J. Field Ornithol. 77(1):67–70, 2006

A nondamaging blood sampling technique for waterfowl embryos

Nicolas Lecomte,[1,4] Gilles Gauthier,[1] Louis Bernatchez,[2] and Jean-François Giroux[3]

[1] *Département de Biologie and Centre d'Études Nordiques, Université Laval, Québec, Québec, G1K7P4 Canada*
[2] *Département de Biologie, Université Laval, Québec, Québec, G1K7P4, Canada*
[3] *Département des Sciences Biologiques, Université du Québec à Montréal, 141 Président-Kennedy, SB-R880, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada*

Received 28 January 2005; accepted 8 June 2005

ABSTRACT. Development of minimally invasive techniques to collect blood in free-living birds is desirable for both ethical and conservation reasons. We describe a new, simple technique for collecting blood samples from waterfowl eggs that involves recovering blood from a small hemorrhage after puncturing the shell of eggs showing the first signs of hatching. This technique allowed us to obtain a large number of blood samples from hatchling Greater Snow Geese (*Chen caerulescens atlantica*) and did not require the sacrifice or retention of any individuals, thus minimizing stress. High-quality blood samples for genetic studies were obtained, with no adverse effects on either embryos or the post-hatch survival of young geese.

SINOPSIS. **Una nueva técnica para obtener muestras de sangre de embriones de gansos**
Es ético y deseable, en aras de conservar, el desarrollar técnicas poco invasivas para tomar muestras de sangre de aves vivas. Describimos una técnica para tomar muestras de sangre de huevos de gansos que consiste en tomar sangre de la pequeña hemorragia producida por una punción del cascaron cuando se observan lo primeros signos de eclosionar. Este técnica permitió tomar una muestra considerable de sangre de huevos de ganso (*Chen caerulescens atlantica*) sin requerir sacrificar o retener a ningún individuo, minimizando de esta forma el estrés causando por lo último. Las muestras tomadas fueron de alta calidad para análisis genético, sin que se observara efecto adverso alguno en los embriones o la supervivencia de los gansitos producto de los huevos manipulados y que eclosionaron.

Key words: blood sampling, egg, genetic, hatching, incubation, Snow Goose

Blood samples are routinely collected in genetic or ecophysiological studies to analyze a wide range of parameters in wild birds such as molecular markers, hormones, or stable isotopes (Colwell et al. 1988, Hoysak and Weatherhead 1991, Schmoll et al. 2004). The most common method for the collection of blood in birds is venipuncture (American Ornithologists' Union 1988, Resources Inventory Branch 1998), a method described by Hoysak and Weatherhead (1991). However, some training and skill are required to be proficient with this method. Smith et al. (2003) advocated the collection of blood samples from feathers rather than by venipuncture to minimize negative impacts, but both techniques require the handling of birds and lead to some level of stress (Cockrem and Silverin 2002). Despite widespread use of these blood collection techniques, few investigators have examined possible impacts on the behavior and survival of free-living birds (Stangel 1986, Hoysak and Weatherhead 1991, Smith et al. 2003, Schmoll et al. 2004). Ethical committees encourage alternative methods that minimize impacts on the studied individuals (e.g., American Ornithologists' Union 1988). In species with special conservation status, such as endangered species, requirements to use methods that have no adverse effect on populations are stringent (e.g., Gaunt et al. 1997). These factors led Dutton and Tieber (2001) to describe a technique to collect blood from eggs using small syringes and fluorescent lamps. However, success varied with species, and their technique was not tested in the field.

Here we report a new method for collecting blood from goose eggs with minimal handling and stress on the birds. We describe the technique, the quality of recovered blood samples, and possible effects on the survival of tagged goslings.

[4] Corresponding author. Email: nicolas.lecomte@bio.ulaval.ca

METHODS

The study was conducted on Greater Snow Geese (*Chen caerulescens atlantica*) breeding on Bylot Island (73°N, 80°W; Sirmilik National Park, Nunavut, Canada). We conducted field work during the hatching periods in 2003 and 2004 (5 July–15 July).

Our method is adapted from the technique developed by Alliston (1975) to web-tag young waterfowl (*Anatidae*) in hatching eggs. His method entails making a small hole in the eggshell (directly below the opening made by the hatching young) to access the hatchling's leg and placing a tag on its foot webbing. In doing so, one has to break through the chorioallantois membrane, which is rich in capillaries (Deeming and Ferguson 1991). This causes a small hemorrhage from which a few drops of blood can be collected. We recovered blood by placing the egg hole over a 1.5 mL Eppendorf collection tube filled with 0.5 mL of Queen's lysis buffer (Seutin et al. 1991) and allowed blood to drip in. Immediately after collection, we closed the tube and shook it to mix the sample with the buffer. Samples were kept at ~4°C for several weeks before analysis in the laboratory. The sampled blood volume was calculated as the total volume in the tube minus the volume of buffer.

To examine the possible effect of our blood sampling technique on the survival of tagged young, we web-tagged 235 goslings in the egg at the same time that we took a blood sample. We also had a control group of goslings (N = 647) that were tagged (Alliston 1975), but not sampled for blood. In both groups, we used only one gosling per nest to insure independence of the data. We revisited many nests shortly after tagging (typically 3 d later) to check for the presence of dead embryos or young. Approximately five weeks after hatching, families were captured during mass banding drives (Menu et al. 2001), and captured goslings were checked for web tags.

We examined the quality of genomic DNA in the blood samples with restriction ligation followed by agarose gel electrophoresis (Quiagen Inc., Chatsworth, CA, USA). A smear occurring during migration in the gel indicated low-quality DNA, with impurities or degraded DNA hindering migration out of the wells and causing nonuniform electrophoretic mobility.

RESULTS

We collected 1–5 drops of blood from each egg for a mean volume of 0.4 ± 0.1 (SE) mL per sample (N = 98). Blood coagulated in less than a minute, making it essential to quickly mix it with the buffer solution. After puncturing an egg, collecting a blood sample took on average of 15 ± 4 (SE) s (N = 79). We recovered blood from 215 hatchlings showing the first signs of hatching (small cracks in the shell, or star-pipped egg), but from only 20 hatchlings that had fully ruptured shell membranes.

We found no dead embryos or goslings during nest visits in either the blood sample (N = 103) or control (N = 151) groups. During banding, we caught 12 of 235 goslings (5.1%) from whom blood samples had been collected in the egg, and 25 goslings from the control group (N = 647; 3.9%). We found no significant difference between the survival of tagged goslings' blood sampled and web-tagged in the egg and those only web-tagged in the egg ($\chi_1^2 = 1.69$; P = 0.19).

All blood samples not in direct contact with the eggshell surface during collection yielded high quality DNA (N = 78). However, all blood samples that ran over the eggshell (N = 20) yielded low-quality DNA.

DISCUSSION

Our blood sampling technique offers several advantages. First, the technique is easy to use in the field, is not time-consuming, and requires little material beyond what is routinely used for nest surveys. Second, sampled individuals are still in eggs and experience little effect from handling, thus reducing stress and possible injury due to physical restraint. Third, our technique is compatible with most behavioral and ecological research because it does not require direct manipulation of individuals, as do other blood sampling techniques (Colwell et al. 1988). The volume obtained with our technique was <7% of the total blood volume of individuals and represents about 8% of total body mass (Sturkie 1986), a percentage well below the limit established by ethical committees (American Ornithologists' Union 1988, Gaunt et al. 1997, Resources Inventory Branch 1998). The sampled blood volume is comparable to that obtained with other sampling protocols: about

0.5 mL from the jugular vein of several passerine birds (Hoysak and Weatherhead 1991), 0.4 mL from the brachial vein of shorebirds (Colwell et al. 1988), and 0.05 mL (SE = 0.02; N = 90) per quill of growing wing coverts in Greater Snow Geese (N. Lecomte, unpubl. data). This volume is also above the minimum usually required for DNA extraction (0.1 mL; Quiagen, Inc., Chatsworth, CA, USA), hormonal assays (0.25 mL; Lipar et al. 1999), or stable isotope analyses (0.1–0.2 mL; Herrera et al. 2003). Finally, risk of injury for the handler should be minimal during the sampling procedure.

Our technique is adapted from the web-tagging method described by Alliston (1975), who recommended that hatchlings should have completely ruptured their shell membranes prior to puncturing a second hole in the egg. However, we found it difficult to obtain sufficient blood at this stage, presumably because the chorioallantois membrane began to dry as the embryo resorbed much of the blood flowing in that membrane prior to hatching. In contrast, the hemorrhage was greater when the hole was made at the first evidence of hatching activity, which made blood collection easier. Thus, we favored this stage in our sampling. Nonetheless, our results indicate that collecting a small amount of blood at an early hatching stage had no detrimental effects on the survival of birds web-tagged in the egg. However, sampling should not be attempted in the absence of physical signs of hatching (e.g., small cracks) because breaking the membranes or blood sampling at this stage may lead to desiccation of the embryo and eventually its death (Alliston 1975).

Although our method generally yielded high-quality blood samples for genetic analyses, quality depended on conditions during collection. In particular, DNA from blood samples that ran over the eggshell was of lower quality, presumably due to contamination from soiled eggshells. The potential for blood contamination with our method may thus be higher than with other techniques such as venipuncture because of the higher risk of contact with the eggshell or human fingers during sampling. Moreover, instruments used to open the egg shells should be cleaned between uses (e.g., with ethanol) to prevent contamination of samples by the blood of other individuals. An additional limitation of our method is that precise knowledge of hatching dates is necessary because nests should be visited at the early hatching stage.

Our method should be useful for species with highly synchronized hatching or colonial nesters. To our knowledge, only young waterfowl have been marked inside the egg. Although other nonwaterfowl species have been web-tagged (e.g., Sabine's Gulls, *Xema sabini*, Abraham 1986; American White Pelicans, *Pelecanus erythrorhynchs*, Evans and McMahon 1987; Common Loon, *Gavia immer*, Fournier et al. 2002), we found no evidence of web-tagging in the egg. Therefore, we do not know to what extent our blood sampling technique could be used with other groups of birds, especially species with small eggs (Deeming and Ferguson 1991). To our knowledge, the smallest eggs in which young have been web-tagged is about 53 × 36 mm (Bufflehead, *Bucephala albeola*; G. Gauthier, unpubl. data).

Although web-tagging in the egg was proposed three decades ago (Alliston 1975), an extensive literature search (>500 papers) revealed no evidence that the bleeding that results from this marking technique has been used as an opportunity to collect blood samples. Our new blood sampling technique can provide high-quality samples with minimal stress on individuals and has no detrimental effects on sampled birds. Our technique may be used in species where web-tagging already occurs because it requires little effort and no additional training or skill.

ACKNOWLEDGMENTS

Funding was provided by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to G. Gauthier, the Arctic Goose Joint Venture (Canadian Wildlife Service), and the Fonds Québécois sur la Nature et les Technologies (FQRNT, Ministère de l'Éducation du Québec). Université Laval provided additional financial assistance to N. Lecomte. Logistical support was generously provided by the Polar Continental Shelf Project (PCSP, Natural Resources Canada). Thanks to all who participated in the fieldwork, especially N. Ouellet, A. Calvert, G. Szor, M-A. Giroux, A. Hargreaves, O. Mathieu, G. Picard, and V. Préfontaine. We are grateful to A.-M. Gale, M.-C. Cadieux, B. Audet, N. Ouellet, and J. Mainguy for their comments on the manuscript. We are indebted to the Hunters and Trappers Association of Pond Inlet, Nunavut Territory, for assistance and support. K. A. Abraham and R. F. Rockwell improved the quality of this paper with their insightful comments. Our study followed the rules of the Canadian Council for the Animal Protection and was approved by the Committee for Animal Protection of the Université Laval (permit numbers 2003-010 and 2004-017).

LITERATURE CITED

ABRAHAM, D. M. 1986. Observations on the breeding biology of Sabine's Gulls (*Xema sabini*). Canadian Journal of Zoology 64: 898–903.

ALLISTON, W. G. 1975. Web-tagging ducklings in pipped eggs. Journal of Wildlife Management 39: 625–628.

AMERICAN ORNITHOLOGISTS' UNION. 1988. Report of committee on the use of wild birds in research. Auk 105: lA–41A.

COCKREM, J. F., AND B. SILVERIN. 2002. Variation within and between birds in corticosterone responses of Great Tits (*Parus major*). General and Comparative Endocrinology 125: 197–206.

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.

DEEMING, D. C., AND M. W. J. FERGUSON. 1991. Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge, UK.

DUTTON, C. J., AND A. TIEBER. 2001. A modified protocol for sex identification of in ovo avian embryos and its application as a management tool for endangered species conservation programs. Journal of Zoo and Wildlife Medicine 32: 176–180.

EVANS, R. M., AND B. F. MCMAHON. 1987. Within-brood variation in growth and condition in relation to brood reduction in the American White Pelican. Wilson Bulletin 99: 199–201.

FOURNIER, F., W. H. KARASOV, M. W. MEYER, AND K. P. KERROW. 2002. Daily energy expenditure of free-ranging Common Loon (*Gavia immer*) chicks. Auk 119: 1121–1126.

GAUNT, A. S., L. W. ORING, K. P. ABLE, D. W. ANDERSON, L. F. BAPTISTA, J. C. BARLOW, AND J. C. WINGFIELD. 1997. Guidelines to the use of wild birds in research. Ornithological Council, Washington, D.C.

HERRERA, G. L., K. A. HOBSON, M. RODRIGUEZ, AND P. HERNANDEZ. 2003. Trophic partitioning in tropical rain forest birds: insight from stable isotopes analysis. Oecologia 136: 439–444.

HOYSAK, D. J., AND P. J. WEATHERHEAD. 1991. Sampling blood from birds - a technique and an assessment of its effect. Condor 93: 746–752.

LIPAR, J. L., E. D. KETTERSON, V. NOLAN, AND J. M. CASTO. 1999. Egg yolk layers vary in the concentration of steroid hormones in two avian species. General and Comparative Endocrinology 115: 220–227.

MENU, S., G. GAUTHIER, AND A. REED. 2001. Survival of juvenile Greater Snow Geese immediately after banding. Journal of Field Ornithology 72: 282–290.

RESOURCES INVENTORY BRANCH. 1998. Live animal capture and handling guidelines for wild mammals, birds, amphibians and reptiles. Standards for Components of British Columbia's Biodiversity No. 3. Version 2.0. Ministry of Environment, Lands and Parks, Victoria, BC, Canada.

SCHMOLL, T., V. DIETRICH, W. WINKEL, AND T. LUBJUHN. 2004. Blood sampling does not affect fledging success and fledgling local recruitment in Coal Tits (*Parus ater*). Journal of Ornithology 145: 79–80.

SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90.

SMITH, T. B., P. P. MARRA, M. S. WEBSTER, I. LOVETTE, H. L. GIBBS, R. T. HOLMES, K. A. HOBSON, AND S. ROHWER. 2003. A call for feather sampling. Auk 120: 218–221.

STANGEL, P. W. 1986. Lack of effects from sampling blood from small birds. Condor 88: 244–245.

STURKIE, P. D. 1986. Body fluids: blood. In: Avian physiology (P. D. Sturkie, ed.), pp. 102–129. Springer-Verlag, New York, NY.