BREEDING PHENOLOGY AND NESTLING MORPHOLOGY OF BALD EAGLES IN THE UPPER MIDWESTERN UNITED STATES[1]

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Abstract.—We report on egg-laying and hatching dates ($n = 656$), and morphological characteristics ($n = 642$) of nestling Bald Eagles (*Haliaeetus leucocephalus*) from six study areas in Minnesota and Wisconsin, USA, from 2006 to 2011, 2014, and 2015. Nestling age was estimated from length of the eighth primary feather using sex-specific equations from the literature. Egg-laying advanced from south to north beginning in late January and continuing through mid-April; hatching began in late February and ended in mid-May. Mean annual hatching dates were correlated with the average dates of ice break-up each spring at two ice-monitoring sites ($n = 8$ yr, $r^2 = 0.90$). We found no significant long-term trend or shift in hatching dates over the 10 yr. Male and female nestlings overlapped in weight, footpad length, bill depth, and culmen and hallux claw chord length; however, by 35 d these features had diverged sufficiently to allow estimation of the sex of nestlings due to the larger size of females ($P < 0.05$ all contrasts). Our models suggest that most of the nestling traits we measured continued to grow beyond the 70 d for which we had measurements, except for footpad, which reached predicted full size by about 46 d after hatching. Footpad length and bill depth were important traits for determining the sex of nestlings $\geq$35 d old. We provide a classification tree that uses footpad length and bill depth to estimate the sex of nestlings. Overall accuracy of this classification tree was 91%. Individuals with footpad length $\geq$132 mm and bill depth $\geq$29 mm (46% of our sample) had a 0.95 probability of being female and individuals with footpad length $<$132 mm and bill depth $<$30 mm (41% of our sample) had a 0.92 probability of being male, making our classification tree unbiased and robust for assigning sex for most nestlings $\geq$35 d old.

Key Words: *Bald Eagle*; Haliaeetus leucocephalus; *nestling morphology*; *phenology*; *sexual dimorphism*; *weather*.

FENOLOGÍA REPRODUCTIVA Y MORFOLOGÍA DE LOS POLLUELOS DE *HALIAEETUS LEUCOCE-PHALUS* EN EL MEDIO OESTE SUPERIOR DE ESTADOS UNIDOS

RESUMEN.—Presentamos las fechas de puesta y eclosión ($n = 656$) y las características morfológicas ($n = 642$) de polluelos de *Haliaeetus leucocephalus* provenientes de seis áreas de estudio en Minnesota y Wisconsin entre 2006–2011, 2014 y 2015. La edad de los polluelos fue estimada a partir de la longitud de la octava pluma primaria usando ecuaciones específicas para cada sexo existentes de la literatura. La puesta de huevos avanzó de sur a norte a partir de finales de enero y continuó hasta mediados de abril; la eclosión comenzó a finales de febrero y terminó a mediados de mayo. Las fechas medias anuales de eclosión se correlacionaron con las fechas medias de ruptura del hielo cada primavera en dos lugares de monitoreo del hielo ($n = 8$ años, $r^2 = 0.90$). No encontramos una tendencia significativa a largo plazo ni un cambio en las fechas de eclosión durante los diez años. Los polluelos machos y hembras se superpusieron en peso, longitud de la almohadilla plantar, altura del pico, y longitud de la cuerda de la uña del culmen y del hálux; sin embargo, a los 35 días estas características habían divergido lo suficiente como para permitir la estimación del sexo de los polluelos debido al mayor tamaño de las hembras ($P < 0.05$ en todos los contrastes). Nuestros modelos sugieren que la mayoría de los rasgos de los polluelos que medimos continuaron creciendo más allá de los 70 días para los que teníamos mediciones, excepto para la almohadilla plantar, que alcanzó el tamaño completo previsto alrededor de los 46 días después de la eclosión. La longitud de la almohadilla plantar y la altura del pico fueron características importantes para determinar el sexo de los polluelos de ≥ 35 días de edad. Proporcionamos un árbol de clasificación que utiliza la longitud de la almohadilla plantar y la altura del pico para estimar el sexo de los polluelos. La precisión general de este árbol de clasificación fue del 91%. Los individuos con una longitud de almohadilla plantar ≥ 132 mm y altura del pico ≥ 29 mm (46% de nuestra muestra) tuvieron una probabilidad de 0.95 de ser hembras y los individuos con una longitud de almohadilla plantar <132 mm y altura del pico <30 mm (41% de nuestra muestra) tuvieron una probabilidad de 0.92 de ser machos, haciendo que nuestro árbol de clasificación sea imparcial y robusto para asignar el sexo a la mayoría de los polluelos de ≥ 35 días de edad.

[Traducción del equipo editorial]

INTRODUCTION

Bald Eagle (*Haliaeetus leucocephalus*) populations declined across North America in the 1950s and 1960s due largely to the indiscriminate use of dichlorodiphenyltrichloroethane (DDT), other pesticides, and industrial pollutants such as polychlorinated biphenyls (PCBs; Grier 1982, Wiemeyer et al. 1984). Following the ban on DDT and PCBs in the mid-1970s, Bald Eagle populations increased across North America, including in the upper Midwest (Madsen et al. 1985, US Fish and Wildlife Service 2022). Throughout the population low and subsequent recovery, Bald Eagle nestlings have been routinely captured and banded to assess demographics, causes of death, contaminant levels, effects of disturbance and many other factors important for understanding the underlying causes of decline and recovery. Bald Eagles continue to be used as biosentinels with nestlings being particularly useful because they are indicators of local stressors (Bowerman et al. 2002, DeSorbo et al. 2020).

Bald Eagle nestlings can be most safely sampled when they are 5–8 wk old. Cain (1985) showed that when nestlings are 5 wk old, the adults spend <20% of their time at the nest. By this time second-down and body feather growth is substantial enough to allow nestlings to thermoregulate without a parent to brood or shade them. After about 9 wk they can quickly move to the edge of the nest where they could fall or prematurely fledge when investigators attempt to capture them (Bortolotti 1984a). Prior knowledge of breeding phenology, especially the dates of egg-laying and hatching, is therefore important for investigators planning studies. Moreover, a baseline of egg-laying and hatching dates is useful for comparing and understanding variation among regions and for assessing long-term change in breeding phenology.

When raptor nestlings are sampled, they are individually marked and it is common for researchers to take standard measurements to estimate age and sex, and to assess general health. These standard morphological measurements have proven useful for a variety of assessments. For example, size and growth of different morphological traits have been used to investigate adaptive modifications that may differ slightly by region (e.g., Ricklefs 1968, O'Connor 1977). Determining a reasonably precise age of nestlings is necessary for calculating breeding phenology and for assessing the effects of weather and climate, which can influence the timing and

success of some breeding raptors (Dykstra et al. 2021). For Bald Eagles in northern areas, the timing of ice-breakup on area rivers and lakes each spring can be particularly important for adults that often depend on aquatic prey to feed nestlings (Grim and Kallemeyn 1995).

Determining the sex of nestlings allows investigators to assess sex-related life history traits, causes of mortality, patterns of migration, and differential effects of contaminants. However, correctly determining the sex of Bald Eagle nestlings can be problematic. Like most raptors, Bald Eagles express reverse sexual size dimorphism as adults, but the sexes overlap extensively in size throughout the early nestling period. Historically many investigators relied on footpad length, bill size, and general appearance to judge the sex of nestling Bald Eagles (Bortolotti 1984b). Today, PCR-based genetic techniques can be used to determine sex with near 100% accuracy (Morinha et al. 2012); however, budget limitations, time constraints, lost samples, and unplanned events can still result in the need for estimating sex based on morphological measurements.

We captured and measured Bald Eagle nestlings at three national parks and adjacent areas in Minnesota and Wisconsin, USA, to monitor the levels of environmental contaminants in aquatic systems (Route et al. 2019) and those results are reported elsewhere. Herein we report on the egg-laying and hatching phenology (collectively, breeding phenology), the timing of ice-breakup in relation to breeding phenology, and the morphological characteristics of the nestlings we sampled. Specifically, our objectives were to: (1) document the patterns and trends in egg-laying and hatching dates, (2) examine the relationship between ice-breakup each spring and Bald Eagle breeding phenology, (3) compare nestling growth and morphological characteristics at six study areas, and (4) determine the best morphological traits to use for estimating the sex of nestlings when genetic samples are not available.

METHODS

Study Areas and Nest Surveys. We recorded the number of Bald Eagle nests and nestling physical characteristics at six study areas in Minnesota and Wisconsin from 2006 to 2011, 2014, and 2015 (Fig. 1). Our focus was on four core study areas that are part of the US National Park Service system: Apostle Islands National Lakeshore (APIS), upper St. Croix

National Scenic Riverway (USACN), lower St. Croix National Scenic Riverway (LSACN), and the Mississippi National River and Recreation Area (MISS). When funding allowed, we also partnered with the Wisconsin Department of Natural Resources to sample along Wisconsin's Lake Superior South Shore (LSSS) and with the Minnesota Pollution Control Agency to sample downstream from MISS in Pools 3 and 4 of the Mississippi River (Pools3+4). The approximate center of the most northerly study area (APIS) is 46°58′N, 90°41′W, and the most southerly study area (Pools3+4) is 44°42′N, 92°42′W (see Supplemental Material Table S1 for all study area coordinates).

To identify Bald Eagle nests for sampling, the National Park Service, Wisconsin Department of Natural Resources, Ramsey County (MN) Parks and Recreation Department, and Prairie Island Indian Community Department of Land and Environment collaborated to locate occupied Bald Eagle nests within the study areas using fixed-wing aircraft, helicopter, and ground-based observations. Each year from April through mid-June, we located and mapped all occupied nests and counted the number of live nestlings, dead nestlings, and addled eggs. We define occupied nests as those being recently maintained (new nest material) with at least one adult nearby, or having egg(s) or nestling(s) present (Postupalsky 1983). The timing of surveys was similar to the productivity surveys traditionally conducted in Wisconsin (Wisconsin Department of Natural Resources 2016) and Minnesota (Baker and Monstad 2006).

From mid-May through late June 2006–2011, 2014, and 2015 (2012 and 2013 were planned years off), when nestlings were 2–10 wk old we used nest survey results to locate all accessible nests with live young. Experienced tree-climbing biologists accessed these nests, hand-captured young, and brought them to the ground for measurements and sampling. For the four core study areas (APIS, MISS, LSACN, and USACN), all known nests with young were sampled unless the trees were unsafe to climb, access was denied through private property, or we observed that nestlings were too old or young to safely handle. For the two additional study areas (LSSS and Pools3+4), we sampled as time and partner funding allowed.

To assess how ice cover might influence Bald Eagle breeding phenology, we used National Park Service records of ice-out dates on the St. Croix River near Grantsburg, Wisconsin (USACN study area) and
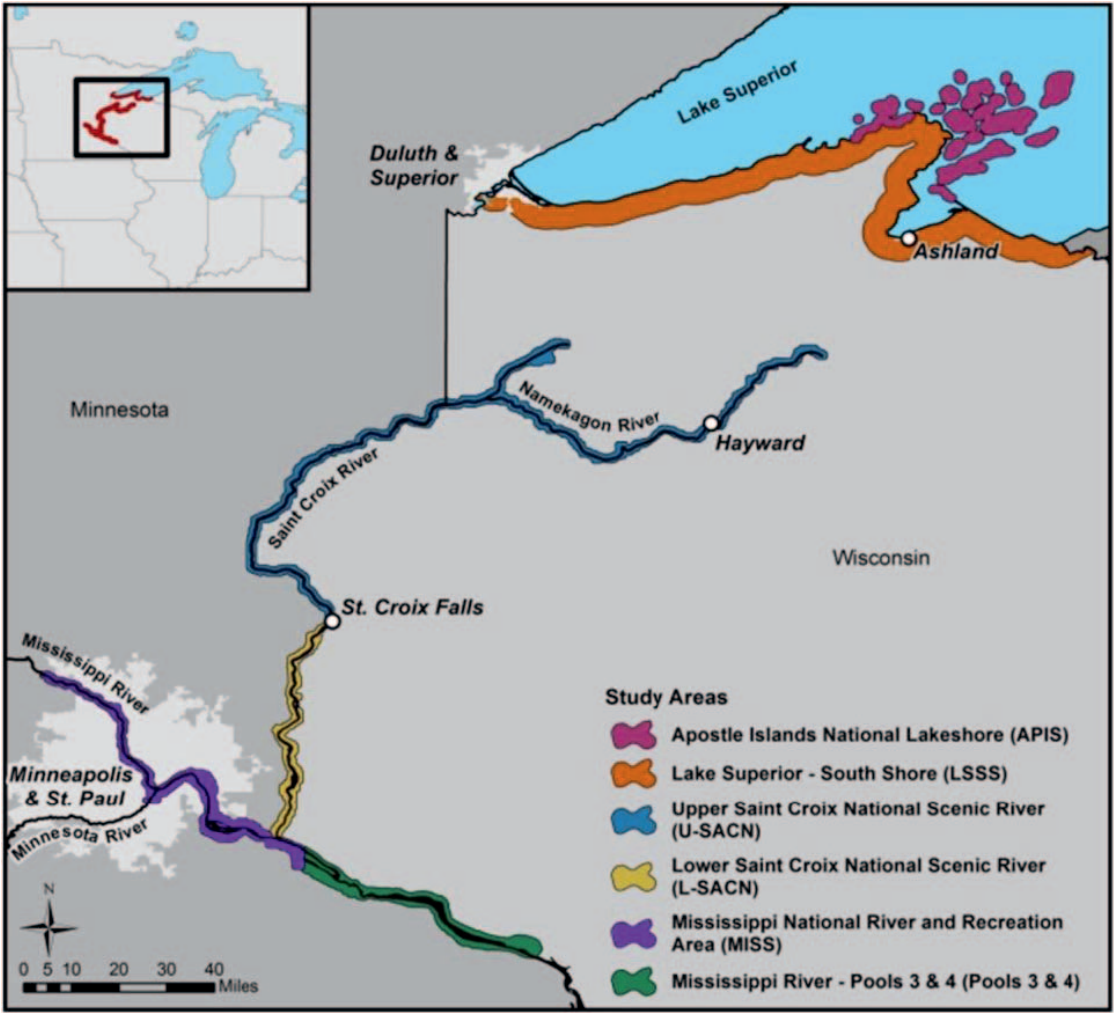
Figure 1. Locations of the six study areas where Bald Eagle nestlings were measured in Minnesota and Wisconsin, USA.

reviewed daily MODIS (Moderate Resolution Imaging Spectroradiometer) satellite imagery of the Apostle Islands (APIS study area; imagery available at: worldview.earthdata.NASA.gov/). When MODIS imagery did not reveal an exact date of ice-out due to cloud cover, we estimated the date as the midpoint between the last known date with ice and first date of ice-free conditions. We used the Spearman Rank procedure to compare the average date when ice left these two locations each year with the mean annual hatching date for Bald Eagle nestlings across the six study areas (Table S2).

Nestling Measurements. We sampled nestlings that were old enough (>5 wk) to thermoregulate but young enough (<9 wk) to minimize the danger of premature fledging. To do this, we sampled each year starting in the most southerly study areas and moved northward, a latitudinal distance of approximately 300 km, to account for earlier nesting in the southern areas.

We banded nestlings with individually numbered US Geological Survey aluminum leg bands; weighed nestlings with a spring-scale (nearest 0.1 kg); measured bill depth, culmen, and hallux claw chord lengths with a digital caliper (nearest 0.1 mm); and measured footpad (nearest 0.1 mm) and eighth primary feather length (P8; nearest 0.1 mm) with a metal rule (see Table S3 for detailed descriptions of

measurements). We examined each nestling for external parasites and malformations of the bill, feet, wings, and feathers. We made a preliminary judgement of sex based on length of the footpad (Bortolotti 1984b) and visually estimated age in weeks based on feather color and growth patterns (Bortolotti 1984a). Each nestling was placed back in the nest after we collected samples.

All measurements followed Bortolotti (1984a, 1984b), except that footpad measurements were done with nestlings positioned on their bellies. We initially placed nestlings on their backs as did Bortolotti (1984b), feet facing upward, but quickly learned that nestlings placed on their bellies with feet drawn back allowed a more relaxed foot that could be measured with less need to physically restrain them. To assess whether position influenced footpad measurements, two of us (WTR and JCS) independently measured 57 nestlings both on their back and on their belly. We found that measurements of nestlings placed on their belly averaged 2.1 mm shorter than the same nestlings when positioned on their back (mean ± SE: 133.8 ± 1.1 vs. 135.9 ± 1.1 mm for belly and back, respectively; paired t-test: $t = 7.22$, $P < 0.0001$).

We also assessed the potential for measurement bias by the three primary handlers (WTR, MM, and JCS). We compared data collected at the MISS study area where, after training, the three handlers worked in separate crews to measure or supervise measurements of all nestlings. Over the 8 yr at MISS ($n = 263$ nestlings), we found no significant difference in measurements obtained by the three primary handlers (AOV, 2 df, $P < 0.05$ for all morphometric measures; Table S4). The remaining study areas were typically sampled by a single crew, with two of the handlers (WTR and JCS) taking or supervising all measurements.

In the lab, the sex of each nestling was determined using PCR-based DNA analysis (Morinha et al. 2012) on a sample of blood. With this DNA-verified sex, we assigned a more precise estimate of age, in days, using sex-specific growth rates of the eighth primary feather (Bortolotti 1984b). The sex-specific growth rates were derived from 64 known-age Bald Eagle nestlings measured in north-central Saskatchewan, Canada (55° latitude), where P8 growth was found to be highly linear through 72 d with low variability (± 2.9 d; Bortolotti 1984b). We lacked known-age nestlings to assess this measure, but we expect a similar level of variation because the linear growth of P8 in our study was similar to that described in

Bortolotti (1984b). Other researchers have reported that raptor flight feathers show little variation in growth rate (Scharf and Balfour 1971, Olsen et al. 1982, Penak et al. 2013) and are not highly dependent on environmental factors or brood size (Olsen and Olsen 1987, Penak et al. 2013). Nonetheless, the potential exists for differences in P8 growth between studies; hence we considered our age estimates to be an index with a small, unknown error around true age.

All data for this study were collected and managed under peer-reviewed protocols (Route et al. 2009, 2019), a quality assurance plan (Route and Vander-Meulen 2018), and with review and approval from the National Park Service Institutional Animal Care and Use Committee.

Statistical Analyses. To compare study areas and trends over time we used data from 642 nestlings for which we had complete information on age, sex, and all morphological measurements. All siblings were included in our analyses and territory was included as a random variable in models to account for nonindependence of siblings in territories. All analyses were conducted in R (R Core Team 2020).

We assessed sexual dimorphism and growth patterns in nestlings by fitting a logistic growth model using function *nls* in package *stats* (R Core Team 2020). We used a self-starting function to estimate initial parameter estimates, then fit the model for each of the morphological variables as K/(1+exp(Po +r*X)) where K is the asymptote, Po is the starting size, r is the rate of growth, and X is either age in d or P8 length in mm. We fit the models with sex as a grouping factor.

To examine age bias at time of sampling, we used a general linear model (GLM) and we found significant differences in the age at time of measurement by study area ($F_{6,646} = 7.17$, $P < 0.001$, $R^2_{adj} = 0.05$). We accounted for this sampling bias by including age in subsequent models. We also used a GLM approach with binomial distribution to test for differences in sex ratio among study areas and years.

To determine if there were significant differences in the morphological traits among study areas, we used a GLM with each trait (weight, footpad length, bill depth, culmen chord length, and hallux claw chord length) as the response variable; however, to provide a scale-less measurement with mean of 1.0 and variance proportional to the original variance of that trait, we performed mean-scaling of measurements by dividing each value by the grand mean of the respective trait (Berner 2011). The grand mean

Table 1. Estimated earliest, mean, median, and latest egg-laying and hatching dates for 656 nestling Bald Eagles from six study areas in Minnesota and Wisconsin, 2006–2015. Study areas are sorted earliest (left) to latest (right). Number in parentheses under study area abbreviation is the number of nestlings with estimated egg-laying and hatching dates.

BREEDING PARAMETER	MEASURE	STUDY AREA[1]					
		MISS (265)	LSACN (114)	POOLS3+4 (72)	USACN (88)	LSSS (26)	APIS (91)
Extrapolated egg-laying dates[2]	Earliest	24 January	9 February	21 February	17 February	9 March	12 March
	Mean	6 March	8 March	8 March	14 March	28 March	30 March
	Median	5 March	8 March	9 March	15 March	28 March	31 March
	Latest	2 April	2 April	24 March	7 April	14 April	18 April
Estimated hatching dates[3]	Earliest	26 February	15 March	27 March	23 March	12 April	15 April
	Mean	9 April	11 April	11 April	17 April	1 May	3 May
	Median	8 April	11 April	12 April	18 April	1 May	4 May
	Latest	6 May	6 May	27 April	11 May	18 May	22 May
Span for both periods		69 d	52 d	31 d	49 d	36 d	37 d

1 = Study areas and sample size (n): MISS = Mississippi National River and Recreation Area; LSACN = Lower St. Croix National Scenic Riverway; Pools3+4 = Pools 3 and 4 of the Mississippi River; USACN = Upper St. Croix National Scenic Riverway; LSSS = Lake Superior South Shore in Wisconsin; APIS = Apostle Islands National Lakeshore.
2 = Extrapolated from hatching date based on a 35-d incubation period (Herrick 1932, Stalmaster 1987).
3 = Back-calculated from estimated age at time of handling each nestling.

of each trait was used to preserve the differences in study area means. The full model included the mean-scaled morphological trait as the response variable with age, study area, year, and study area by year interaction as predictors and the polynomial of age (age + age^2). We compared the full fixed effects models with reduced models and used AIC$_c$ to select the most supported model. When significant differences in morphology among study areas were found, pairwise post hoc tests using package *lsmeans* (Lenth 2016) with Tukey's adjustment were used to determine which study areas differed. We also used a GLM to determine if there were differences in the mean-scaled traits between Lake Superior nestlings (APIS and LSSS) and nestlings from river study areas (MISS, LSACN, USACN, Pools3+4) using age as a polynomial (age + age^2) with year as a covariate.

To evaluate a method of predicting the sex of nestlings we used classification and regression tree analysis using R function *rpart* (Therneau and Atkinson 2019, Milborrow 2021). This analysis is nonparametric, which is more appropriate than discriminant analyses when assumptions of balanced sample size are not strictly met (Karels et al. 2004). Within *rpart* we used method = "class" for footpad length, bill depth, and culmen chord length of known-sex nestlings with P8 measurements ≥102 mm (relating to an indexed-age ≥35 d). We used a matrix of classification success to measure overall accuracy (the proportion of correct predictions).

RESULTS

We captured, measured, and determined sex, age, and hatching dates for 656 Bald Eagle nestlings from 162 nesting territories at six study areas over eight years (49–111 nestlings/yr; Table 1, Fig. 2). For logistic growth models and comparing mean-scaled traits we omitted 14 nestlings that had incomplete data, leaving $n = 642$ for those analyses at the six study areas: APIS ($n = 91$), LSSS ($n = 26$), MISS ($n = 253$), Pools3+4 ($n = 71$), LSACN ($n = 113$), USACN ($n = 88$).

Sex, Age, and Hatching Phenology. DNA-verified sex ratios were near parity (344 females, 312 males) with no significant difference among study areas or years (GLM $X^2 = 4.68$, $P = 0.7$, df = 7). Age at time of sampling averaged 41 d (range = 19–71 d) and varied among study areas and years ($F_{6,646} = 7.17$, $P < 0.001$, $R^2_{adj} = 0.05$), likely reflecting our sampling schedules, but also local and regional weather patterns (see Discussion). Because age differed by study area, we included age in our models when testing for differences in morphological traits.

We back-calculated hatching date from age at time of handling for the 656 nestlings with measured P8 length (Table 1). From these hatching dates we then extrapolated the dates when eggs were laid using an average incubation period of 35 d (Herrick 1932, Stalmaster 1987). We estimated that Bald Eagles laid eggs as early as 24 January at MISS and as late as 18 April at APIS, a span of 85 d across the region. The
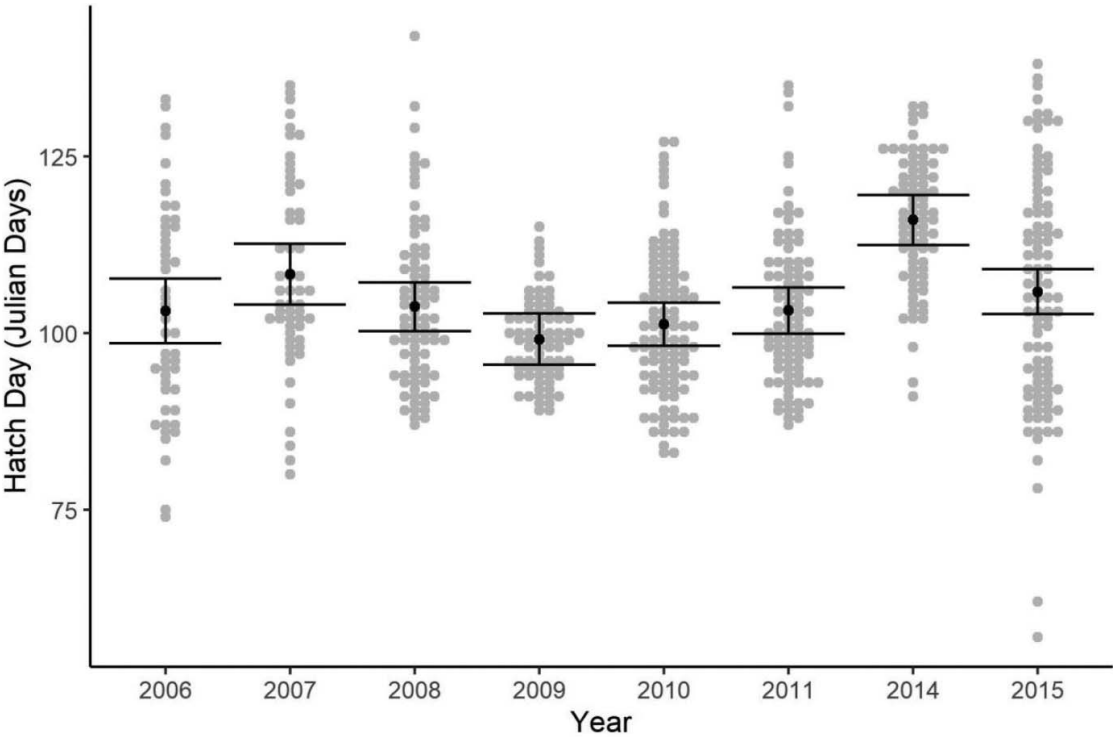
Figure 2.   Estimated mean Julian date of hatching with 95% CI for 656 nestlings at six study areas from 2006 through 2015 in Minnesota and Wisconsin. Each gray dot represents the estimated date of hatching for a nestling in that year. For reference: Julian day 75 = 16 March, Julian day 100 = 10 April, and Julian day 125 = 5 May.

hatching period, also spanning 85 d, began as early as 26 February at MISS and lasted through 22 May at APIS. The span of hatching and egg-laying dates within study areas ranged from 31 d at Pools3+4 to 69 d at MISS. As expected, mean egg-laying and hatching dates were earliest in southern study areas and later in the more northerly study areas (Table 1).

We found no annual trend in hatching dates over the 10-yr span of the study, though hatching dates were highly variable (Fig. 2). In 2009, all nestlings ($n = 77$) hatched within a 29-d period across the region while in 2015 hatching was spread across 81 d ($n = 99$). Mean hatch date for 2014 appeared later than for other years, but this difference was not statistically significant.

We found a strong correlation between the mean annual hatching date for nestlings across the six study areas and the average date of ice-out at the USACN and APIS ice monitoring locations ($n = 8$ yr; Spearman Rank $r^2 = 0.90$; Table S2).

Nestling Morphology. We constructed logistic growth curves for four morphological traits using P8 length as an index to age (Table 2, Fig. 3). Each nestling was measured once for each trait so that the curves do not reflect individual growth; rather they describe the average change in size for successively older nestlings. Measurements for both sexes increased at nearly the same rate for all four traits, and both sexes reached 90% of their footpad length, bill depth, and culmen chord length size by age 27 d, 41 d, and 52 d, respectively. However, males reached 90% of their full weight 3 d prior to females (52 d vs. 55 d, respectively; Fig. 3). We provide estimates for average size at three important ages: 35 d and 56 d, which bound the lower and upper ages we targeted for safe handling of nestlings, and 70 d, which we used as a lower bound for potential fledging age (Table 2).

For comparing morphological measurements among study areas, we excluded LSSS due to small sample size ($n = 26$). For the remaining study areas, measurements were highly variable (Table S5), but

Table 2. Predicted size of Bald Eagle nestlings at 35 d (5 wk), 56 d (8 wk), and 70 d (10 wk) and associated equations for four morphological traits. The eighth primary (P8) is a measured value used to predict age ($\pm \sim$3 d; Bortolotti 1984b). Other traits are predicted from logistic growth curves ($n = 642$ known-sex nestlings) using either age (d) or length of P8 (mm) to predict trait size for female and male nestlings from Wisconsin and Minnesota, 2006–2015.

SEX	MORPHOLOGICAL TRAIT	PREDICTED TRAIT SIZE AT INDEX AGES			PREDICTIVE EQUATIONS
		35 d	56 d	70 d	
Females	8th primary length (mm)[1]	103	253	353	Measured value[1]
	Weight (kg)	3.4	4.4	4.7	$Wt^F_{Age} = 4.83/(1+\exp(1.62 + -0.07*age))$
					$Wt^F_{P8} = 4.83/(1+\exp(0.32 + -0.010999*P8))$
	Footpad length (mm)	133.6	138.7	139.0	$Fp^F_{Age} = 139.035/(1+\exp(1.35 + -0.13*age))$
					$Fp^F_{P8} = 139.196/(1+\exp(-1.1737 + -0.0196*P8))$
	Bill depth (mm)	29.4	32.7	33.5	$Bd^F_{Age} = 34.167/(1+\exp(0.27 + -0.06*age))$
					$Bd^F_{P8} = 34.21/(1+\exp(-0.9177 + -0.00895*P8))$
	Culmen chord length (mm)	41.1	48.1	50.3	$Cl^F_{Age} = 52.53/(1+\exp(0.58 + -0.053*age))$
					$Cl^F_{P8} = 52.39/(1+\exp(-0.4409 + -0.0078*P8))$
Males	8th primary length (mm)[1]	113	263	363	Measured value[1]
	Weight (kg)	2.8	3.7	3.9	$Wt^M_{Age} = 3.98/(1+\exp(1.91 + -0.079*age))$
					$Wt^M_{P8} = 3.9/(1+\exp(0.31 + -0.010999*P8))$
	Footpad length (mm)	122.8	127.5	127.8	$Fp^M_{Age} = 127.86/(1+\exp(1.36 + -0.13*age))$
					$Fp^M_{P8} = 127.41/(1+\exp(-1.12898 + -0.0196*P8))$
	Bill depth (mm)	27.4	30.4	31.2	$Bd^M_{Age} = 31.835/(1+\exp(0.27 + -0.06*age))$
					$Bd^M_{P8} = 31.61/(1+\exp(-0.9123 + -0.00895*P8))$
	Culmen chord length (mm)	38.7	45.3	47.4	$Cl^M_{Age} = 49.48/(1+\exp(0.58 + -0.053*age))$
					$Cl^M_{P8} = 48.74/(1+\exp(-0.4350 + -0.0078*P8))$

[1] The 8th primary feather (P8) is measured and age is estimated from sex-specific equations presented by Bortolotti (1984b), where female age = 20.6+(0.14*P8); and male age = 19.2+(0.14*P8).

there was evidence that nestlings at USACN weighed less on average than nestlings at APIS, MISS, and Pools3+4, but not LSACN (mean-scaled weight contrasts: $t = 3.69$, $P = 0.003$; $t = 3.06$, $P = 0.03$; and $t = 4.00$, $P = 0.001$, respectively). We also found evidence that nestlings at APIS had larger footpads on average than at MISS, LSACN, and USACN, but not Pools3+4 (mean-scaled measurement contrasts: $t = -4.63$, $P < 0.001$; $t = -2.4$, $P = 0.02$; and $t = -2.75$, $P = 0.006$, respectively). Mean-scaled measurements of bill depth, culmen chord length, and hallux claw chord length differed among years ($t = -2.82$, $P = 0.005$; $t = -2.25$, $P < 0.03$; and $t = -3.97$, $P < 0.001$, respectively), but these three traits did not differ among study areas.

We further examined these morphological traits by grouping nestlings from the two Lake Superior study areas (APIS and LSSS) and comparing them to nestlings of the four river study areas (LSACN, MISS, Pools3+4, USACN). We found no difference in mean-scaled weight ($t = 1.25$, $P = 0.21$), bill depth ($t = -0.05$, $P = 0.96$), hallux claw chord length ($t = -0.94$, $P = 0.35$), or culmen chord length ($t = -1.89$, $P = 0.06$); however, the footpad length of Lake

Superior nestlings was larger than those from river study areas ($t = -3.94$, $P < 0.001$).

Criteria for Determining Sex. We determined the sex of nestlings genetically, but if we had used the single criteria that nestlings ≥35 d with footpads ≤135 mm were males and those with footpads >135 mm were females (i.e., Bortolotti 1984b), we would have misclassified the sex of 21.3% of the 524 nestlings older than 34 d. Moreover, this misclassification would have been biased toward males, with only 1.1% of males being misclassified as female, but 31.6% of females misclassified as male.

To improve upon the criteria for assigning sex from morphological measurements, we used data from 248 females and 276 males that were verified genetically and that had P8 length ≥102 mm. The 102-mm P8 length is associated with our indexed age of 35 d, our targeted lower age for handling. Moreover, like Bortolotti (1984a, 1984b), we found that size differences between males and females began to diverge after 35 d. We found that footpad length and bill depth were useful for developing a classification tree (Fig. 4). The classification tree correctly identified the sex of 477 of the 524
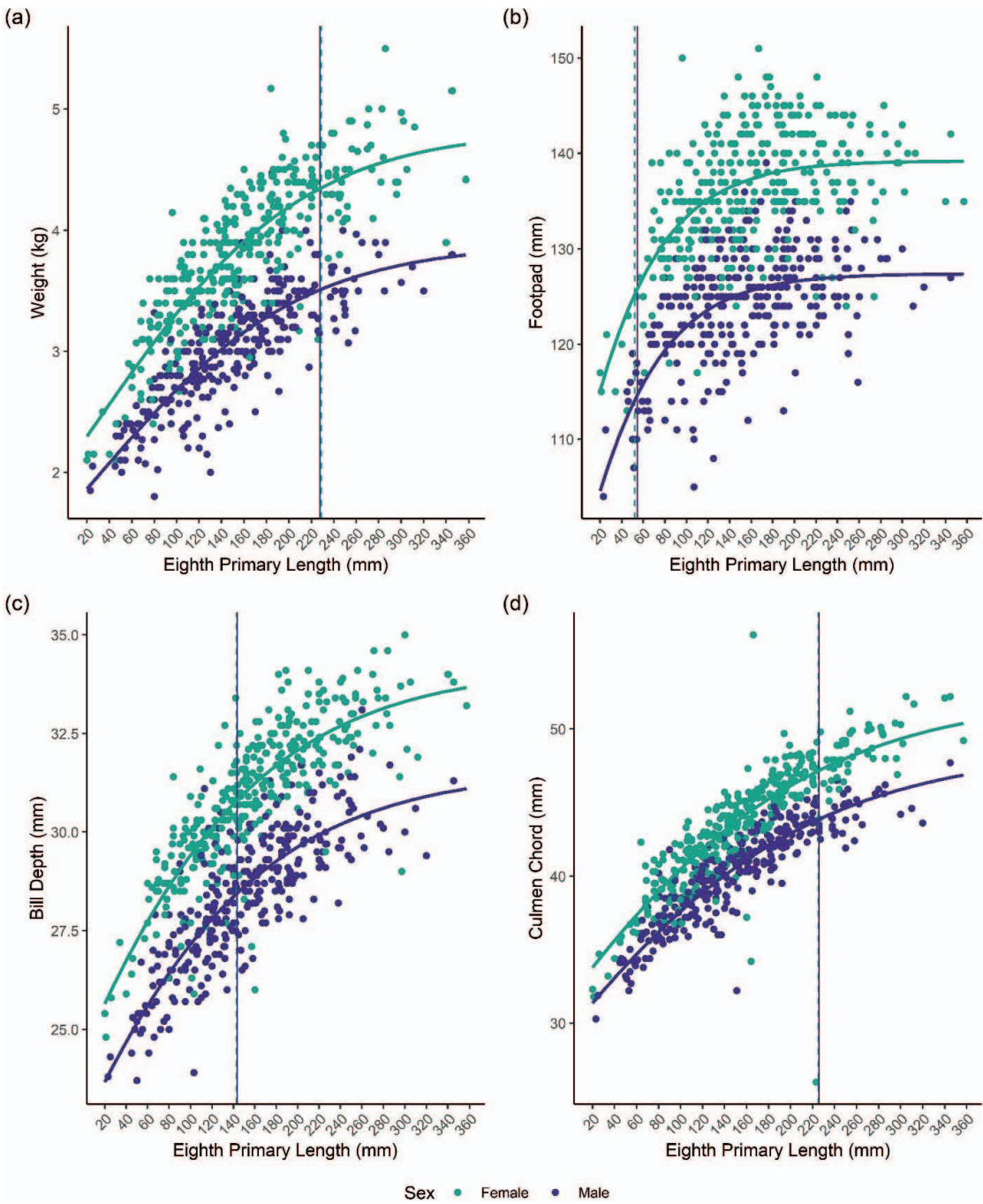
Figure 3.   Logistic growth curves showing increases for male (blue) and female (green) Bald Eagle nestlings in weight (panel a), footpad length (panel b), bill depth (panel c), and culmen chord length (panel d) against growth of the eighth primary feather as an index to age. Vertical line indicates the age at which the population reaches 90% of growth for that trait; solid is male, dashed for female.
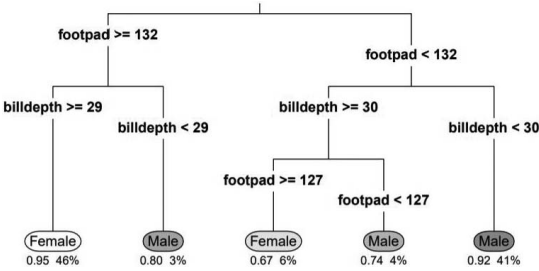
Figure 4. Classification tree to estimate the sex of Bald Eagle nestlings with P8 ≥ 102 mm (≥ ∼35 d) and up to P8 = 350 mm (∼77 d) in Wisconsin and Minnesota. The bottom row is the probability of a correct sex classification and the percent of observations that fell into each category for 524 nestlings (n = 248 females, 276 males) in six study areas. The footpad measurements are most appropriate for nestlings positioned on their bellies when measurements are made.

nestlings (91.0%) and was unbiased (90.9% of females and 91.1% of males were correctly identified). On the left side of Fig. 4, individuals with footpad length ≥132 mm and bill depth ≥29 mm had a 0.95 probability of being female and accounted for 46% of our sample. Another 3% on the left side had footpads ≥132 mm, but bill depths <29 mm and were males with a probability of 0.80. On the right side of Fig. 4, individuals with footpad length <132 mm and bill depth <30 mm had a 0.92 probability of being male and accounted for 41% of the nestlings. On the right side, we also found 10% of the nestlings had footpads <132 mm, but bill depths ≥30 mm and required another application of footpad length to separate females with footpads ≥127 mm (6% of nestlings with probability of 0.67) from males that had footpads <127 mm (4% of nestlings with a probability of 0.74; Fig. 4). Overall, footpad length and bill depth explained 73% and 3.6% of the variation, respectively.

DISCUSSION

Breeding Phenology. We found no linear trend or shift in breeding phenology based on egg-laying and hatching dates, though the short time span of our study and high annual variation may have masked any longer-term trends. Long, north-south oriented study areas had the greatest variation in breeding phenology and the east-west oriented study areas the shortest, reflecting the effect of latitude and the return of warm weather each spring. Access to open (i.e., ice-free) water is important for Bald Eagles due to their high reliance on the aquatic food web (Grim and Kallemeyn 1995) and we found the onset of the breeding season each year was associated with the timing of ice-breakup at two locations. The USACN ice-monitoring location is central to all of our study areas and the ice around the Apostle Islands (APIS location) on Lake Superior is fairly stable because it is buffered from winds that can breakup and disperse ice on this large body of water. Others have found that ice cover is a good integrator of mean winter temperatures over broad areas (Quinn et al. 1978); thus, these two ice monitoring locations are likely indicative of the duration and severity of winter across our study region, which in turn influences Bald Eagle breeding.

Weather extremes during our study provide examples of the synchrony between Bald Eagle breeding phenology and ice conditions. The latest mean hatching date occurred in 2014 (Fig. 2) when the ice did not break up on the St. Croix River until 10 April, 15 d later than the 32-yr average at the USACN ice monitoring site. Moreover, the ice left the Apostle Islands that year on 13 May, 45 d later than the average of the 8 yr of satellite imagery we reviewed. The late hatching in 2014 followed a winter and spring (January through May) of temperatures that were below the 30-yr average each month at the Minneapolis/St Paul Airport adjacent to the MISS study site (Fig. S1). By contrast, the earliest mean hatching date in our study was in 2009, when the ice left the St. Croix River at Grantsburg on 21 March, 5 d earlier than the 32-yr USACN ice-out average, and left the Apostle Islands on 17 April, about 2 wk later than the 8-yr average and nearly a month earlier than in 2014.

Early arrival to nesting areas is important for gaining or retaining prime territories (Turrin and Watts 2014); however, early nesting can also increase exposure to weather extremes. The two earliest hatching dates in our study corresponded to egg-laying dates of 23 and 28 January 2015 at a nest in MISS. In 2015, a year of above-average winter and spring temperatures (Fig. S1), nestlings from this nest survived to fledging, but in two of five years following our study, egg-laying in January resulted in breeding failure due to late winter storms that caused the eggs to freeze (Minnesota Department of Natural Resources, unpubl. data).

Morphology. As expected, female Bald Eagle nestlings reached significantly greater size than males for all four traits we measured, but there was overlap, especially up to 35 d (Fig. 3). Males attained

90% of their highest weight slightly earlier than females (about 3 d) while footpad size, bill depth, and culmen chord length increased at nearly the same rate in both sexes. Our models suggest that nestlings continued to grow beyond the 70-d period for which we had measurements, except for their footpad length, for which both sexes reached predicted full size by about 46 d. Our findings of sexual size dimorphism, the extent and timing of divergence in size between sexes, the similarity in growth rates between sexes, and the continued growth of all nestlings beyond 70 d mirror the findings of Bortolotti (1984a, 1984b).

We found no difference in nestlings' bill depth, culmen chord length, or hallux claw chord length among the different study areas. These traits differed by age as would be expected for growing nestlings, but we also found they differed by year. Differences among years could indicate that these traits are affected by stochastic events that cause variation in size for an annual cohort. Data from both Bortolotti's (1984b) study and ours show that the hallux claw and the bill (both length and depth) grow slowly (Fig. 3c, 3d). Bortolotti (1984b), who also cited work by Ricklefs (1979), theorized that the bill and hallux claw have low priority for growth during the nestling stage. By comparison, growth of the footpad (important for stability in the nest and grasping food provided by the adults) and primary feathers (necessary for their first flight) receive higher priority. Hence, it could be hypothesized that lower priority for growth of the bill and hallux claw make these traits more vulnerable to annual variations in food availability.

We found evidence that nestlings at USACN weighed less than nestlings at other study areas, though this difference was not statistically significant between USACN and LSACN, which are adjacent segments of the same river. Food habits and detailed habitat characteristics were beyond the scope of our study, but potentially, the type and size of prey and/or the narrow riverine habitat of the upper St. Croix Riverway (USACN) could have an effect on nestling weight. Similarly, Bald Eagles nesting in mesohaline reaches of Lower Chesapeake Bay (USA) provided more energy-rich prey to their nestlings compared to nearby eagles in tidal-fresh waters, resulting in higher growth rates for the better-fed nestlings (Markham and Watts 2008).

We also found evidence that nestlings from Lake Superior (APIS and LSSS) had larger footpads than nestlings on river study areas (USACN, LSACN,

MISS, and Pools3+4; $t=-3.94$, $P<0.001$), though no other traits differed significantly between these two groups. We dropped LSSS from the individual study area comparisons due to small sample size, but in that analysis, we also found the footpad lengths of nestlings at APIS to be larger than those of nestlings at MISS, LSACN, and USACN, but not Pools3+4. Grouping nestlings by Lake Superior vs. river study areas allowed us to use the LSSS data and examine more thoroughly the potential difference in footpad size. Others have shown that intraspecific differences in trait size of raptors can occur across a species' geographic range and these differences are often attributed to type of food available, which in turn is influenced by habitat, climate, migratory behaviors, and geography. For example, museum specimens of Northern Goshawks (*Accipiter gentilis*) from Finland collected between 1960–1990 showed that wing and tail lengths increased for both sexes of adults, and the increase was attributed to shifts in the size of their prey over the 10–30 yr span (Tornberg et al. 1999). Bald Eagles began repopulating the Apostle Islands (APIS study area) in about 1983 (Kozie 1986), providing 23–33 yr (6–7 generations) for adaptive change in morphological features to occur. However, there is currently no indication that Lake Superior Bald Eagles are genetically distinct from inland populations. Future research would need to consider genetic relatedness as well as the type and size of prey available to eagles at different study areas.

We caution that there was a great deal of variation and overlap in morphological measurements among study areas and years, which suggests some uncertainty in the above comparisons. The differences we found in all four traits could be influenced by small sample size for some age classes in some study areas and years. Moreover, we necessarily used P8 length as a proxy for age, and we assumed P8 growth to be consistent among study areas and years. Although many raptor ecologists use flight feathers as an index to age (Penak et al. 2013 and references therein), there remains a need for additional study to confirm these assumptions. Hence, we believe the morphological differences we found need additional verification.

Sex Determination. Bald Eagle nestlings should be sexed genetically, but for times when this is not possible, we provide a classification tree using footpad length and bill depth to give an unbiased estimate of sex for nestlings ≥35 d. Both Bortolotti's (1984a, 1984b) and our data showed that the early

divergence in size of the footpad, coupled with the fact that footpad length reaches its asymptote well before fledging (Fig. 3b), makes footpad measurement the most useful for discriminating between the sexes. However, we found that 132 mm, rather than the 135 mm used by Bortolotti (1984b) was a better cutoff for determining sex in our study areas. The 3-mm difference could reflect an overall smaller size of nestlings in our population. Our study region was 1045 km south of Bortolotti's study area in north-central Saskatchewan and others have shown (e.g., Stalmaster 1987) that adult Bald Eagles in northern, cooler environments are usually larger than those in more southerly, warmer areas of North America.

It is also likely that the method of measuring the footpad contributed to the difference between our study and that of Bortolotti (1984b). The position of Bald Eagle nestlings during footpad measurement varies by handler and is not well documented (W. W. Bowerman, M. Meyer, J. Papp, and S. Strom pers. comm.). We found that footpad measurements average 2.1 mm shorter when nestlings are positioned on the belly. When nestlings are placed on their back, the handler must overcome the tendency for nestlings to clench their feet, which becomes increasingly difficult as the nestling grows. The effort may result in the footpad being slightly overextended compared to a more relaxed foot of a nestling placed on its belly—or alternatively, the footpad could be less extendable from the belly position. Regardless, it is difficult to get consistent measurements of flexible, muscled body parts and we found that consistent positioning of nestlings for footpad measurements is important for minimizing measurement bias. We found no significant difference in morphometric measurements among our three crews over the 8 yr. Our three primary observers trained together each year and agreed early in the study on the position of nestlings for footpad measurements.

There may be other morphological traits for determining sex that we did not measure. Some researchers have used length and/or width of the tarsus as a trait for sexing adult Bald Eagles (e.g., Garcelon et al. 1985), although Bortolotti (1984b) found high measurement variance in this trait for Bald Eagle nestlings. Trait size criteria for discriminating between sexes could also differ by area. Our classification tree is most appropriate for Bald Eagle nestlings in the upper Midwestern USA and may not apply elsewhere. For example, Pitzer et al. (2008) found that the effectiveness of a discriminant function using tarsus width, hallux claw chord length, and wing chord length to estimate the sex of nestling Red-tailed Hawks (*Buteo jamaicensis*) varied at two study areas in California with the percent incorrectly sexed ranging from 8.7% to 20.7%.

Conclusions. We found that annual Bald Eagle egg-laying and hatching dates were correlated with ice-breakup during spring at two locations. Open (ice-free) water is necessary for adults to access the aquatic prey they require for feeding nestlings. Moreover, the timing of spring ice-thaw can reflect the severity and duration of winter weather. One of the hallmarks of a warming climate is increased variability in weather at local scales (Kunkle et al. 2014). Hence the timing of ice-breakup can be an important metric for monitoring eagle breeding activities and how weather might affect them.

When possible, genetic analyses should be used to determine the sex of Bald Eagle nestlings. However, if genetic samples are unavailable, a classification tree based on footpad length and bill depth allows investigators to estimate of sex of Bald Eagle nestlings 35 d or older in the upper Midwestern USA.

SUPPLEMENTAL MATERIAL (available online). Table S1: Geographic coordinates of approximate center of the six study areas and the furthest points in the four cardinal directions across the study region in Minnesota and Wisconsin. Table S2: Average annual Julian hatching dates for Bald Eagle nestlings at six study areas and the region (average of the six) compared to the corresponding dates when ice was estimated to leave APIS and USACN and the average of these two ice-out dates. Table S3: Methods for taking physical measurements of Bald Eagle nestlings. Table S4: Quality assurance test showing no significant differences in morphological measurements obtained by three primary handlers. Table S5: Descriptive statistics for six morphological measurements and estimated age of 642 nestling Bald Eagle nestlings sampled at six study areas over eight years in Minnesota and Wisconsin. Figure S1: Monthly maximum and minimum temperatures (°F) and precipitation (% of average) departures from the 30-year average (1971–2000) from January 2006 through June 2015 as measured at the Minneapolis / St Paul International Airport.

LITERATURE CITED

Baker, R., and Y. A. Monstad (2006). Minnesota Bald Eagle Surveys. Nongame Wildlife Program, Minnesota Department of Natural Resources Report, St. Paul, MN, USA. http://files.dnr.state.mn.us/eco/nongame/projects/eagle_report_2005.pdf.

Berner, D. (2011). Size correction in biology: How reliable are approaches based on (common) principal component analysis? Oecologia 166:691–971.

Bortolotti, G. R. (1984a). Physical development of nestling Bald Eagles with emphasis on the timing of growth events. Wilson Bulletin 96:524–542.

Bortolotti, G. R. (1984b). Criteria for determining age and sex of nestling Bald Eagles. Journal of Field Ornithology 55:467–481.

Bowerman, W. W., A. S. Roe, M. J. Gilbertson, D. A. Best, J. Sikarskie, R. S. Mitchell, and C. Summer (2002). Using Bald Eagles to indicate the health of the Great Lakes' environment. Lakes and Reservoirs Research and Management 7:183–187.

Cain, S. L. (1985). Nesting activity time budgets of Bald Eagles in southeast Alaska. MS thesis, University of Montana, Missoula, MT, USA.

DeSorbo, C. R., N. M. Burgess, P. E. Nye, J. J. Loukmas, H. A. Brant, M. E. H. Burton, C. P. Persico, and D. C. Evers (2020). Bald Eagle mercury exposure varies with region and site elevation in New York, USA. Ecotoxicology 29:1–15.

Dykstra, C. R., J. L. Hays, M. M. Simon, and A. R. Wegman (2021). Breeding phenology of Red-shouldered Hawks (Buteo lineatus) is related to snow cover and air temperature during the pre-laying period. Frontiers in Ecology and Evolution 9:658390. doi: 10.3389/fevo.2021.658390.

Garcelon, D. K., M. S. Martell, P. T. Redig, and L. C. Buøen (1985). Morphometric, karyotypic, and laparoscopic techniques for determining sex in Bald Eagles. Journal of Wildlife Management 49:595–599.

Grier, J. W. (1982). Ban of DDT and subsequent recovery of reproduction in Bald Eagles. Science 218:1232–1235.

Grim, L. H., and L. W. Kallemeyn (1995). Reproduction and Distribution of Bald Eagles in Voyageurs National Park, Minnesota, 1973–1993. US Department of the Interior, National Biological Service Biological Science Report 1. Washington, DC, USA.

Herrick, F. H. (1932). Daily life of the American eagle: Early phase. The Auk 49:307–323.

Karels, T. J., A. A. Bryant, and D. S. Hik (2004). Comparison of discriminant function and classification tree analyses for age classification of marmots. Oikos 105:575–587.

Kozie, K. D. (1986). Breeding and feeding ecology of Bald Eagles in Apostle Islands National Lakeshore. MS thesis, University of Wisconsin–Stevens Point, Stevens Point, WI, USA.

Kunkle, K. E., R. S. Vose, L. E. Stevens, and R. W. Knight (2014). Is the monthly temperature climate of the United States becoming more extreme? Geophysical Research Letters 42:629–636.

Lenth, R. (2016). Least-squares means: The R package lsmeans. Journal of Statistical Software 69:1–33.

Madsen, C. R., T. J. Sheldrake, J. T. Leach, and J. Engel (1985). Bald Eagle Production in the Great Lakes States 1973–1985. US Fish and Wildlife Service, Twin Cities, MN, USA.

Markham, A. C., and B. D. Watts (2008). The influence of salinity on provisioning rates and nestling growth in Bald Eagles in the lower Chesapeake Bay. The Condor 110:183–187.

Milborrow, S. (2021). An enhanced version of plot.rpart. R package version 3.1.0. https://CRAN.R-project.org/package=rpart.plot.

Morinha, F., J. A. Cabral, and E. Bastos (2012). Molecular sexing of birds: A comparative review of polymerase chain reaction (PCR)-based methods. Theriogenology 78:703–714.

O'Connor, R. J. (1977). Differential growth and body composition in altricial passerines. Ibis 119:147–166.

Olsen, P. D., and J. Olsen (1987). Estimating the age of nestling raptors. Australian Bird Watcher 12:130–131.

Olsen, P. D., J. Olsen, and N. J. Mooney (1982). Growth and development of nestling Brown Goshawks, Accipiter fasciatus, with details of breeding biology. Emu – Austral Ornithology 82:189–194.

Penak, B. L., C. R. Dykstra, S. J. Miller, and D. M. Bird (2013). Using morphometric measurements to estimate age of nestling Red-shouldered Hawks in two eastern populations. Wilson Journal of Ornithology 125:630–637.

Pitzer, S., J. Hull, H. B. Ernest, and A. C. Hull (2008). Sex determination of three raptor species using morphology and molecular techniques. Journal of Field Ornithology 79:71–79.

Postupalsky, S. (1983). Techniques and terminology for surveys of nesting Bald Eagles: Appendix D. In

Northern States Bald Eagle Recovery Plan (J. W. Grier, J. B. Elder, F. J. Gramlich, N. F. Green, J. V. Kussman, J. E. Mathisen, and J. P. Mattsson, Editors). US Department of Interior, Fish and Wildlife Service, Twin Cities, MN, USA.

Quinn, F. H., R. A. Assel, D. E. Boyce, G. A. Leshkevich, C. R. Snider, and D. Weisnet (1978). Summary of Great Lakes Weather and Ice Conditions, Winter 1976–77. US Department of Commerce, National Oceanic and Atmospheric Administration. Technical Memorandum ERL GLERL-20. Great Lakes Environmental Research Laboratory, Ann Arbor, MI, USA.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ricklefs, R. E. (1968). Patterns of growth in birds. Ibis 110:419–451.

Ricklefs, R. E. (1979). Adaptation, constraint, and compromise in avian postnatal development. Biological Reviews 54:269–290.

Route, B., W. Bowerman, and K. Kozie (2009). Protocol for Monitoring Environmental Contaminants in Bald Eagles, Version 1.2: Great Lakes Inventory and Monitoring Network. Natural Resource Report NPS/GLKN/NRR—2009/092. National Park Service, Fort Collins, CO, USA.

Route, B., and D. VanderMeulen (2018). Quality Assurance Plan for Monitoring Environmental Contaminants in Bald Eagle Nestlings. Great Lakes Inventory and Monitoring Network, Natural Resource Report NPS/GLKN/NRR-2018/1848. National Park Service, Fort Collins, CO, USA.

Route, B., D. VanderMeulen, R. Key, W. Bowerman, and K. Kozie (2019). Protocol for Monitoring Environmental Contaminants in Bald Eagles, Version 2.0: Great Lakes Inventory and Monitoring Network, Natural Resource Report NPS/GLKN/NRR-2019/1983. National Park Service, Fort Collins, CO, USA.

Scharf, W. C., and E. Balfour (1971). Growth and development of nestling Hen Harriers. Ibis 113:323–329.

Stalmaster, M. V. (1987). The Bald Eagle. Universe Publications, New York, NY, USA.

Therneau, T., and B. Atkinson (2019). rpart: Recursive partitioning and regression trees. R package version 4.1–15. https://CRAN.R-project.org/package=rpart.

Tornberg, R., M. Mönkkönen, and M. Pahkala (1999). Changes in diet and morphology of Finnish goshawks from 1960s to 1990s. Oecologia 121:369–376.

Turrin, C., and B. D. Watts (2014). Intraspecific intrusion at Bald Eagle nests. Ardea 102:71–78.

US Fish and Wildlife Service (2022). Eagle permits: Updated Bald Eagle population estimates and take limits. Federal Register 87:5493–5495.

Wiemeyer, S. N., T. G. Lamont, C. M. Bunick, C. R. Sindelar, F. J. Gramlich, and J. D. Fraser (1984). Organochlorine pesticide, polychlorobiphenyl, and mercury residues in Bald Eagle eggs, 1969–79, and their relationships to shell thinning and reproduction. Archives of Environmental Contamination and Toxicology 13:529–549.

Wisconsin Department of Natural Resources (2016). Bald Eagle and Osprey Nest Survey Protocol – Version 1.1. Wisconsin Department of Natural Resources, Madison, WI, USA.