The Condor 99:224–228
© The Cooper Ornithological Society 1997

VARIATION IN INCUBATION PERIODS AND EGG METABOLISM IN MALLARDS: INTRINSIC MECHANISMS TO PROMOTE HATCH SYNCHRONY[1]

MARGARET C. MACCLUSKIE
Department of Biology and Wildlife, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775, e-mail: ftmcm@aurora.alaska.edu

PAUL L. FLINT
National Biological Service, Alaska Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503

JAMES S. SEDINGER
Institute of Arctic Biology, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775

Abstract: We investigated factors affecting incubation time and metabolic rates of Mallard (*Anas platyrhynchos*) eggs incubated under constant environmental conditions. Time required to reach the star-pipped stage of hatch varied significantly among females, but not with laying sequence or egg size. Metabolic rate of eggs varied positively with position in the laying sequence and tended to vary among females. Metabolic rate did not vary with egg volume or incubation length. Our results indicate metabolic rate may act as one synchronization mechanism for hatch. The role of maternal effects in development time should be considered in subsequent studies of incubation time in ducks.

Key words: *Mallard,* Anas platyrhynchos, *metabolism, egg, incubation, incubation length.*

INTRODUCTION

Waterfowl typically begin incubation before the clutch is complete (Caldwell and Cornwell 1975, Cooper 1978, Afton and Paulus 1992), which results in developmental asynchrony of eggs within clutches at the end of laying (Caldwell and Cornwell 1975, Afton 1979, Kennamer et al. 1990). However, asynchrony at hatching is less than that observed at the end of laying (Caldwell and Cornwell 1975, Afton 1979, Cargill and Cooke 1981), suggesting that some mechanisms exist to synchronize hatch (Afton 1979, Davies and Cooke 1983). Such mechanisms could include developmental retardation of eggs laid early in the laying sequence or developmental acceleration of eggs laid after the onset of incubation (Davies and Cooke 1983). Regardless of how such mechanisms work, eggs within clutches must have variable incubation lengths.

Synchronization mechanisms could be either extrinsic to the egg or intrinsic to the egg. Extrinsic mechanisms could include behavior of the parent. For example, eggs in the center of the nest have higher temperatures (Caldwell and Cornwell 1975), thus females may alter development time of specific eggs by regulating egg position in the nest. Also, vocal stimulation decreases time to hatching (Vince 1966, Orcutt and Orcutt 1976) and may accelerate development of later laid eggs (Davies and Cooke 1983). Intrinsic factors include inherent properties of the egg, such as egg composition, egg size, or physiology. Smaller eggs require less incubation, both among and within species (Worth 1940, Rahn and Ar 1974, Arnold 1993). Martin and Arnold (1991) found

effects of egg size on incubation length were relatively weak when studied within species, however their analysis did not control for among female factors other than egg size. Egg composition also varies among (Alisauskas 1986, Hepp et al. 1987) and within clutches (Alisauskas 1986), which could influence development rate. Alternatively, inherent differences in metabolic rate might result in differences in development time. Several studies have demonstrated greater variation in lipid reserves among newly hatched chicks as compared to freshly laid eggs, suggesting differential lipid reserve use among eggs (Alisauskas 1986, Hepp et al. 1987, Slattery and Alisauskas 1995).

The combination of these studies led us to hypothesize that relationships may exist among metabolic rate, egg size, and the time required for embryo development. Our goal in this study was to examine the potential for among female variation in intrinsic factors to influence egg development in a controlled incubation environment (i.e., incubator), thereby eliminating female behavior as an extrinsic factor. Factors we considered were maternal contribution to eggs, egg size, egg metabolic rates, and egg sequence in the clutch.

METHODS

We conducted this study at the Delta Waterfowl and Wetlands Research Station, Portage la Prairie, Manitoba, Canada from April–July, 1994. Thirty, 1-year-old, captive, wild stock Mallards (*Anas platyrhyncos*) were used for this study. We placed each pair in 2 × 4 m breeding pens on 30 April. All pairs were fed Delta Waterfowl Turkey Starter ad libitum and had free access to water for bathing and swimming. A covered, wooden box filled with straw was placed in each pen for a nesting structure. Appropriate animal use and care permits were obtained from the Canadian Wildlife Service (Permit # WS-M25) and the University of Alaska, Fairbanks (IACUC Protocol # 94-019) for all portions of this study.

Beginning 1 May, nest boxes were checked twice daily for eggs. Nest checks were conducted between 09:00 and 11:00 and again between 18:00 and 20:00. Fresh eggs were removed from the nest and replaced with a plastic egg of similar size, weight and color. Our goal was to remove eggs as soon as possible so the eggs would be minimally exposed to female behavior. Eggs were individually numbered on the blunt end with an indelible marker and placed in a commercial grade incubator (Humidaire 550) that maintained a constant temperature of 37.5°C and relative humidity of 84–87%. Eggs were assigned random locations in the incubator to randomize microclimate effects. Eggs were candled every 7 days to determine fertility (Weller 1956). Infertile eggs were removed from the incubator, and fertile eggs were replaced in their location.

DEVELOPMENT TIME

Eggs that were judged to be within 12 hours of initiating hatch, based on candling (Weller 1956), were removed from the incubator and placed in a hatching machine (Humidaire Hatcher 50) that maintained a relative humidity of 87–89% and a temperature of 37.2°C. We assigned eggs to random locations in the hatching machine to randomize microclimate effects. Eggs in the hatching machine were examined every 8 hours to determine timing of hatch, which we defined as occurring when shells were star-pipped. A star-pipped egg was one for which the shell had cracked enough to create a small diameter circle (approximately 3 mm). We used the star-pipped stage to define hatch because variable humidity in the hatching machine may have artificially increased shell exodus time for some ducklings. The star-pipped stage of hatch is the first externally observable stage of hatch, and was the most unaffected by humidity fluctuations in this study.

METABOLIC MEASUREMENTS

We randomly selected one egg from early, middle and late in the laying sequence of each clutch for metabolic measurement. Egg position in the laying sequence was determined using all fertile and infertile eggs in a clutch. We measured metabolic rate of these eggs two or three times during the incubation period. Metabolic rates were measured using a closed-system respirometry apparatus consisting of two, 240-ml glass jars, each fitted with an airtight injection port and connected to each other by a manometer filled with colored water (Scholander 1950). One jar served as the chamber to hold the egg, the bottom of which was covered with Ascarite to absorb CO_2 produced by the embryo, while the other jar served as a pressure buffer and completed the closed system. Eggs were placed in the respirometry chamber on a piece of wire mesh 1.5 cm over the Ascarite. Both jars were then placed with the upper 2 cm exposed in a circulating water bath (40°C) which maintained a chamber temperature of 37°C. Lids were placed loosely on the jars and we allowed eggs to equilibrate in the apparatus for 10 minutes before beginning a trial. The manometer was not submerged at any time and rested outside the water bath so that water level in the manometer could be monitored. All steps preceding submersion of the chambers were completed as quickly as possible to minimize temperature fluctuations experienced by the egg. After equilibration, the pre-injection level of the manometer was recorded, lids were secured on both chambers and 1 ml of pure, ambient temperature and pressure O_2 was injected into the chamber using a 3 ml gas-tight syringe. The plunger of the syringe was pumped three times after O_2 injection to ensure an even mixture of gas in the chamber. After the third time the plunger was pumped, a stopwatch was started and time required for the manometer level to return to the equilibration point was recorded. Three trials were conducted for each metabolic measurement. Barometric pressure and chamber temperature were recorded at the beginning of each trial and true volume of oxygen (ml) consumed by the embryo was corrected to standard temperature and pressure (STP). Measurements of oxygen consumption were converted to a rate of oxygen consumption, defined as ml of oxygen consumed/hour. Length (L) and width (W) of all eggs were measured to the nearest 0.01mm using vernier calipers and egg volume was

CALDWELL, P. J., AND G. W. CORNWELL. 1975. Incubation behavior and temperatures of the Mallard duck. Auk 92:706–731.

CAREY, C. 1983. Structure and function of avian eggs. Current Ornithol. 1:69–102.

CARGILL, S. M., AND F. COOKE. 1981. Correlation of laying and hatching sequences in clutches of the Lesser Snow Goose (*Anser caerulescens caerulescens*). Can. J. Zool. 59:1201–1204.

COOPER, J. A. 1978. The history and breeding biology of the Canada Goose of Marshy Point, Manitoba. Wildl. Monogr. 61.

DAVIES, J. C., AND F. COOKE. 1983. Intra-clutch hatch synchronization in the Lesser Snow Goose. Can. J. Zool. 61:1398–1401.

FLINT, P. L., LINDBERG, M. S., M. C. MACCLUSKIE, AND J. S. SEDINGER. 1994. The adaptive significance of hatching synchrony of waterfowl eggs. Wildfowl 45:248–254.

FLINT, P. L., AND J. S. SEDINGER. 1992. Reproductive implications of egg size variation in Black Brant. Auk 109:896–903.

HEPP, G. R., D. J. STANGOHR, L. A. BAKER, AND R. A. KENNAMER. 1987. Factors affecting variation in the egg and duckling components of Wood Ducks. Auk 104:435–443.

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

HOYT, D. F., BOARD, R. G., RAHN, H. AND C. V. PAGANELLI. 1979. The eggs of the Anatidae: conductance, pore structure, and metabolism. Physiol. Zool. 52:438–50.

KENNAMER, R. A., W. F. HARVEY, AND G. R. HEPP. 1990. Embryonic development and nest attentiveness of Wood Ducks during laying. Condor 92:587–592.

MARTIN, P. A., AND T. W. ARNOLD. 1991. Relationships among fresh mass, incubation time, and water loss in Japanese Quail eggs. Condor 93:28–37.

ORCUTT, F. S., JR., AND A. B. ORCUTT. 1976. Nesting and parental behavior in domestic Common Quail. Auk 93:135–141.

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

RHYMER, J. M. 1988. The effect of egg size variability on thermoregulation of Mallard (*Anas platyrhynchos*) offspring and its implications for survival. Oecologia 75:20–24.

RICKLEFS, R. E. 1984. Variation in the size and composition of eggs of the European Starling. Condor 86:1–6.

SAS INSTITUTE, INC. 1990. SAS user's guide: statistics. Ver. 5. SAS Institute, Inc. Cary, NC.

SCHOLANDER, P. F. 1950. Volumetric plastic micro respirometer. Rev. Sci. Instruments 13:32–33.

SLATTERY, S. M., AND R. T. ALISAUSKAS. 1995. Egg characteristics and body reserves of the neonate Ross' and Lesser Snow Goose. Condor 97:970–984.

STUNDEN, C. E. 1996. Studies on reproductive characteristics and artificial insemination in captive Mallard ducks (*Anas platyrhyncos*). M.Sc. thesis, Univ. British Columbia, Victoria, BC, Canada.

VINCE, M. A. 1966. Artificial acceleration of hatching in quail embryos. Anim. Behav. 14:389–394.

VLECK, C. M., AND D. VLECK. 1980. Patterns of metabolism and growth in avian embryos. Am. Zool. 20:405–416.

WELLER, M. W. 1956. A simple field candler for waterfowl eggs. J. Wildl. Manage. 20:111–113.

WORTH, B. C. 1940. Egg volumes and incubation periods. Auk 57:44–60.

TABLE 1. General linear models describing variation in egg development time and metabolic rate for Mallard eggs.

	df	F	P
Egg development time (n = 49)			
Female	9	2.26	0.03
Laying sequence	2	0.12	0.88
Egg volume	1	1.24	0.27
Egg metabolic rate (n = 45)			
Female	12	1.98	0.07
Laying sequence	2	4.63	0.02
Egg volume	1	0.24	0.63
Day of incubation	1	18.95	<0.001

calculated as $0.515LW^2$ following the methods of Hoyt (1979).

STATISTICAL ANALYSES

We used analysis of covariance (ANCOVA) to examine variation in incubation length. We used egg development time (from laying to star pipped) in days as the dependent variable, female and laying sequence (early, middle or late) as class variables, and egg volume as a covariate in the analysis.

We also used ANCOVA to examine variation in metabolic rates of eggs. We used metabolic rate/hour as the dependent variable, female and laying sequence in the clutch as class variables, and egg volume and day of incubation as covariates. Day of incubation was included because metabolic rate varies with stage of incubation in several species of *Anas* (Hoyt et al. 1979, Vleck and Vleck 1980).

We tested for a direct relationship between development time and metabolic rate using an ANCOVA model that included egg size, metabolic rate, day of incubation, and laying sequence in the clutch. For this analysis, we used days to star-pipped stage of hatching as the response variable and the last metabolic measurements taken from each egg as the independent variable. Day of incubation was a covariate and laying sequence was a class variable in this test. Only eggs that were observed in the star-pipped stage of hatch and had a metabolic rate measurement were used for this analysis.

To determine if metabolic rates of eggs varied with egg volume, we regressed the $\log_{10}$ metabolic rate from the last measurements taken on each egg against $\log_{10}$ egg volume (Ricklefs 1984) while controlling for laying sequence and female effects. We specifically were interested in testing the null hypothesis that the slope of this line would equal 1, indicating isometry. A slope different from 1 would indicate a proportional increase or decrease in size-specific metabolic rates.

All statistical analyses were conducted using the General Linear Models (GLM) Procedure of the SAS statistical package (SAS Institute 1990). Type III sums of squares were used to evaluate the contribution of individual variables to models for all analyses. A significance level of $P < 0.05$ was used for all tests.

RESULTS

Only 17 of the 30 pairs of Mallards used for the study produced fertile clutches (defined as clutches with at least two viable eggs). Cause of infertility in these 1-year-old birds was unknown, but we suspect inexperience of males and possibly low sperm count (Stunden 1996).

We measured time from laying to star pipped for 49 eggs from 10 females. Average development time from laying to star pipped was 22.5 days (range 20.9–24.4) among females. Egg incubation period varied significantly among females, but not with position in the laying sequence. Likewise incubation period was not significantly related to egg volume, and the correlation coefficient was negative (slope = −0.02) (Table 1).

We measured metabolic rates on 45 eggs from 13 females. Egg metabolic rate varied with rank in the laying sequence, tended to vary among females, but did not vary significantly with volume (Table 1). Average egg metabolic rates (±SE) for the entire incubation period after controlling for the covariates female, volume and laying sequence were 9.46 ± 1.08, 11.09 ± 1.07, and 13.24 ± 1.07 ml · oxygen · hr^{-1} (STP) for eggs from early, middle, and late in the laying sequence, respectively (Fig. 1). Day of incubation (Fig. 1) explained a significant amount of variation in metabolic rate (Table 1). Log egg-metabolic rate did not increase significantly with log egg-volume (slope = 0.578, 95% CI = −1.33–2.48, r = 0.31, P = 0.54).

We detected no relationship between time to reach star pipped stage of hatch and metabolic rate ($F_{1,14}$ = 0.79, P = 0.38), egg volume ($F_{1,14}$ = 1.10, P = 0.31), day of incubation ($F_{1,14}$ = 0.40, P = 0.53), or position in the clutch ($F_{2,14}$ = 0.20, P = 0.81); however, our sample size was only 20 eggs for this analysis.

DISCUSSION

The goal of our study was to determine if, after removing female behavioral effects, there was an intrinsic mechanism influencing egg incubation period. We found no relationship between intrinsic variables we measured and incubation length within clutches. However, our data suggest that variation in egg metabolic rate is one mechanism by which hatch synchrony occurs because late sequence eggs tended to have higher metabolic rates. In a natural setting intrinsic factors may be important to, and work in concert with, extrinsic factors to promote hatch synchrony, but was not observed in this study because of the experimental design.

We also hypothesized that within females, intraclutch variation in egg size may be an adaptation to synchronize hatch (Flint and Sedinger 1992, Arnold 1993, Flint et al. 1994) and specifically that late laid eggs would be smaller and require less incubation time. Our data do not support this hypothesis however, because egg size explained an insignificant proportion of variation in egg development time and

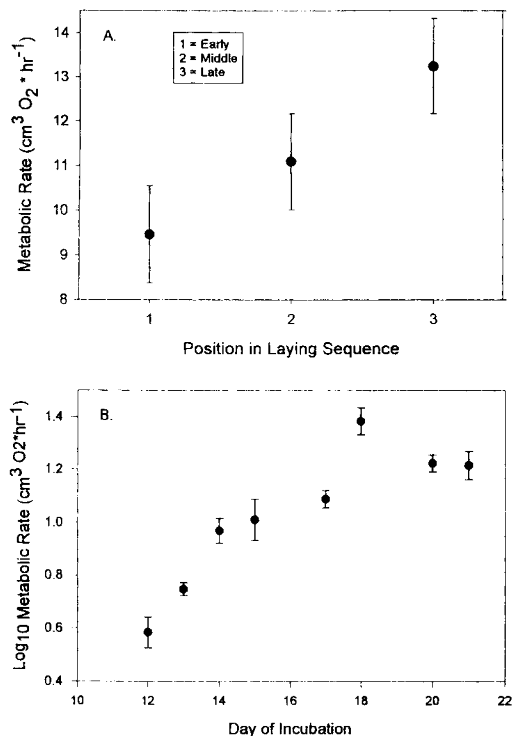

FIGURE 1. A. Mean metabolic rate of eggs early, middle and late in the laying sequence. Points for each position (n = 45) are mean rates (±1 SE) of all eggs within each category. B. Mean change in metabolic rate of Mallard eggs during incubation. Points for each day of incubation (n = 42) are mean rates (±1 SE) of all eggs from all females.

metabolic rate. Small sample size reduced our power to detect a relationship between egg size and development time.

Variation in egg metabolism may represent a tradeoff between lipid used during development and amount of lipid reserves remaining at hatch. For example, high egg metabolism would result in faster embryo development and slower egg cooling rate, but fewer lipid reserves would remain at hatch. This could be important because small lipid reserves at hatch may influence early juvenile survival (Ankney 1980). Alisauskas (1986) found that rate of lipid metabolism varied among eggs for American Coots (*Fulica americana*) and Hepp et al. (1987) suggested a similar result for Wood Ducks (*Aix sponsa*). Additionally, Slattery and Alisauskas (1995) showed that metabolism of lipid stores did not increase isometrically with egg size (but see Rhymer 1988). Our finding that egg metabolism did not increase isometrically with egg size is consistent with their results. We believe that lipid use by embryos during development is likely correlated with egg metabolism.

Egg metabolic rate was not directly linked to incubation length in our data set. It may be that late sequence eggs, while having higher metabolic rates, would hatch at a relatively less mature condition. Other studies have shown variation among hatchlings in tissue water content (Ricklefs 1984, Alisauskas 1986) and related that to tissue maturity. We did not account for this variation in our study. Therefore, we recommend controlling for tissue water content of hatchlings in subsequent studies.

We observed significant variation in incubation period among females. Similarly, Arnold (pers. comm. reanalysis of Arnold et al. 1993) also found significant variation among females in length of incubation of wild Blue-winged Teal (*Anas discors*) but detected no among-female variation in incubation length in Mallards and Northern Shovelers (*Anas clypeata*). The risk of nest predation should favor shorter incubation period for females (Arnold et al. 1987). However, there may be costs associated with shorter incubation length if eggs hatch with reduced lipid reserves as a result (see above).

To our knowledge, this study is the first that has attempted to control for maternal behavioral and genetic effects on egg development time and metabolic rate in wild-strain ducks. We detected significant variation in length of the incubation period and metabolic rate of eggs among females. Our data suggest higher metabolic rates in later laid eggs may aid in synchronization of hatch in ducks. The mechanism by which metabolic rates vary with position in the clutch is unknown (Carey 1983). Overall, our findings indicate that the maternal component of eggs should be considered when examining questions involving incubation aspects of life history of the Anatidae.

Funding for this project was provided by Delta Waterfowl and Wetlands Research Station. We thank Julie Campbell, Lea Craig, Dean Jeske, and Kim Wright for assistance collecting data. We also thank the staff of Delta Waterfowl and Wetlands Research Station for logistical support. Todd Arnold, Frank Rohwer and Brian Barnes provided valuable reviews that greatly improved this manuscript.

LITERATURE CITED

AFTON, A. D. 1979. Incubation temperatures of the Northern Shoveler. Can. J. Zool. 57:1052–1056.

AFTON, A. D., AND S. L. PAULUS. 1992. Incubation and brood care, p. 62–108. *In* B. Batt, A. Afton, M. Anderson, C. D. Ankney, D. Johnson, J. Kadlec, and G. Krapu [eds.], Ecology and management of breeding waterfowl. Univ. Minnesota Press, Minneapolis, MN.

ALISAUSKAS, R. T. 1986. Variation in the composition of the eggs and chicks of American Coots. Condor 88:84–90.

ANKNEY, C. D. 1980. Egg weight, survival, and growth of Lesser Snow Goose goslings. J. Wildl. Manage. 44:174–182.

ARNOLD, T. W., F. C. ROHWER, AND T. ARMSTRONG. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. Am. Nat. 130:643–653.

ARNOLD, T. W. 1993. Factors affecting egg viability and incubation time in prairie ducks. Can. J. Zool. 71:1146–1152.