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A Comparative Analysis of Embryonic Growth and Incubation Length in Dabbling Ducks

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Alicia May Wells

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Harold C. Prince
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A COMPARATIVE ANALYSIS OF EMBRYONIC GROWTH AND INCUBATION LENGTH IN DABBLING DUCKS

By

Alicia May Wells

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ABSTRACT

COMPARATIVE ANALYSIS OF EMBRYONIC GROWTH AND INCUBATION LENGTH IN DABBLING DUCKS

By

Alicia May Wells

Embryonic growth and incubation length of Blue-winged Teal, Northern Shoveler, Mallard, and Gadwall were examined. Nesting chronologies as compiled from daily nest searches on Minnedosa, Manitoba study area for Northern Shovelers, Mallards, and Gadwalls are similar to previous studies while Blue-winged Teal initiated nesting earlier. Unincubated eggs were collected throughout the breeding season and incubated at 37.5°C and 70% relative humidity until pipped, and 85% relative humidity until hatch. There was a positive linear increase in oxygen consumption (ml/hr) as embryonic wet weight increased for all four species. Embryonic wet weight can be estimated based on embryonic oxygen consumption. For estimated wet weights based on oxygen consumption, Gadwall and Mallard embryos grew faster than Northern Shoveler embryos and Blue-winged Teal grew the slowest. Mallards had the longest incubation length followed by Gadwalls and Blue-winged Teal respectively, with Northern Shovelers having the shortest incubation length. Incubation lengths of Mallard and Blue-winged Teal eggs that were artificially incubated were significantly shorter as the breeding season progressed.

ACKNOWLEDGEMENTS

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Introduction

Incubation is defined as the process by which heat is applied to an egg causing embryonic development to occur (Beer 1964, Tullet 1985). Avian incubation evolved in relation to 1) physical requirements of the embryos for development, 2) metabolic requirements of the parents, and 3) predation on the eggs and the parents (Afton and Paulus 1992). Incubation strategies should balance requirements of the embryo with those of the parent. Requirements of the embryos must be met through incubation behavior that compensates for fluctuating environmental conditions, allows the parents to maintain and acquire sufficient energy to support body metabolism, and minimizes the probability of predation on the eggs and the parents (Afton and Paulus 1992).

Embryonic Growth

Embryonic development has been studied on a variety of species such as the domestic chicken (*Gallus domesticus*; Okuda and Tazawa 1988), Japanese Quail (*Coturnix coturnix japonica*; Spiers and Baummer 1990), and Mallard and Pekin ducks (*Anas platyrhynchos*; Prince et al. 1968). Prince et al. (1968) found that embryonic weight increased exponentially with a high degree of correlation between wet and dry weight. Present methods used to measure embryonic growth requires opening the egg and terminating development thus necessitating large numbers of eggs for samples of individual embryos representing different embryonic stages. The relationship between metabolic rate and stage of incubation has been studied on the embryos of the chicken (Romijn and Roos 1938, Romijn and Lokhorst 1951, Barott 1937, Wangensteen and Rahn 1970), domestic duck (Khaskin 1961), House Wren (*Troglodytes aedon*; Kendeigh 1940), the Herring Gull (*Larus argentatus*; Drent 1970), and the Ostrich (*Struthio*

camelus; Hoyt et al. 1978). Oxygen consumption of the domestic duck embryos increases exponentially during the first 80% of the incubation period and remains relatively constant during the remaining "plateau" phase (Rahn et al. 1974). In addition, embryo mass approaches hatchling mass as early as 80% of the way through incubation (Vleck et al. 1980). Inherent differences in oxygen consumption have been positively correlated with development time thus creating the potential to estimate the size of the embryo based on oxygen consumption (MacCluskie et al. 1997). The relationship between oxygen consumption and embryonic wet weight needs to be quantified to document a more precise representation of growth.

A potential to relate avian embryonic growth and oxygen consumption exists. This non-invasive measure of estimation of embryonic size follows individual embryos through hatching and results in a significant reduction of sample sizes of eggs for studies of embryonic growth. This technique could provide an opportunity to use a wider variety of avian species and be applied to a comparative study of incubation length in dabbling ducks.

Incubation Length

Incubation length for waterfowl species is defined as the time from when the last egg is laid until it hatches (Drent 1970). Feldheim (1997) documented incubation lengths under field conditions for nesting Blue-winged Teal (*Anas discors*), Mallard (*Anas platyrhynchos*), Northern Shoveler (*Anas clypeata*), Gadwall (*Anas strepera*), and Northern Pintail (*Anas acuta*) females. Field based incubation lengths such as Feldheim's (1997) as well as those reported by Bellrose (1980) and Baicich and Harrison (1997) are subject to variability caused by the changing environment and the incubation

rhythms of the hens. When dealing with incubation lengths the ambient and incubation temperature could greatly affect the results. Lack (1968) suggested that incubation period in waterfowl has evolved as a function of temperature. Prince et al. (1969) showed that embryonic growth in Mallards increased with temperature and resulted in a predictable decrease in incubation length. To accurately document incubation lengths that eliminates natural variation caused by hens incubating at different temperatures, unincubated eggs should be collected from laying females and incubated in a constant environment.

Egg Collection

The two most common nest searching techniques were flushing hens with cable-chain or chain drags pulled by all-terrain vehicles. The chain-cable drag (Higgins et al. 1969) was developed to locate ducks nesting in grassland habitats. Most investigators believe the cable-chain drag is more efficient in dense, herbaceous cover, but some prefer the chain drag because it is easier to maintain, covers a large area per sweep, and is less likely to tangle or hang up on obstacles than the cable-chain drag (Klett et al. 1986). Drags pulled by vehicles are most effective in grassland, cropland, and short brush (Klett et al. 1986). Areas of tall brush and trees or wetlands must be searched by walking or wading. Hand pulled drags with cans or sections of chain attached can be used when access with vehicles is not feasible (Klett et al. 1986). Some studies require repeated searches of the same field. Best results are obtained when the subsequent searches are conducted by the same people following the same drag pattern as before (Klett et al. 1986). To identify the hen while searching, one individual must look for distinguishing features such as the hen size or the coloration of the plumage while the other individual

spots the location where the hen flushed (Klett et al. 1986). If the hen can not be identified in flight, species can usually be determined by the size and color of the eggs, down, or breast feathers found at the nest (Klett et al. 1986). Once a nest has been located, only one person should revisit the nest to avoid leaving scent and creating excessive damage to the vegetation that may attract predators (Klett et al. 1986). Once at a nest, for collection of unincubated eggs, all eggs in the nest should be candled to determine stage of incubation (Weller 1956) and if unincubated all eggs should be marked with a waterproof marker (Arnold 1993). After recording the necessary information, the eggs should be covered with down and other nest materials for warmth and concealment from predators. Tall, slender willow stakes flagged with short strips of cloth or fluorescent tape are used to mark the nest site for relocation (Klett et al. 1986). Stakes should be firmly anchored at least 15cm into the soil, at a standard distance and direction from the nest (Klett et al. 1986). When planning search schedules study objectives, nesting chronologies, manpower and other resource limitations need to be considered (Klett et al. 1986). Duck nesting activity in the Prairie Pothole region lasts about 4 months. When prolonged nesting activity occurs during years of favorable wetland conditions, an additional search in late June or early July is usually needed (Klett et al. 1986). The amount of area that can be searched in a day depends on the cover type, terrain, and number of nests found (Klett et al. 1986). Nest searches should be conducted from 0800 to 1400 central standard time (Gloutney et al. 1993). If collecting unincubated eggs from laying hens the nest should be revisited the next evening to collect any new egg that was laid (Arnold 1993). The best time to visit a nest without flushing the hen

from her nest is between 1600 and 2100 hours for Gadwalls, Northern Shovelers, and Blue-winged Teal, and between 1800 and 2100 hours for Mallards (Gloutney et al. 1993).

Goals of Study

This study has been divided into three phases: egg collection, embryonic growth and incubation length. Egg collection activities are reported in the first section. Fresh laid eggs were used for the embryonic growth and incubation length parts of this study. The goal of the egg collection component was to document nesting chronologies of Mallard, Blue-winged Teal, Northern Shoveler, and Gadwall hens nesting in Minnedosa, Manitoba study area. The goal of the embryonic growth component was to quantify the relationship between embryo oxygen consumption and wet weight as a non-invasive technique to measure growth of an individual through incubation. The goal of the incubation length component was to clarify perceived interspecific differences in incubation length for Mallard, Blue-winged Teal, Northern Shoveler, and Gadwall eggs incubated in a constant environment.

Egg Collection

Methods

Egg collection and fieldwork were completed at Minnedosa, Manitoba, Canada (50° 10'N, 99° 47'W) using dense nesting cover areas developed by Ducks Unlimited and some hay fields (Figure 1). The area consists of native aspen (*Populus* spp.) parkland community prior to agricultural development. The landscape varied from flat to rolling, and contained numerous glacial depressions with ponds (Leonard et al. 1996). Searches were made 6 days each week starting the first week of May and ending the third week of July with a goal of 160 acres per day in 1999 and 2000. Unincubated Blue-winged Teal, Mallard, Gadwall, and Northern Shoveler eggs were collected throughout the 1999 and 2000 breeding season. Eggs found were candled to determine their stage of development (Weller 1956). If the clutches appeared to be incomplete and unincubated, the eggs were marked with a waterproof marker (Arnold 1993). The nest was also marked with a stake approximately 5 meters north from the nest. The nest was revisited the next evening to see if any new eggs had been laid. All new eggs were collected and weighed and this procedure was replicated until no new eggs were found.

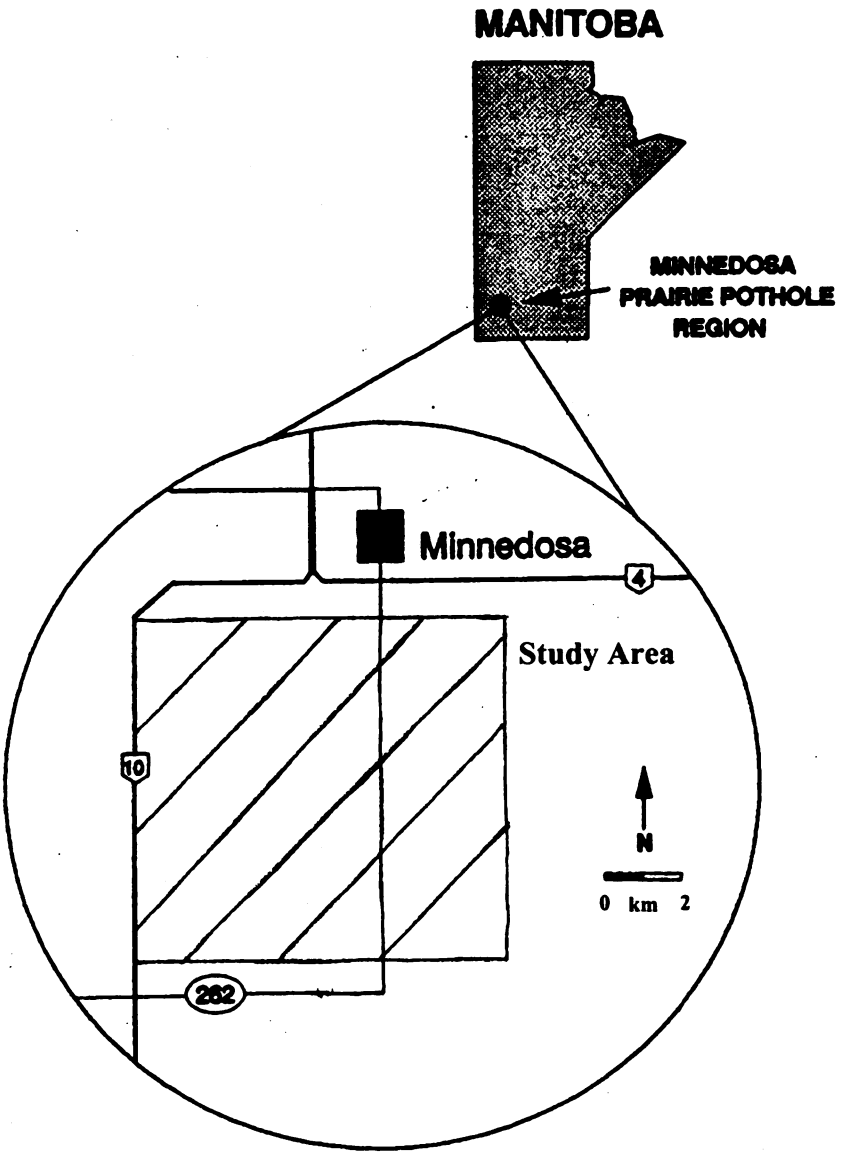

Figure 1. Location of the Minnedosa Prairie pothole region in southwestern Manitoba, Canada, and location of the study area south of Minnedosa.

Results

A total of 1,600 and 3,680 acres were searched in 1999 and 2000. Approximately 160 acres were searched per day and some of this area was dragged multiple times (Appendix A). The primary focus in 1999 was to collect eggs needed for the next two components of the study. In 2000 field season nesting data was collected so the results below are from 2000 field season (Appendix B). Approximately 3000 acres consisted primarily of native prairie grasses (Ducks Unlimited dense nesting cover fields) and 680 acres consisted of alfalfa.

A total of 142 Blue-winged Teal, 47 Northern Shoveler, 80 Mallard, and 24 Gadwall nests were found during the 2000 field season and stage of incubation was recorded (Figure 2). Blue-winged Teal nests were found between days 137 and 202 with the majority of Blue-winged Teal nests having unincubated eggs being found between days 143 and 163. From day 173 to day 202, the majority of the Blue-winged Teal nests found contained incubated eggs. I began to find Gadwall nests on day 158, and most of the nests found between days 167 and 179 contained unincubated eggs. The majority of Gadwall nests with incubated eggs were found between days 179 and 205. Mallard nests were found throughout the entire period of nest searching (days 137 to 205). The majority of nests containing unincubated eggs were found between days 146 and 175. There were a number of nests found between days 137 and 155 with incubated eggs. After day 179 of the breeding season, all nests found contained incubated eggs. Northern Shoveler nests were found between days 140 and 202. The number of Northern Shoveler nests containing unincubated eggs was highest between days 146 and 154 and declined

progressively until day 173. Most of the nests containing incubated eggs were found between days 161 and 202 and by day 191 all nests found contained incubated eggs.

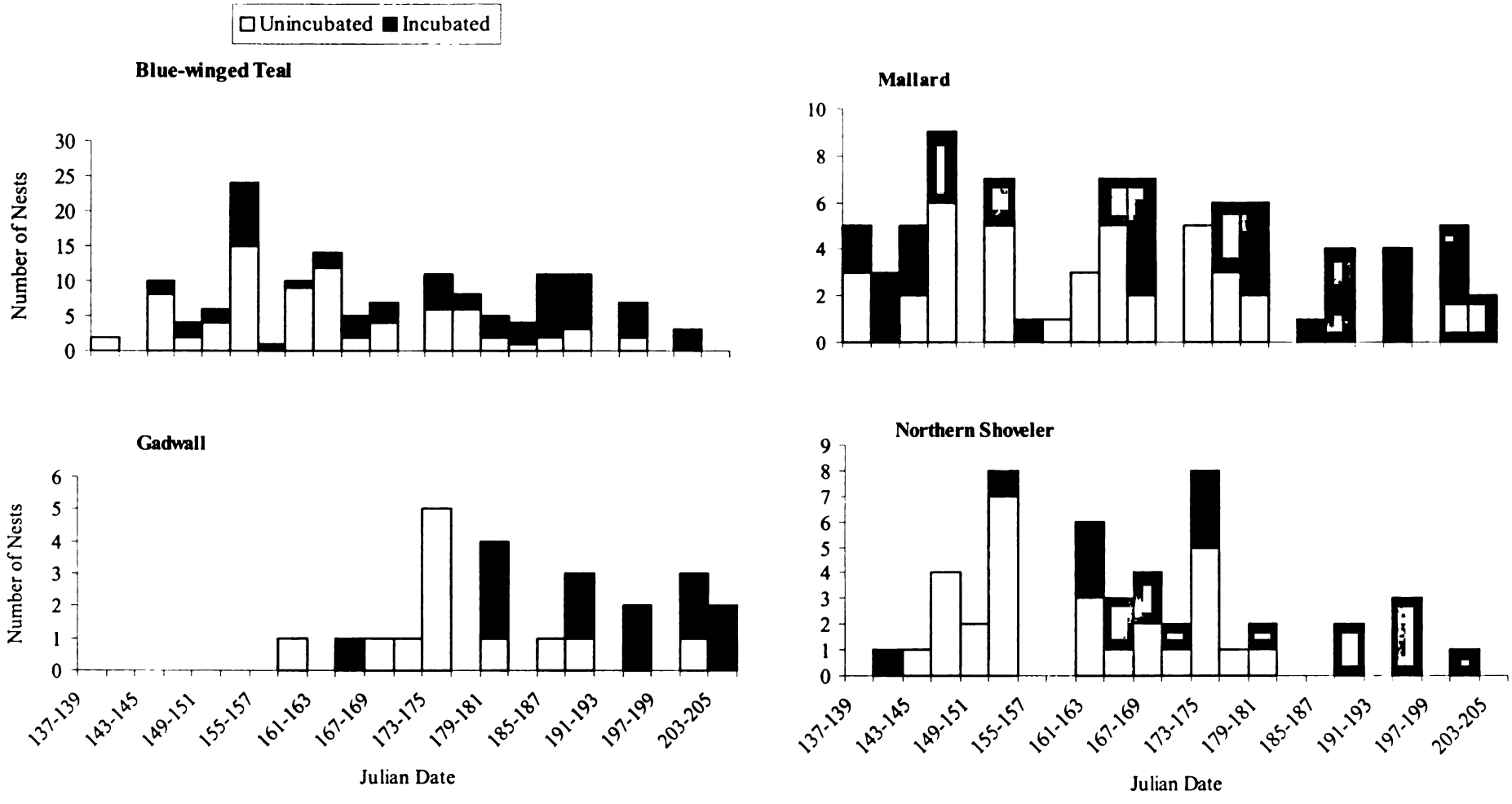

Figure 2. Chronological status (unincubated and incubated) and number of Blue-winged Teal, Gadwall, Mallard, and Northern Shoveler nests found per day from 9 May to 16 July on Minnedosa, Manitoba study area.

In 1999, we collected 181 Blue-winged Teal, 73 Gadwall, 64 Mallard, and 65 Northern Shoveler eggs from nests with unincubated eggs (Table 1). In 2000, we collected 59 Blue-winged Teal, 82 Gadwall, 49 Mallard, and 47 Northern Shoveler eggs. Sixty of the Gadwall eggs were collected in Ceylon, Saskatchewan during days 187 to 198. Blue-winged Teal eggs were collected throughout the breeding season in 2000 and the majority of those eggs were collected between days 146 and 166. For the Minnedosa area, the majority of Gadwall eggs were collected between days 167 and 179. The number of eggs collected for Mallards peaked between days 146 and 179 in 1999 and 2000. We collected most of our Northern Shoveler eggs between days 146 and 166 for both years.

Table 1. Number of eggs collected throughout the 1999 and 2000 breeding seasons for four dabbling duck species that were used in embryonic growth and incubation length experiments.

	Species							
	BWTE		GADW		MALL		NOSH	
Date	1999	2000	1999	2000	1999	2000	1999	2000
132-138	4	2	0	0	2	0	0	0
139-145	29	8	0	0	1	2	3	0
146-152	132	17	0	0	15	10	16	8
153-159	16	1	10	0	15	12	12	17
160-166	0	10	6	5	6	7	28	14
167-172	0	2	31	0	18	7	5	1
173-179	0	5	26	15	7	11	1	5
180-186	0	7	0	0	0	0	0	2
187-192	0	5	0	23	0	0	0	0
193-198	0	2	0	39	0	0	0	0
Total	**181**	**59**	**73**	**82**	**64**	**49**	**65**	**47**

Discussion

The main goal of this section was to report recent nesting chronologies for dabbling ducks nesting in the Minnedosa, Manitoba area. Nesting chronologies are similar to Sowls (1955), Dane (1966), Bellrose (1980), and Baicich and Harrison (1997). Sowls (1955) and Dane (1966) both noted that peak nest initiation for the Blue-winged Teal in southern Manitoba was between day 144 to day 157 which corresponds with the pattern I found. However, I observed another later peak in nest initiation, which is probably related to a cold front that occurred at the end of May. Bellrose (1980) noted that a cold spell on 9-11 May (days 137-139), 1966 slowed the nesting activity of Blue-winged Teals in Minnedosa causing a peak in nest initiation to occur on 22 May (day 150). In addition, Klett et al. (1986) found 82% of their Blue-winged Teal nests in south-central North Dakota when they searched from the end of April to the beginning of July with most of their nests found from the beginning of May to mid-June. Although Blue-winged Teal are considered a late nesting species (Bellrose 1980), our results suggest their season is longer and they should be considered a mid-season nesting species like the Northern Shoveler.

Mallards first arrived in southern Manitoba early in April (Sowls 1955, Bellrose 1980). Nest initiation usually began between 10 April (day 107) and 30 April (day 128) with peak initiation occurring between 5 May (day 133) and 20 May (day 148)(Bellrose 1980). Batt and Prince (1979) found that nest initiation dates for captive Mallards in Manitoba ranged between 9 April and 7 June. Mallards usually initiated nesting over a span of about 65 days in southern Manitoba (Bellrose 1980). My results showed a similar trend with a peak between days 149 and 151. Klett et al. (1986) found 77% of

their Mallard nests in south-central North Dakota from then end of April to the beginning of July with the most nests found from the beginning of May to mid-June. My results showed another peak between days 155 and 157 which may have been caused by the cold front. Cowardin et al. (1985) found a significant relationship between nest initiation date and temperatures in April and May of Mallards in the agricultural environment of North Dakota. The high number of nests found with incubated eggs when we started nest searching indicates that we did not begin searching for Mallard nests early enough to collect fresh eggs.

In North America, the Gadwall reaches its greatest breeding numbers in the mixed prairie of the Dakotas and the Prairie Provinces (Bellrose 1980). Gadwalls are the last species to arrive in numbers on the study area and begin nesting efforts the last two weeks in May (days 150 to 163; Bellrose 1980). Peak nest initiation occurs during the first two weeks of June (days 162 to 177; Sowls 1955, Bellrose 1980). My results showed that peak nest initiation was between days 173 and 175. Klett et al. (1986) found 87% of their Gadwall nests from the end of May to the beginning of July with most of the nests found in June. Lokemoen et al. (1990) found that younger hens nested earlier than older hens in North Dakota.

Northern Shovelers commenced nesting on 2 May (day 130) and reached peak activity between 13 to 19 May (days 141 to 147), 1949 and 27 May to 2 June (days 155 to 161), 1950 and started their last nests about 4 July (Sowls 1955, Bellrose 1980). My results are similar to Sowls (1955) results in 1950. Peak nest initiation occurred between days 145 and 157. It seems for the Minnedosa, Manitoba area nesting chronologies have changed very little from the studies conducted in the 1950's.

Embryonic Growth

Methods

Embryonic Oxygen Consumption & Wet Weight

Approximately 20 unincubated Blue-winged Teal (*Anas discors*), Mallard (*Anas platyrhynchos*), Gadwall (*Anas strepera*), and Northern Shoveler (*Anas clypeata*) eggs were collected daily from nests of laying females on Minnedosa, Manitoba study area. These eggs were stored at 7.2°C for no longer than 3 days and incubated at 37.5°C with 70% humidity. At day 10, 13, 16, and 19 of incubation, equal numbers of eggs (5) were selected and removed from the incubator and their rate of oxygen consumption at 37.5°C was measured. The five eggs selected to be measured for each incubation stage were chosen based on date laid to represent the entire breeding season to eliminate any seasonal biases. Once the oxygen consumption was measured, the egg was opened and wet weight of the embryo determined.

Embryonic Growth based on Oxygen Consumption

Ten eggs from different females of each species (Mallard, Blue-winged Teal, Gadwall, and Northern Shoveler) were removed from the incubator and their rate of oxygen consumption measured on days 10, 13, 16, and 19 of incubation. Oxygen consumption of individual embryos in eggs from different females selected for this experiment were measured throughout the incubation period repeatedly to determine growth as indicated by oxygen consumption.

Respirometry Apparatus

Oxygen consumption was estimated using a closed-system maintained in a water bath at 37.5°C (Figure 3). The respirometry apparatus consists 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, MacCluskie et al. 1997). One jar served as the chamber to hold the egg, and the bottom was covered with Ascarite to absorb the CO_2 the embryo produced. The other chamber served as a pressure buffer and completed the closed system (MacCluskie et al. 1997). Eggs were placed in the chamber on a piece of wire mesh above the Ascarite. Lids were placed on the jars and the egg was allowed 10 minutes to equilibrate with its surroundings. After equilibration, the pre-injection level of the manometer was recorded and 1 ml of pure O_2 was injected into the chamber using a 1-ml gas-tight syringe (MacCluskie et al. 1997). The plunger was then pumped three times to ensure the gas mixed well. After the third time the plunger was pumped, a stopwatch was started and the time required for the manometer to reach equilibrium was recorded. Barometric pressure and chamber temperature were recorded at the beginning and end of each trial and true volume of oxygen (ml) consumed by the embryo was corrected to standard temperature and pressure (STP)(MacCluskie et al. 1997).

Analysis

I used Fisher's LSD to compare least square means of measured and estimated wet weights. To determine relationship between measured and estimated wet weights as a function of stage of incubation (days) I regressed $\log_{10}$ measured and estimated wet weights against stage of incubation for each species. I also used analysis of covariance (ANCOVA) to examine variation within measured and estimated wet weights. I used log transformed measured and estimated wet weight as the dependent variables, species (BWTE, MALL, NOSH, GADW) as class variables, and stage of incubation (days) as a covariate in the analysis.

To determine relationship between wet weight as a function of oxygen consumption I regressed oxygen consumption (ml/hr) against embryonic wet weight (g). I also used ANCOVA to examine variation in oxygen consumption rates of eggs. I used oxygen consumption as the dependant variable, species as class variables, and wet weight as a covariate in the analysis.

To compare measured and estimated wet weights residual analysis was completed. I also used ANCOVA to examine variation between measured and estimated wet weights. I used type ($\log_{10}$ measured and estimated wet weight) as the dependent variable, species as class variables, and stage of incubation as a covariate in the analysis.

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

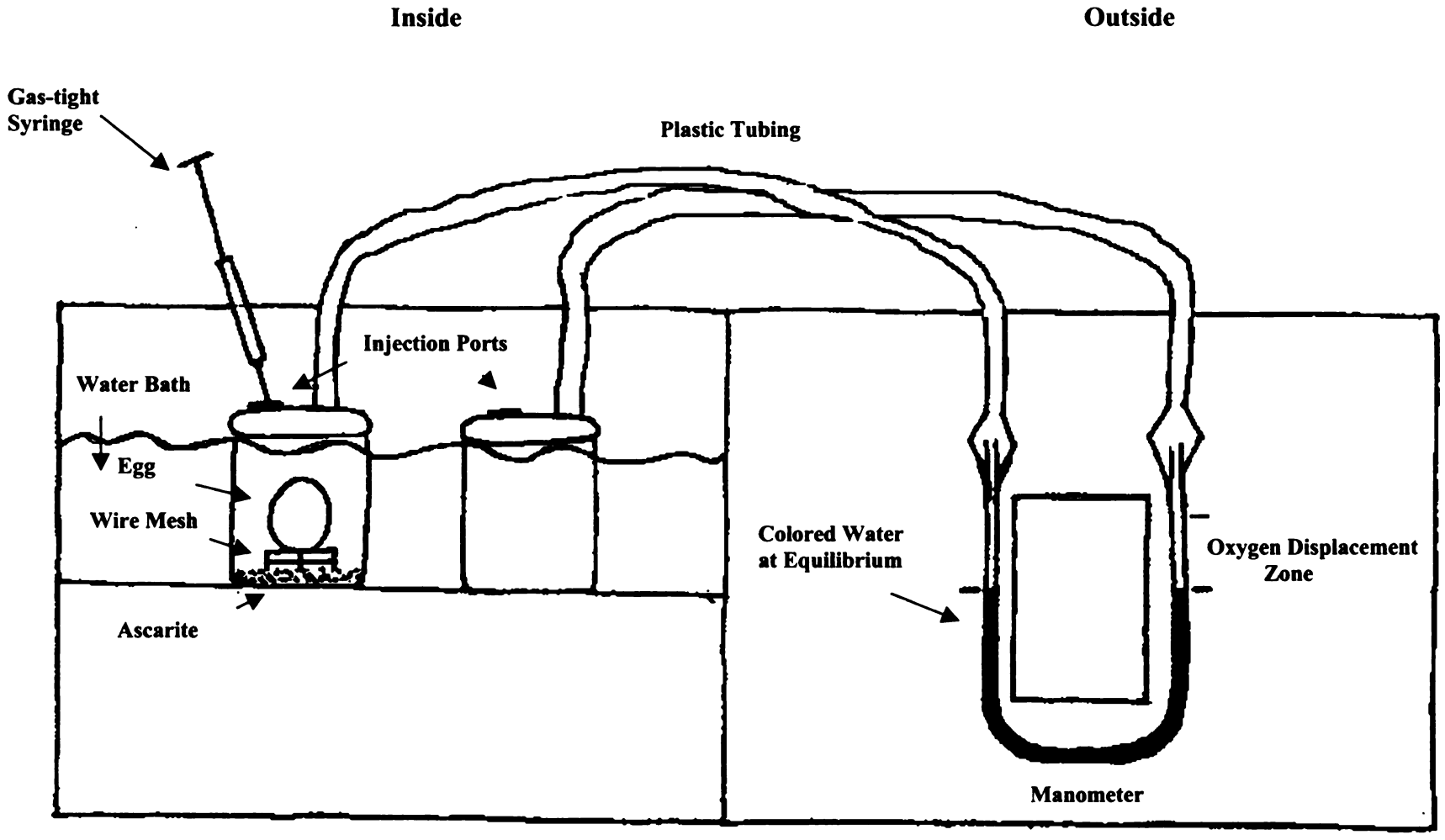

Figure 3. The respirometry apparatus used to measure oxygen consumption of avian embryos.

Results

Embryonic Oxygen Consumption & Wet Weight

Wet weights (g) of Northern Shoveler, Gadwall, and Mallard day 10 embryos were significantly higher than Blue-winged Teal embryos (Table 2). There were no significant weight differences on day 13 and 16 embryos for all four species. Mallard embryos on day 19 were heavier than Northern Shoveler and Blue-winged Teal embryos while Gadwall embryos were heavier than Blue-winged Teal embryos. Measured wet weight increased exponentially as incubation progressed for all four species (Figure 4). A slope heterogeneity test indicated that the slopes of the measured wet weight regressions for each species were not significantly different from each other. Analysis of covariance revealed a significant difference between intercepts showing that Northern Shoveler embryos were the smallest. Comparatively, Blue-winged Teal embryos were 4.8% larger, Gadwall embryos were 13.2% larger, and Mallard embryos were 15.4% larger than Northern Shoveler embryos for the same age.

Table 2. Wet weights ($\bar{x}$ ± SE) of Blue-winged Teal, Northern Shoveler, Gadwall, and Mallard embryos from eggs incubated at 37.5°C on day 10, 13, 16, and 19 of incubation.

	Wet Weight (g) ($\bar{x}$ ± SE)[a]							
Incubation Days	10		13		16		19	
Species	**n**		**n**		**n**		**n**	
BWTE	5	1.14 ± 0.09**a**	5	4.86 ± 0.56**a**	5	7.17 ± 0.92**a**	2	9.66 ± 1.72**a**
NOSH	5	1.45 ± 0.09**b**	4	3.45 ± 0.63**a**	5	7.84 ± 0.92**a**	5	11.17 ± 1.09**ab**
GADW	5	1.49 ± 0.09**b**	5	3.17 ± 0.56**a**	5	9.04 ± 0.92**a**	4	14.43 ± 1.22**bc**
MALL	10	1.55 ± 0.06**b**	12	3.70 ± 0.36**a**	11	8.51 ± 0.62**a**	7	15.73 ± 0.92**c**

[a] For each wet weight, values followed by the same letter do not differ significantly ($p>0.05$) as determined by ANOVA (LS Means).

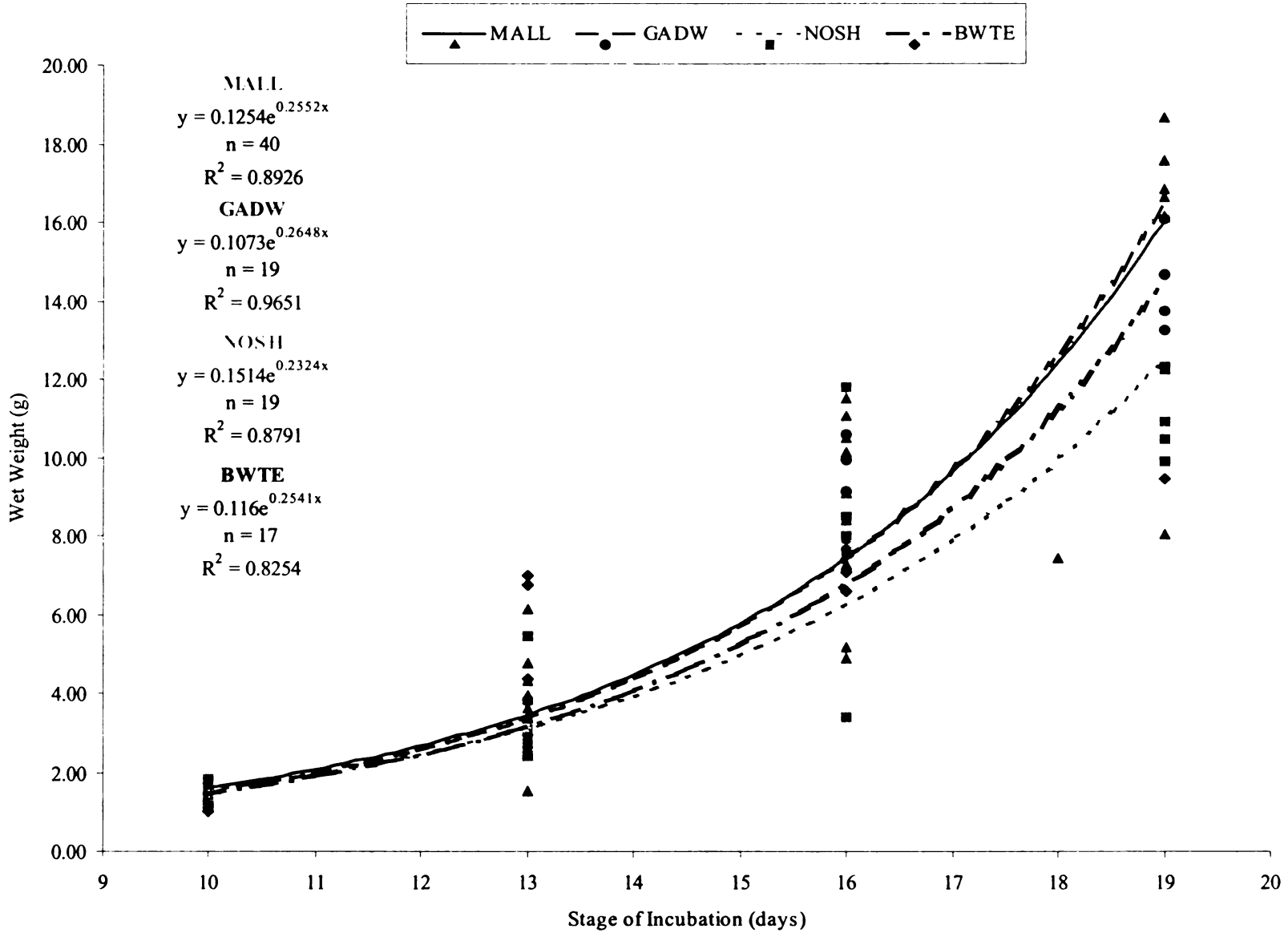

Figure 4. Growth curves for Mallard, Gadwall, Northern Shoveler, and Blue-winged Teal embryos from eggs incubated at 37.5°C.

Oxygen consumption and wet weights were measured for 67 Blue-winged Teal, 19 Gadwall, 41 Mallard, and 19 Northern Shoveler embryos. There was a positive linear increase in oxygen consumption (ml/hr) as embryonic wet weight (g) increased for all four species (General Linear Model, $p = 0.0001$)(Figure 5). Slope heterogeneity test indicates the coefficients for Gadwall, Mallard, and Northern Shoveler embryos similar ($p = 0.958$, $p = 0.228$, $p = 0.255$) while intercepts are significantly different by species (ANCOVA, $p = 0.0001$). The slope of the Blue-winged Teal equation was significantly higher than the other three species ($p = 0.0002$, $p = 0.0001$, $p = 0.0222$). Although different slopes makes it difficult to detect significant differences between the intercept values, when the slopes were adjusted to be equal the intercept for the Blue-winged Teal equation was significantly different from the other three species ($p = 0.0001$). Mallard, Gadwall, and Northern Shoveler embryos grew faster compared to Blue-winged Teal embryos.

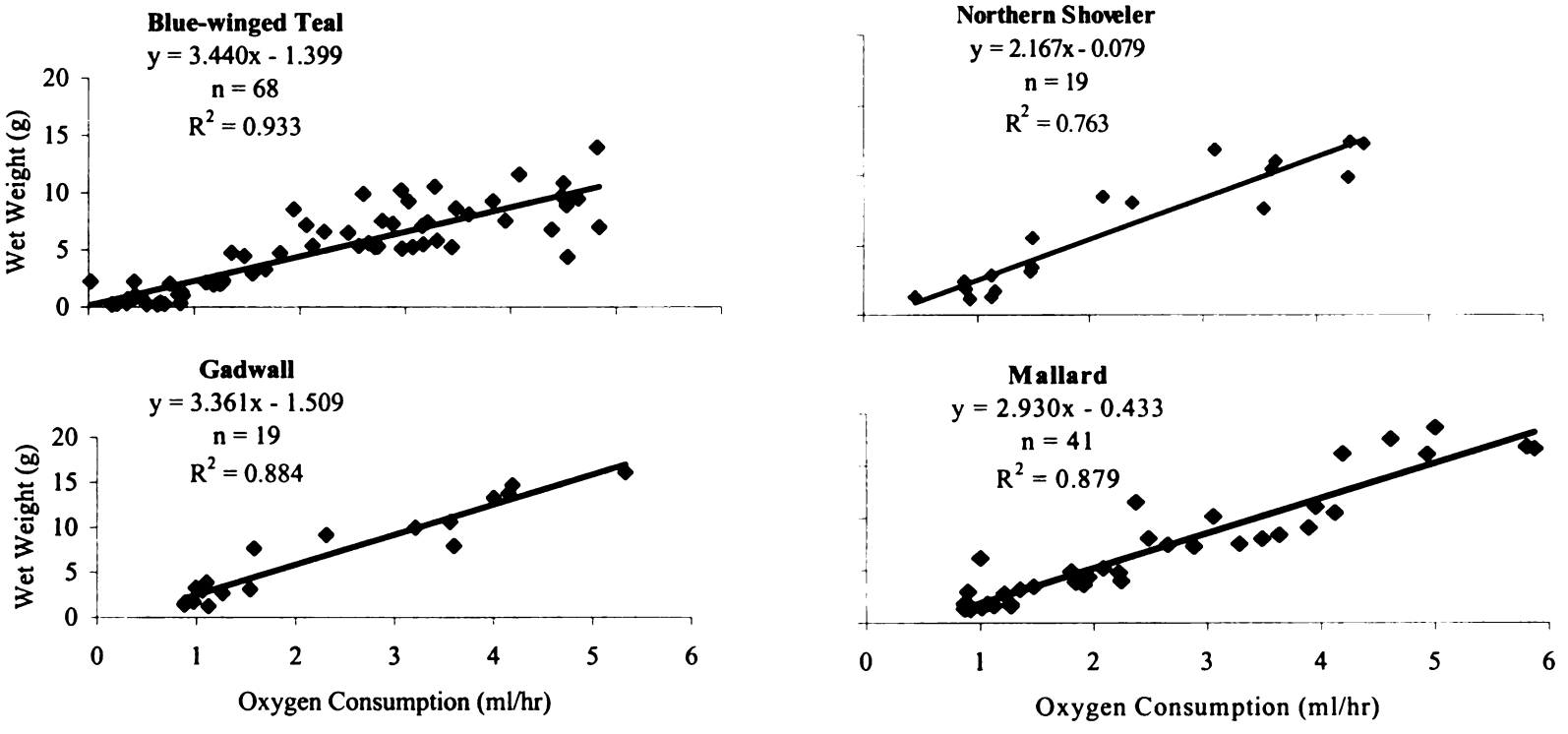

Figure 5. Relationship between oxygen consumption and wet weight for Blue-winged Teal, Northern Shoveler, Gadwall, and Mallard embryos from eggs incubated at 37.5°C.

Embryonic Growth based on Oxygen Consumption

On day 10, estimated wet weights of Blue-winged Teal embryos were significantly different from Northern Shoveler and Gadwall embryos while Gadwall embryos were significantly different from the other three species (Table 3). On day 13, Mallard embryos were heavier than the other three species and Northern Shoveler embryos were heavier than Blue-winged Teal and Gadwall embryos. Mallard embryos were again heavier than the other three species on day 16 and Gadwall embryos were heavier than Blue-winged Teal embryos. Blue-winged Teal embryos were smaller than the other three species on day 19 and Northern Shoveler embryos were smaller than Gadwall embryos, but similar to Mallard embryos.

Table 3. Estimated wet weights ($\bar{x} \pm SE$) based on oxygen consumption of Blue-winged Teal, Northern Shoveler, Gadwall, and Mallard embryos from eggs incubated at 37.5°C on day 10, 13, 16, and 19 of incubation.

	Estimated Wet Weight (g) ($\bar{x} \pm SE$)[a]							
Incubation Days	10		13		16		19	
Species	**n**		**n**		**n**		**n**	
BWTE	9	2.04 ± 0.08**c**	9	2.73 ± 0.25**c**	9	6.20 ± 0.71**c**	9	9.51 ± 0.73**a**
NOSH	5	2.42 ± 0.11**b**	5	4.18 ± 0.33**b**	5	7.59 ± 0.96**bc**	5	13.79 ± 0.98**b**
GADW	8	1.66 ± 0.09**a**	8	3.29 ± 0.26**c**	8	8.41 ± 0.76**b**	8	17.39 ± 0.76**c**
MALL	3	2.28 ± 0.14**bc**	3	5.54 ± 0.43**a**	3	11.44 ± 1.24**a**	3	16.37 ± 1.27**bc**

[a] For each estimated wet weight, values followed by the same letter do not differ significantly ($p>0.05$) as determined by ANOVA (LS Means).

Estimated wet weight increased exponentially as incubation progresses (Figure 6). A slope heterogeneity test indicated that the slopes of the estimated wet weights were significantly different between four species ($p = 0.0001$). Blue-winged Teal embryos grew the slowest with Northern Shoveler embryos being slightly faster. Gadwall embryos grew at the fastest rate followed by Mallard embryos.

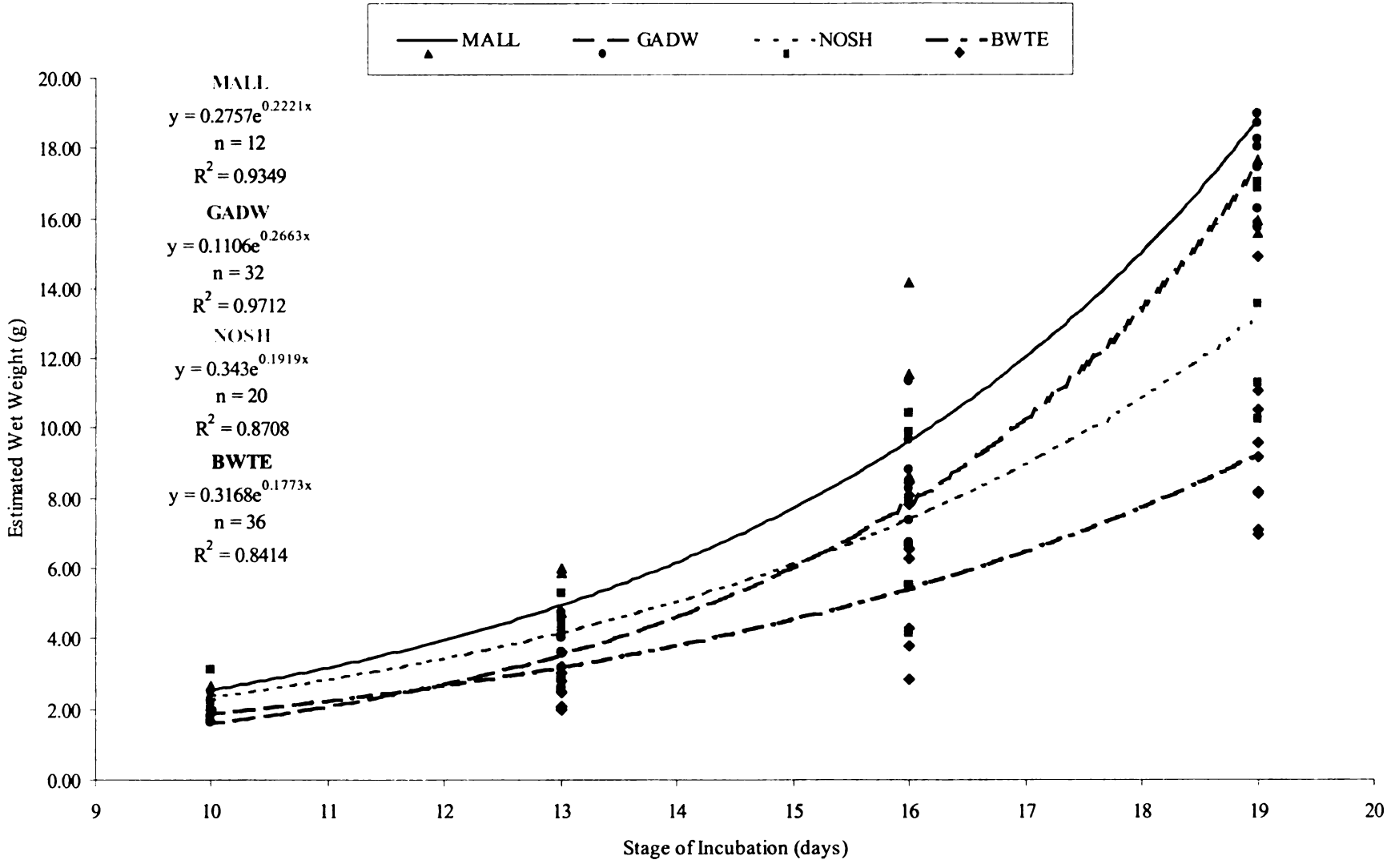

Figure 6. Growth of Mallard, Gadwall, Northern Shoveler, and Blue-winged Teal embryos based on oxygen consumption.

Residual analyses showed that the estimated wet weights corresponded to the measured wet weights with no obvious biases for all four species. Slope heterogeneity tests comparing estimated and measured wet weight regressions showed that there was no significant difference for all species except Blue-winged Teal embryos ($p = 0.0102$). Based on the measured wet weights the estimated wet weights were higher for Gadwall (5%), Mallard (36%), and Northern Shoveler embryos (26%). Since the slopes were significantly different for Blue-winged Teal comparison between measured and estimated wet weight were calculated for each stage of incubation. On day 10, the estimated wet weights were 26% larger than the measured wet weights. On day 13, both estimated and measured wet weights were the same, but by day 16 the estimated wet weights were 20% smaller and by day 19 they were 37% smaller than the measured wet weights.

Discussion

The purpose was to quantify the relationship between embryo oxygen consumption and wet weight as a non-invasive technique to measure growth of an individual through incubation. Oxygen consumption (ml/h) was significantly related to embryonic wet weight (g) for all four species. So embryonic wet weight can be estimated based on oxygen consumption. In addition, there was an exponential increase in estimated wet weight based on oxygen consumption as incubation progressed. Gadwall embryos grew at a faster rate followed by Mallard embryos and Blue-winged embryos were the slowest based on oxygen consumption. The late breeding chronology of the Gadwall can be related to a faster rate of embryonic development.

Estimated wet weights based on oxygen consumption were consistently higher than measured wet weights of Gadwall, Mallard, Northern Shoveler, and day 10 Blue-winged Teal embryos. The increased handling of these eggs may have caused embryos to become more active resulting in an increased rate of oxygen consumption which caused higher estimates of their wet weights. Also removing the eggs from the incubator and putting them into the chamber may have altered temperatures to be lower on the average for the 37.5°C target for brief periods. The effects of handling on embryonic development need to be studied in more depth. Future use of this technique requires that the results be adjusted accordingly for each species to find a more accurate estimation of wet weight and growth. The main advantage of this technique is it allows individual eggs to be measured throughout the incubation length to create more precise growth curves.

Incubation Length

Methods

Approximately 70 unincubated Mallard, Gadwall, Blue-winged Teal, and Northern Shoveler eggs were collected at Minnedosa, Manitoba. Once the eggs were collected, they were packed into plastic egg trays and placed in small plastic crates with foam rubber for cushion and support. The eggs were transported to the hatchery located at Delta Waterfowl Research Station in Portage la Prairie, Manitoba, marked for identification, stored in a refrigerator at 7.2°C for at most three days, and rotated twice a day. Equal numbers of stored eggs were set twice a week and incubated at 37.5°C and 70% humidity until pipped. Once pipped, all eggs were transformed to a new environment, incubated at 37.5°C and 85% humidity. Regular checks of the incubator were made every 8 hours to determine hatch time, defined as the moment the duckling emerged from the shell. To eliminate hatch synchrony effects, eggs of the same clutch were placed in different areas of the incubator and eggs of the same species were never set next to each other.

I used Fisher's LSD to compare mean incubation lengths between species. To determine if incubation length varied with date laid, I regressed the time to hatch (h) against date laid. All statistical analyses were conducted using the General Linear Models (GLM) Procedure of the SAS statistical package (SAS Institute 1990). A significance level of $p < 0.05$ was used for all tests.

Results

A total of 33 Mallard, 37 Gadwall, 81 Blue-winged Teal, and 35 Northern Shoveler eggs were incubated at 37.5°C and 70% humidity (Table 4). Mallard eggs had

the longest incubation length (604-614 h) and Northern Shoveler eggs had the shortest incubation length (551-562 h). Gadwall and Blue-winged Teal eggs had incubation lengths of 586-598 hours and 575-600 hours, respectively. Mallard eggs were significantly longer at time to pip (h) than the other three species. Gadwall and Blue-winged Teal eggs were not significantly different from each other at time to pip (h) and time to hatch (h). For time to hatch (h), Northern Shoveler eggs were significantly different from the other three species except for Blue-winged Teal eggs in 2000. Hatchabilities ranged from 27% to 68%.

Table 4. Incubation periods ($\bar{x}$ ± SE) and hatchabilities of fresh laid eggs collected in 1999 and 2000 from nesting Blue-winged Teal, Northern Shoveler, Gadwall, and Mallard hens incubated at 37.5°C.

	1999					2000				
Species	n_0	% Hatch	n	Time to Pip (h)[a]	Time to Hatch (h)[a]	n_0	% Hatch	n	Time to Pip (h)[a]	Time to Hatch (h)[a]
MALL	40	68	27	573 ± 5.2**c**	604 ± 6.0**b**	16	38	6	578 ± 8.6**b**	614 ± 7.1**c**
GADW	35	66	23	557 ± 9.3**b**	598 ± 9.7**b**	40	35	14	552 ± 5.1**a**	586 ± 5.5**b**
BWTE	129	55	71	554 ± 1.7**b**	600 ± 2.0**b**	20	50	10	534 ± 6.1**a**	575 ± 7.4**ab**
NOSH	56	55	31	529 ± 2.6**a**	562 ± 2.9**a**	15	27	4	525 ± 3.0**a**	551 ± 2.2**a**

[a]For each time to pip and hatch (hrs.) values followed by the same letter do not differ significantly ($p>0.05$) as determined by ANOVA (LS Means).

Incubation periods of Mallard and Blue-winged Teal eggs that were artificially incubated were significantly shorter as laying date increased ($p = 0.01$; Figure 7). For Mallards in 1999 and Blue-winged Teal in 2000 incubation period declined at a rate of 1.7 hours per day as a function of day laid. For Blue-winged Teal eggs in 1999 incubation period declined at a rate of 2.4 hours per day as a function of day collected. Mallard eggs collected in 2000 did not show a decline due to very low sample size. None of the collected Gadwall and Northern Shoveler eggs showed a seasonal decline in incubation period.

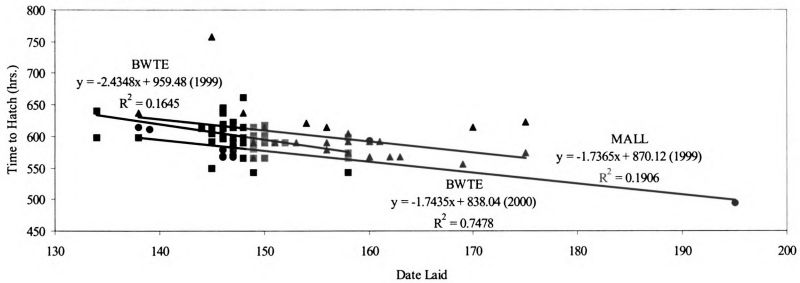

Figure 7. Incubation length (time to hatch (h)) for Mallard and Blue-winged Teal eggs collected from 2 May to 11 July and incubated at 37.5°C.

Discussion

The goal of this experiment was to complete a comparative analysis on incubation lengths of eggs artificially incubated in a constant environment. Feldheim (1997) documented incubation lengths for Mallard (24.45 ± 4.12 days), Gadwall (24.05 ± 2.26 days), Northern Shoveler (23.83 ± 3.17 days), and Blue-winged Teal (23.23 ± 2.94 days in 1996 and 23.13 ± 2.24 days in 1997). Estimates of incubation length based on field observations are subject to environmental variability, incubation temperature, and incubation rhythms of the hens. When dealing with incubation lengths, the ambient and incubation temperature could greatly affect the results. For example, Felheim's (1997) incubation length results for Blue-winged Teal ranged from 19 to 28 days. Mallard incubation length ranged from 20 to 31 days. To accurately document incubation lengths that eliminates natural variation caused by hens incubating at different temperatures, unincubated eggs from laying hens should be collected and incubated in a constant environment. Arnold (1993) collected eggs from laying dabbling duck hens and incubated them in a constant environment. Arnold (1993) noted that Northern Shoveler eggs hatched relatively quickly, whereas Blue-winged Teal and Mallard eggs hatched relatively slowly. The primary goal of his study was to document the affects of pre-incubation delay on egg viability and incubation length, actual incubation lengths for each species are not reported (Arnold 1993). Prince et al. (1969) evaluated the influence of temperature and humidity on the development of Mallard embryos and estimated hatch time to be 650 hours at 37.5°C. My mean incubation lengths at 37.5°C in 1999 and 2000 for Mallards were 604 to 614 hours. When mean incubation lengths were compared to Felheim's (1997) means documented in the field, all our incubation lengths were about a

day longer except for Northern Shovelers. This would suggest that hens in the field are incubating at a higher temperature except for Northern Shovelers. However, the range of incubation lengths that he documented included our mean incubation lengths. These incubation lengths can be used as a baseline for future comparative studies on factors affecting incubation length in dabbling ducks in a controlled environment.

We also wanted to document the rate of change in incubation length of eggs incubated in a constant environment as a function of date collected for these four species of dabbling ducks. We observed a decline for Mallards and Blue-winged Teal, but not for Northern Shovelers or Gadwalls. Small sample size reduced our power to detect a relationship for Northern Shovelers however Arnold (1993) found a decline in incubation length with nest initiation date. No relationship was detected for Gadwalls which may be due to the short length of their breeding period.

Various explanations have been proposed to explain the factors causing a decline of incubation length over the breeding season, such as differences in nutrient reserves of early and late females (Krapu 1981, Alisauskas et al. 1990, Elser and Grand 1994), ambient temperature (Biebach 1984, Haftorn and Reinertsen 1985, Arnold 1993), egg size (Martin and Arnold 1991, Arnold 1993, Flint and Sedinger 1992, Rohwer 1986), clutch size (Rohwer 1992, Feldheim 1997), incubation constancy (Afton and Paulus 1992), and embryonic development (Arnold 1993). Eggs in this study were incubated in a constant environment, the exogenous factors were controlled. So one possible explanation for this decline could be that there are mechanisms inherent in late season eggs that may facilitate increased development rates and shorter incubation lengths (Arnold 1993). If late season eggs do develop more quickly than early season eggs, a

female would benefit by laying fewer eggs (Arnold 1993, Flint et al. 1994) and development to hatch could further be expedited by smaller egg size (Flint et al. 1994; Feldheim 1997). Growth rates of Mallard and Blue-winged Teal embryos from early and late eggs need to be investigated under constant incubation environments to understand this decline more thoroughly.

Further, late nesting individuals may be limited by season length. Shorter incubation lengths coupled with smaller clutch sizes may be an adaptation that helps late nesting females complete reproduction earlier (Hohamn et al. 1992, Feldheim 1997). Significant variation in incubation length among females has been observed for wild Blue-winged Teal and captive Mallards, but not for wild Mallards and Northern Shovelers (Arnold 1993, MacCluskie et al. 1997). The eggs from these females were incubated in a constant environment eliminating any environmental factors influencing their incubation. This suggests there may be a genetic basis relative to incubation length which could be a basis for further research.

Appendix A. Nest searching frequencies for fields in the Minnedosa, Manitoba study area.

Intensity[a]	Location[b]				
1	SW 16-14-17	SW 17-14-18	SW 20-14-17	NE 19-14-17	SE 18-13-18
	NW 12-14-18	SE 17-13-18	SW 23-14-18	SE 19-14-18	
2	NE 2-14-17	NE 18-14-17	SW 3-14-18	SW 18-14-18	SE 17-14-17
	SW 2-14-18	NE 12-14-18	NW 15-14-17		
3	NW 3-14-18	SE 19-14-17	SE 23-14-18	NE 32-14-18	
4	SE 36-14-18				
5	SW 6-14-17				

[a] Intensity is defined as the number of time a field was searched from early May to mid-July.

[b] quarter - section - township - range

Appendix B. Number of nests found for each species on square mile quarter sections in Minnedosa, Manitoba study area in 2000.

Location[c]	Species						
	BWTE	NOSH	MALL	LESC	NOPI	GADW	AMWI
NE 2-14-17	6	0	1	0	0	0	0
NE 18-14-17[b]	1	1	0	1	0	0	0
NW 3-14-18	1	5	5	1	1	1	0
SW 3-14-18	5	2	9	1	0	1	0
SW 6-14-17	16	6	3	0	0	0	0
SW 16-14-17[b]	3	1	0	0	0	0	0
SW 17-14-18	3	1	1	0	0	1	0
SW 18-14-18	15	2	1	0	0	0	0
SE 19-14-17	7	0	10	0	0	0	0
SW 20-14-17[b]	6	0	1	0	0	0	0
SE 17-14-17[b]	21	6	8	0	0	3	0
SE 23-14-18	9	0	4	0	0	3	1
SW 2-14-18	10	1	2	0	0	0	0
NE 19-14-17	2	3	0	0	0	0	0
SE 36-14-18	12	3	6	2	0	4	1
NE 32-14-18	5	2	10	0	0	2	0
SE 18-13-18[a]	1	1	1	0	0	0	0
NW 12-14-18	2	1	0	0	0	1	0
NE 12-14-18	4	2	5	0	0	1	0
SE 17-13-18	7	2	0	0	0	5	0
SW 23-14-18	4	6	5	0	0	1	0
SE 19-14-18	0	1	3	0	0	1	0
NW 15-14-17	2	1	5	0	1	0	0
Total	142	47	80	5	2	24	2

[a] not a full quarter section

[b] cover type is alfalfa, all others are Ducks Unlimited dense nesting cover fields

[c] quarter - section - township - range

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