be collected once or twice a day (see below) and the estimates of asynchrony are derived from two linear regression models each with a calculated margin of error (for the regression used to calculate the embryo temperature: MSE=8.94, SD.=2.99; for the regression used to estimate development at different temperatures: MSE=14.04, SD.=3.75). Despite these sources of error, measuring incubation behavior by egg temperature appears to constitute a valid way to estimate parental incubation effectiveness. Other studies have commonly relied on behavioral observation to determine incubation attentiveness (Matthews 1954, Vermeer 1970, Chardine and Morris 1983, Mead and Morton 1985, Morris 1987, Magrath 1992), but attentiveness does not prove that warming is occurring (Lack 1947, Magrath 1990). Using actual incubation temperature removes this potential uncertainty about the effectiveness of early incubation.

It is important to note that my results also show that other variables that might have affected hatch asynchrony were of little or no consequence in ring-billed gulls. Like incubation temperature, some other variables tested were presumably also under parental control, either behaviorally or physiologically. These included laying intervals, egg size, laying sequence, and differential incubation temperature within the completed clutch. None of these variables, however, was found to have significant measurable effect on hatch intervals.

In contrast to the variables discussed above, variation in ambient temperature is not under parental control. Ambient temperature appeared to affect incubation periods only when

temperatures were extreme, as in 1992 when temperatures went below 0°C. In that year most early nests failed, with only 5 of 29 nests studied yielding any hatched eggs and then only partial clutches. Ring-billed gulls in general rarely experience these low temperatures. The study site at Kaweenakumik Lake was at the northern extreme of their range (Ryder 1993), and temperatures below 0°C were rare even for that region and time (Figure 17, 18). There was also snow during the initial laying period in 1992, which may have inhibited breeding (*see* Morris and Chardine 1985). The rarity of snow and freezing ambient temperature during laying suggests that the effect of ambient temperature shown in Figure 16 may not be relevant to the species in most years and locations.

Ambient temperatures to which eggs were exposed during the laying period had little if any impact on the embryos other than to maintain them in viable condition without any advancement in development (Fig. 15). The regression of days left at ambient temperature in relation to the incubation period showed a one-to-one correspondence, with a slope not significantly different from one, indicating that these eggs tended to hatch out about a day later for every day they were left at ambient temperature. There was thus no indication that parents could simply expose a-eggs to ambient conditions to begin their development, independent of parentally applied warmth.

The outliers in Figure 13 represent an unexplained variation in the results. The predictions still correlated with actual asynchrony when these points were included in the analysis, but their presence increased variance about the regression line. One possible explanation for the occurrence of incubation variance arises from my decision to minimize egg predation by making rounds to collect data only once a day during the laying period (Fetterolf 1979). As a result estimates of laying time could be up to 24 hours different from actual laying

time. It was possible, for example, that an egg was laid minutes after I left the colony and not marked as freshly laid until the next day, nearly 24 hours later. It is also possible that an egg may have been accidentally rolled out and been neglected at some point in their incubation, though there was no direct evidence of this.

ADAPTIVE SIGNIFICANCE OF HATCHING ASYNCHRONY:

The evidence in this study suggesting that parents can control the hatching asynchrony of their brood is consistent with, but does not prove the possibility that they are facultatively adjusting asynchrony according to environmental conditions or to any other aspect of their life history strategy . It does, however, support the assumption underlying most adaptive theories explaining hatching asynchrony (Magrath 1990), that parents can exert control over hatch asynchrony. The most widely accepted adaptive explanation for hatch asynchrony is that it facilitates brood reduction (Lack 1954, 1968), enabling parents to adjust brood size to currently available resources (Pijanowski 1992). The underlying assumption that the magnitude of adaptive asynchrony is under control of the parent is supported by all results in this report which indicate that parental incubation intensely alters the asynchrony of their brood. The variation in early incubation temperature also suggests parents would be able to facultatively fine-tune hatch asynchrony in response to current conditions. If resources are low, parents could, for example, incubate earlier and thereby cause more asynchrony to facilitate adaptive brood reduction. Conversely, when food is plentiful, parents could delay incubation and cause less asynchrony to minimize the competitive disadvantage of the last hatched chick. The relevance of this possibility was demonstrated by Bortolotti and Weibe

(1993), who found that American Kestrels (*Falco sparverius*), if supplemented with food, produced broods with reduced hatching asynchrony.

The results from this study are also consistent with some other adaptive hypotheses of hatch asynchrony. This includes Clark and Wilson's (1981) assertion that asynchrony has resulted from an interplay of selection pressures arising from predation on eggs and young. Results are also consistent with the peak load reduction hypothesis (Hussell 1972) which suggests that asynchrony has evolved to reduce the demands on the parent at the time when all chicks require the greatest amount of food from their parents. If they hatched and grew synchronously they would all reach this stage at approximately the same time, which could place an unnecessary burden on the parents. My results are also relevant to the sibling rivalry reduction hypothesis (Hahn 1981), which states that asynchrony reduces the level of energetically costly aggression between siblings. Size differences among nestlings that arise from asynchrony are thought to facilitate a dominance hierarchy. The results from this study are less supportive of the hurry-up hypothesis (Hussell 1972, Slagsvold 1986), which states that parents are selected to get as many eggs to hatch and the young to fledge as soon as possible to reduce their vulnerability to predation. The partial incubation in ring-billed gulls at the start of laying means that there is some delay in hatching of at least the a-egg. An earlier onset of incubation and greater resultant asynchrony is predicted by the hurry-up hypothesis. This has been found, for example, in some pelicaniformes birds that commence incubation immediately after laying the first egg (Anderson 1989, Evans and Knopf 1993)

It has also been suggested (Clark and Wilson 1981) that asynchrony of up to 24 h could arise from random variations in incubation periods within the clutch. Although random variation in incubation period undoubtedly occurred in ring-billed gulls, it was not sufficient to

mask the highly significant correlations found here between early incubation temperature and incubation period of individual eggs.

Mead and Morton (1985) put forth a hypothesis that suggests that asynchrony is a non-adaptive epiphenomenon, tied to adaptive variation in hormone levels. They suggested that if the same hormone, probably prolactin, that causes an adaptive cessation of ovulation, also brings about the onset of incubation then one would expect that birds would initiate incubation after the penultimate egg is laid. Further, they suggested that birds with variable clutch sizes might show more variation in the timing of the onset of incubation. My study supports neither of these predictions from this hypothesis. Most birds began substantial amounts of effective incubation within the first 12 h of temperature readings, up to 2 days before the penultimate egg was laid. Also, there was significant variation in the onset of incubation, even within modal three-egg clutches (Fig. 7). Ring-billed gulls, in general, show little variation in clutch size (Vermeer 1970, Ryder 1993). The variability in the onset of incubation in this study thus does not support Mead and Morton's hypothesis of a close, non-adaptive link between the laying of the penultimate egg and the onset of incubation.

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APPENDIX 1

Various regression models of hours of development versus incubation temperature were examined. One model that was tested was a natural log transformation of a second order polynomial regression of hours of developmental on incubation temperature. This gave a curvilinear relationship in the shape of a typical growth curve, as would be expected when graphing development data. Growth data typically show an exponential curve, slow at the start and then increasing dramatically. Also there is always a point where development begins to level off, which in this case might correspond to the attainment or exceeding of optimum temperatures. The second order log transformation shows these attributes. However, this model was rejected in the end because the residuals showed significant positive autocorrelation (D=.64, P=.0461) rather than normality as required for a valid model (Neter *et al* 1990). It may be that a model of this complexity requires more data than available. The final model arrived at was a simpler second-order polynomial, done without transforming the data. This model, though, had to be adjusted. Examination of the data points near the upper temperature range in Fig. 3 indicates the presence of a constraint not taken into account in the model. This constraint arises above the optimum incubation temperature where the model showed development above 48 hours.

APPENDIX 2

The model that was used to correct temperature readings for probe position, estimates embryo position temperature with a regression that incorporates more of the variables that were measured rather than just the embryo and blunt end temperatures used to plot Figure 5. The model used in the final analysis considers blunt end temperature readings as a dependent variable, as this more closely reflects biological reality. Although corrections for probe location were necessarily done by determining embryo position temperature from blunt end temperature, considered biologically, embryo temperature of an actively incubated egg does not depend primarily on the blunt end temperature. Since the embryo location is directly under the parent's warm brood patch, heat transfer during incubation would normally start from the region of the embryo and emanate out to all less well covered parts of the egg, including the blunt end, thus resulting in the embryo position more nearly acting as an independent variable than blunt end temperature. Also, if parents were incubating and the blunt end were less covered, ambient temperature would act more directly on blunt end temperature, again suggesting that blunt end temperature acts more as a dependent variable, in this instance responding to ambient. This effect would be lessened during periods when the entire egg, including the upper surface, was fully exposed. The final model selected used embryo position temperature, ambient temperature, time of day, and the number of eggs in the nest, as independent variables. All these variables contributed significantly to the variability of the blunt end temperature taken as the dependent variable (Forward stepwise regression, adjusted R2=.85, F=1778.03, P=0.0000). A graphical representation of the final model would require four dimensional axes. Therefore Figure 5 is used to illustrate a more easily represented

relationship between the two main variables, embryo position temperature and blunt end temperature.

To estimate embryo temperatures in experimental nests using the above regression model required data from each nest for blunt end temperature, simultaneous ambient temperatures, the time of day (coded), and the number of eggs in the nest when recordings were taken. The method for coding the time of day, in both the dummy egg experiment and in the field data, was to convert the logged time data to binary or categorical variable, with '0' representing time data from primarily daylight hours between 8:00 am and 8:00 p.m., and a '1' representing readings taken between the hours of 8:00 p.m. and 8:00 am.

As indicated above, estimation of embryo temperatures from nest measurements was done by non-linear interpolation of the independent variable (X) from the dependent variable (Y), rather than estimating Y from X as is usually the case. This is appropriate in situations where it is impossible to measure directly the actual independent variable, (Neter *et al* 1990, p. 173) in this case the embryo temperature.