Tools and Technology Note

Effects of Tissue Collection Methods on Morphometrics and Survival of Captive Neonatal Northern Bobwhite

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ABSTRACT We assessed effects of tissue collection methods (i.e., patagial microbiopsy and down feathers) and chick age at sampling on morphometrics and 21-day survival of 600 captive neonatal northern bobwhite (*Colinus virginianus*). We observed minimal effects on morphometrics and no difference in survival among patagial microbiopsy ($\bar{x} = 0.96 \pm 0.03$), down feathers ($\bar{x} = 0.92 \pm 0.04$), and control ($\bar{x} = 0.86 \pm 0.05$) methods. DNA analysis from patagial microbiopsy, down feather, and egg tooth samples showed greater concentrations of DNA from patagial microbiopsy ($\bar{x} = 10.28 \pm 1.74$ μg/ml) than either down feather ($\bar{x} = 4.10 \pm 1.74$ μg/ml) or egg teeth ($\bar{x} = 2.35 \pm 1.74$ μg/ml). (JOURNAL OF WILDLIFE MANAGEMENT 73(7):1241–1244; 2009)

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When sampling live individuals for genetic analysis, it is essential to collect tissue samples in a manner that minimizes deleterious effects on the subject (Frederick 1986, Colwell et al. 1988, Lukacs and Burnham 2005, Waits and Paetkau 2005). In studies that examine genetic relatedness of precocial chicks within broods and to brood-tending adults, it is necessary that chicks are sampled as soon as possible after hatching to ensure that all chicks in a brood are represented and that brood mixing has not yet occurred. When conducted correctly, procedures including muscle and tissue biopsies and feather removal have been shown to have little or no effect on survival of adult birds (Marsden and May 1984, Westneat 1986, Westneat et al. 1986, Morin et al. 1994). Alternatively, special consideration must be taken when sampling neonates because they are susceptible to handling, exposure, and injury (Stangel and Lennartz 1988). Unfortunately, no studies to date have examined effects of tissue collection methods on the morphological development and survival of precocial neonates.

Success of tissue collection for population genetic analysis is also dependent on the quantity of extractable DNA derived from tissue samples. Feather sampling offers a reasonable alternative to more invasive sampling methods such as muscle biopsy or blood extraction (Taberlet and Bouvet 1991, Ellegren 1992, Morin et al. 1994, Pearce et al. 1997, Segelbacher 2002), and down feathers easily can be removed from precocial chicks. However, the amount of DNA that can be extracted from feather samples varies based on feather size and age and is notably lower than amounts available in tissue or blood samples (Segelbacher 2002). Likewise, the low quantity of DNA extracted from feather samples makes DNA amplification more difficult and subsequent analyses prone to genotyping errors (Segelbacher 2002). Alternatively, tissue microbiopsy is a minimally invasive procedure that allows for extraction of a small core tissue sample from the wing patagium using a cylindrical razor punch, and high levels of genotyping success (99%) have been reported for DNA derived from patagial microbiopsy samples of bobwhite neonates (Faircloth 2008).

Our objective was to quantify effects of 2 tissue collection methods (i.e., patagial microbiopsy and plucking of down feathers) on morphometrics and survival of captive neonatal bobwhite up to 21 days after hatching. To isolate specific effects of tissue collection on morphometrics and survival, we compared data from chicks subject to tissue collection to that of similarly handled chicks from which we collected no tissue samples. We also report a cursory evaluation of DNA quantity derived from 3 tissue types (i.e., patagial microbiopsy, down feathers, and egg tooth) to verify expectations of DNA yield for these tissues based on previous research by Segelbacher (2002) and Faircloth (2008).

METHODS

We assessed effects of tissue collection on measures of morphometrics and survival by collecting patagial tissue microbiopsy and down feather samples from captive neonatal bobwhites in 2001. All bird handling and tissue collection procedures were reviewed and approved by the Mississippi State University Institutional Animal Care and Use Committee (protocol 01-051). We randomly distributed 600 chicks to 12 factorial treatment combinations (50 chicks/combination) of 3 tissue collection methods (i.e., tissue microbiopsy, down feathers, and control) taken at 4 ages (1 day, 3 days, 6 days, and 10 days posthatch). We then assigned groups of 5 chicks from each tissue collection method and age at tissue collection combination (12 treatment combinations) to each of 10 temperature-

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controlled brooders (60 chicks/brooder, total of 600 chicks). We marked chicks with color-coded leg bands denoting specific treatment combinations on day 1 posthatch.

Tissue collection method treatments consisted of skin microbiopsy samples from the left wing patagium (2 mm posterior to the wing tendon and anterior to the humerus) using a 1.5-mm sterile microbiopsy punch (Miltex, Inc., York, PA), removal of 10 down feathers from the ventral tract, and control (handling for morphometric measurements but no tissue collection). We measured mass (g), tarsus length (mm), bill length (mm), wing chord length (mm), and first primary feather length (mm) of each chick at 21 days posthatch. We evaluated the influence of tissue collection method and chick age at tissue collection on mass and morphometrics using measurements at 21 days posthatch as dependent variables.

To qualitatively assess the potential of various tissue types for DNA extraction and amplification, we removed tissue samples of 3 types (i.e., down feather, patagial microbiopsy, and egg tooth) from each of 26 neonatal bobwhites (1–2 days posthatch) in 2001 ($n = 13$) and 2002 ($n = 13$). Sample size was limited by the number of chicks still possessing attached egg teeth upon sampling (the egg tooth is naturally shed by bobwhite neonates the first day after hatching). Each chick was subject to removal of 10 down feathers from the ventral tract, tissue biopsy from the left wing patagium using a 1.5-mm sterile microbiopsy punch, and removal of the egg tooth using sterile surgical gloves and the blunt end of a surgical blade if necessary. We stored feather samples in 0.5 ml of 70% ethanol (AMRESCO, Inc., Solon, OH), and we stored patagial tissue and egg tooth samples in 0.2 ml of Tris buffer solution, pH 8.0 (1 M Tris, 1 M NaCl, 0.5 M ethylenediaminetetraacetic acid, and 10% sodium dodecyl sulfate; AMRESCO, Inc.). We extracted DNA from samples using a DNeasy tissue extraction kit (QIAGEN Sciences, Germantown, MD). We added dithiothreitol (AMRESCO, Inc.) to feather samples to assist in breakdown of keratinized materials.

We arranged factorial combinations of tissue collection method and chick age at tissue collection in a generalized randomized complete block design, with brooders as blocks. We evaluated mass and morphometric measurements at 21 days posthatch using a mixed model analysis of variance (ANOVA) in SAS PROC MIXED (Littel et al. 1996). We treated blocks as a random effect and tissue collection method and chick age at tissue collection as fixed effects. After a significant interaction, we examined effect of tissue collection method within levels of chick age at tissue collection using the SLICE option in SAS PROC MIXED. We only evaluated main effects of tissue collection method and chick age at tissue collection in the absence of a significant interaction. After a significant main effect of tissue collection method, we compared model adjusted means among tissue collection methods, using the least square means (LSMEANS) procedure in SAS PROC MIXED.

We used a Cox proportional hazard model in SAS PROC PHREG (Allison 1995) to test for effect of tissue collection on survival from day 1 to day 21 posthatch. We used mass-at-hatch as a continuous covariate and tissue collection method as a time-varying covariate to control for different ages at sampling (Allison 1995). We estimated treatment combination specific 21-day survival using the Kaplan–Meier product-limit estimator (Allison 1995).

We measured DNA and double-stranded DNA concentrations of 20 randomly selected extractions from each tissue type using a GeneSpec spectrophotometer (Hitachi America, Ltd., Tarrytown, NY; absorption [abs] 260 nm, 280 nm) spectrophotometer and a 96-well microplate fluorometer (PicoGreen reagent [Invitrogen, Carlsbad, CA] at 538-nm abs). We evaluated differences in DNA concentrations (ng/μl) among patagial microbiopsy, down feathers, and egg teeth using a mixed model ANOVA in SAS PROC MIXED (Littel et al. 1996), blocked for individual chicks. We treated blocks as a random effect and tissue collection method as a fixed effect. After a significant main effect, we used measures of least square means (LSMEANS) to compare DNA concentration among sampling methods.

RESULTS

We observed no interaction ($P > 0.05$) between tissue collection method and chick age at tissue collection for mass or morphometric measures of bill length, wing chord, and length of first primary at 21 days of age. We observed an interaction between tissue collection method and chick age at tissue collection for measurements of tarsus length ($F_{6, 533} = 2.57$, $P = 0.02$). Within the group that received feather removal, tarsus length varied among chick age at tissue collection groups ($F_{3, 533} = 2.92$, $P = 0.03$), with mean tarsus length (mm) for chicks sampled at day 1 ($\bar{x} = 21.32$, SE = 0.45) greater than all other ages ($\bar{x}_3 = 19.84$, SE = 0.44; $\bar{x}_6 = 19.76$, SE = 0.44; $\bar{x}_{10} = 21.10$, SE = 0.43). In addition, for the group we sampled at day 1, tarsus length varied among tissue collection methods ($F_{2, 533} = 6.25$, $P = 0.002$), with mean tarsus length (mm) for chicks subject to feather sampling ($\bar{x} = 21.32$, SE = 0.45) greater than all other sampling methods ($\bar{x}_{\text{microbiopsy}} = 20.21$, SE = 0.44; $\bar{x}_{\text{control}} = 9.16$, SE = 0.46).

Neither mean mass, bill length, nor length of the first primary at 21 days differed relative to tissue collection method ($F_{2, 533} = 1.22$, $P = 0.30$; $F_{2, 533} = 0.09$, $P = 0.91$; $F_{2, 530} = 0.58$, $P = 0.55$) or chick age at tissue collection ($F_{3, 533} = 1.28$, $P = 0.28$; $F_{3, 533} = 0.77$, $P = 0.51$; $F_{3, 530} = 0.72$, $P = 0.54$). There was no effect of tissue collection method on wing chord length ($F_{2, 532} = 0.16$, $P = 0.85$); however, we observed an effect of chick age at tissue collection ($F_{3, 532} = 2.90$, $P = 0.03$) on wing chord length. Raw effect sizes (in mm) for chick age at tissue collection on wing chord length ranged from −0.51 to 1.65, with effect sizes between 1 day and 10 days posthatch ($t_{532} = 2.00$, $P = 0.05$) and between 3 days and 10 days posthatch ($t_{532} = 2.91$, $P = 0.004$). Wing chord length at 21 days was least ($\bar{x} = 72.50$, SE = 0.45) for the group we sampled at 10 days and greatest for chicks we sampled at 3 days ($\bar{x} = 74.15$, SE = 0.46).

Table 1. Mean mass-at-hatch (g) of neonatal northern bobwhite in Mississippi State, Mississippi, USA, and effect sizes of 2 sampling methods (i.e., down feather plucking and patagial microbiopsy) and a control, sampled at 4 different ages (in days posthatch), 2001.

Treatment	Age at sampling	*n*	Mean mass	SD	Effect size
Control	1	50	7.67	1.14	
	3	50	7.80	1.13	
	6	50	7.75	1.28	
	10	50	7.90	1.17	
	Overall	200	7.83	1.18	
Down feather	1	50	7.64	1.13	0.03
	3	50	7.86	1.13	0.13
	6	50	7.62	1.00	0.12
	10	50	7.96	1.09	0.05
	Overall	200	7.77	1.09	0.06
Microbiopsy	1	50	7.75	0.92	0.07
	3	50	7.69	0.97	0.29
	6	50	7.78	0.94	0.02
	10	50	7.85	1.02	0.05
	Overall	200	7.77	0.96	0.06

Tissue collection method did not affect survival from day 1 to day 21 posthatch ($\chi_1^2 = 0.04$, $P > 0.84$); however, mass-at-hatch had a strong influence on survival ($\chi_1^2 = 88.00$, $P < 0.001$). The hazard ratio for the covariate mass-at-hatch (0.09) indicated that for each 1-g increase in mass-at-hatch, risk of mortality decreases by an estimated 90.60% (Allison 1995). Thus, we conducted an a posteriori analysis to determine whether, through random assignment of chicks to treatments, the significant effect of mass-at-hatch on survival was unintentionally confounded with treatments by calculating effect sizes of mean mass-at-hatch for each treatment combination (Table 1). Effect sizes (d) were small (d < 0.20 g) for each treatment and age combination, with the exception of patagial microbiopsy taken at 3 days posthatch (d = 0.29 g; Table 1). Therefore, our data suggest no substantive differences among treatments in mass-at-hatch associated with random assignment of chicks to treatment combinations. Kaplan–Meier survival from hatch to 21 days of chicks we sampled at day 1 varied from 0.86 (SE = 0.05) for control to 0.92 (SE = 0.04) for feather collection and 0.96 (SE = 0.03) for tissue microbiopsy (Fig. 1).

Based on GeneSpec analysis, DNA concentrations differed among patagial microbiopsy, down feather, and egg tooth samples ($F_{2, 24} = 5.92$, $P = 0.01$). We extracted a greater quantity of DNA from patagial microbiopsy samples ($\bar{x}$ = 10.28 μg/ml; SE = 1.74) than either down feathers ($\bar{x}$ = 4.10 μg/ml; SE = 1.74) or egg teeth ($\bar{x}$ = 2.35 μg/ml; SE = 1.74). Similarly, double-stranded DNA concentrations also differed among the 3 sampling methods ($F_{2, 38} = 3.42$, $P = 0.04$). Mean double-stranded DNA concentration in patagial microbiopsy samples ($\bar{x}$ = 0.99 μg/ml; SE = 0.18) was nearly twice greater than that of egg tooth samples ($\bar{x}$ =0.48 μg/ml; SE = 0.18) and 2.5 times greater than that of down feather samples ($\bar{x}$ = 0.38 μg/ml; SE = 0.18).

DISCUSSION

Several studies of adult birds (Marsden and May 1984, Westneat et al. 1986, Colwell et al. 1988) and one study of altricial red-cockaded woodpecker (*Picoides borealis*) nestlings (Stangel and Lennartz 1988) reported that collection of different tissue types, including muscle, feathers, and blood, has little effect on survival of individuals. Currently, our study is the only systematic attempt to evaluate effects of tissue sampling using patagial microbiopsy or plucking of down feathers on neonatal northern bobwhite. A critical evaluation of tissue sampling methods for neonatal bobwhite is necessary before implementation of field studies that incorporate this methodology.

Chick age at tissue collection had a small effect on measures of wing chord length, with minimal effects when we collected samples between days 1 and 3 and greatest effect when we collected samples 10 days posthatch. Shorter wing chord lengths of chicks sampled at older ages may suggest earlier sampling (i.e., days 1–3 posthatch) has less effect on morphometrics than sampling at older ages (i.e., day 10 posthatch). However, there was an effect on chicks subject to sampling at day 1 posthatch on measures of tarsus length, indicating that it may be better to remove feathers on or after day 3 posthatch. Measures of mass, bill length, and length of first primary feather were unaffected by tissue collection method or chick age at tissue collection. Collectively, these results suggest that, regardless of tissue collection method, sampling on or approximately 2–3 days posthatch might minimize potential deleterious effects of tissue sampling.

Survival of captive bobwhite neonates was not affected by patagial microbiopsy or collection of down feathers in our

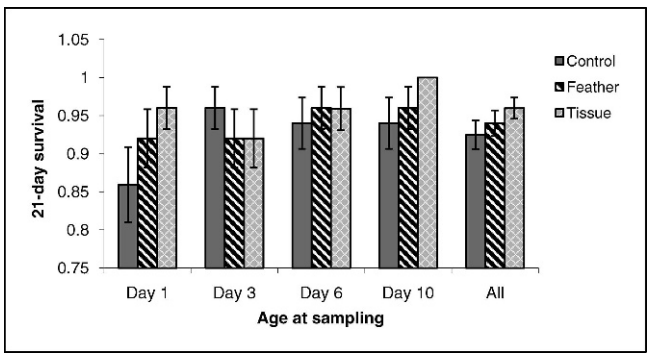

Figure 1. Survival (±SE) of captive northern bobwhite chicks in Mississippi State, Mississippi, USA, from day 1 to day 21 in relation to tissue collection method and chick age at tissue collection, 2001.

study. Mass at hatch was the only important predictor of neonatal bobwhite survival to 21 days. Lusk et al. (2005) also reported mass as a significant covariate for survival of wild bobwhite chicks. When we compared mean mass-at-hatch among treatments, effect sizes were small for all but one treatment combination, suggesting that there was no spurious effect of mass interacting with treatments. Although we randomly assigned chicks to treatment combinations to guard against systematic bias, random sampling error associated with mass at hatch could have inadvertently increased variation in survival among treatment groups. Interestingly, the group with the lowest survival from day 1 (control) also had the greatest mean mass-at-hatch overall (Table 1).

Our experimental design necessitated that we handle all chicks (including chicks receiving no tissue sampling treatment) to assess measures of morphometrics. We therefore cannot conclude that the entire process of handling and collecting tissue had no influence on survival, but rather that survival did not differ between the 2 tissue collection methods and the control group. Recent research suggests handling alone may induce capture myopathy and reduce survival in adult bobwhite (Abbott et al. 2005).

Although we acknowledge that DNA quantity does not translate directly to genotyping success, our analysis of DNA quantity demonstrated that patagial microbiopsy samples produced greater quantities of DNA than did down feather or egg tooth samples. These observations are consistent with those of Faircloth (2008) who used DNA from patagial microbiopsy procedures to determine microsatellite genotypes for 841 wild neonatal bobwhites (obtaining 99% genotyping success) in a study of genetic sex ratios and brood amalgamation in bobwhite broods.

Management Implications

Insofar as we observed no deleterious effects of these 2 tissue collection methods on survival and few effects on morphometric measurements for captive neonatal bobwhite, selection of tissue collection method should be based on quantity and quality of DNA extractable from the tissue sample. As such, we suggest that careful use of patagial microbiopsy tissue sampling can produce DNA samples of sufficient quality for genotyping studies with minimal effects on chick growth and survival. To simultaneously minimize the opportunity for chick loss and brood mixing and effects of tissue sampling on chick growth, we advocate sampling during the first 3 days posthatch.

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LITERATURE CITED

Abbott, C. W., C. B. Dabbert, D. R. Lucia, and R. B. Mitchell. 2005. Does muscular damage during capture and handling handicap radiomarked northern bobwhites? Journal of Wildlife Management 69:664–670.

Allison, P. D. 1995. Survival analysis using the SAS® system: a practical guide. SAS Institute, Inc., Cary, North Carolina, USA.

Colwell, M. A., C. L. Gratto, L. W. Oring, and A. J. Fivizzani. 1988. Effects of blood sampling on shorebirds: injuries, return rates, and clutch desertions. Condor 90:942–945.

Ellegren, H. 1992. Polymerase-chain reaction (PCR) analysis of microsatellites—a new approach to studies of genetic relationships in birds. Auk 109:886–895.

Faircloth, B. C. 2008. An integrative study of social and reproductive systems in northern bobwhite (*Colinus virginianus*): a non-migratory, avian species bearing precocial young. Dissertation, University of Georgia, Athens, USA.

Frederick, P. C. 1986. Parental desertion of nestlings by white ibises (*Eudocimus albus*) in response to muscle biopsy. Journal of Field Ornithology 57:168–169.

Littel, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® system for mixed models. SAS Institute, Inc., Cary, North Carolina, USA.

Lukacs, P. M., and K. P. Burnham. 2005. Review of capture-recapture methods applicable to noninvasive genetic sampling. Molecular Ecology 14:3909–3919.

Lusk, J. J., F. S. Guthery, S. A. Cox, S. J. DeMaso, and A. D. Peoples. 2005. Survival and growth of northern bobwhite chicks in western Oklahoma. American Midland Naturalist 153:389–395.

Marsden, J. E., and B. May. 1984. Feather pulp: a non-destructive sampling technique for electrophoretic studies of birds. Auk 101:173–175.

Morin, P. A., J. Messier, and D. S. Woodruff. 1994. DNA extraction, amplification, and direct sequencing from hornbill feathers. Journal of the Science Society of Thailand 20:31–41.

Pearce, J. M., R. L. Field, and K. T. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology 68:471–481.

Segelbacher, G. 2002. Noninvasive genetic analysis in birds: testing reliability of feather samples. Molecular Ecology Notes 2:367–369.

Stangel, P. W., and M. R. Lennartz. 1988. Survival of red-cockaded woodpecker nestlings unaffected by sampling blood and feather pulp for genetic studies. Journal of Field Ornithology 59:389–394.

Taberlet, P., and J. Bouvet. 1991. A single plucked feather as a source of DNA for bird genetic studies. Auk 108:959–960.

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Westneat, D. F. 1986. The effects of muscle biopsy on survival and condition in white-throated sparrows. Wilson Bulletin 98:280–285.

Westneat, D. F., R. B. Payne, and S. M Doehlert. 1986. Effects of muscle biopsy and breeding success in indigo buntings. Condor 88:220–227.

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