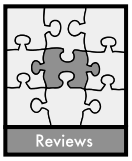

J. Avian Biol. 39: 369–378, 2008
doi: 10.1111/j.2008.0908-8857.04295.x

Received 19 June 2007, accepted 13 October 2007

Effects of blood collection on wild birds: an update

Lani D. Sheldon, Eunice H. Chin, Sharon A. Gill, Gregory Schmaltz, Amy E. M. Newman and Kiran K. Soma

L. D. Sheldon, E. H. Chin, A. E. Newman, and K. K. Soma (correspondence), Depts. of Psychology and Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4. Email: ksoma@psych.ubc.ca. – S. A. Gill, Dept. of Ecology and Evolutionary Biology, Princeton University, Princeton NJ, USA., 08544. – G. Schmaltz, Dept. of Biology, McMaster University, Hamilton, ON., Canada, L8S4K1.

Blood sampling is often critical for answering a variety of questions about wild birds. However, it is important to assess the impacts, if any, of blood collection on wild birds. Here, we examined the effects of blood sampling on adults or nestlings in three species of free-living birds. First, we examined the effects of blood collection on annual survival and reproductive success in adult buff-breasted wrens *Thryothorus leucotis* in Panama. In adult wrens, blood collection from the brachial vein during the breeding season had no effect on annual survival or reproductive success. Second, we examined whether blood collection influenced mass gain in developing smooth-billed anis *Crotophaga ani* in Puerto Rico. In developing anis, blood collection from the femoral or jugular veins had no effect on mass gain of nestlings. Third, in developing European starlings *Sturnus vulgaris* in British Columbia, Canada, blood collection from the brachial vein had no effect of body condition. Blood collection from the jugular vein had a transient effect on body condition during the first week post-hatch, but this effect disappeared by the second week of age. Lastly, we present an extensive up-to-date review of the literature on the effects of blood collection on free-living avian species. Taken together, these data show that blood collection has no major negative effects on developing or adult birds in the wild.

Blood samples are used to answer a variety of proximate and ultimate questions about birds (Stangel 1986, Oring et al. 1988, Gaunt et al. 1997, C.C.A.C. 2003). For example, blood samples can be used to (1) measure reproductive or stress hormones (e.g. Wingfield and Farner 1976, Saldanha and Schlinger 1997, Schew and Ricklefs 1998, Soma and Wingfield 2001, Romero 2004, Dufty and Crandall 2005, Soma 2006), (2) collect DNA for molecular sexing, paternity analysis, or evolutionary studies (e.g. Griffiths et al. 1996, Irwin et al. 2005), (3) measure metabolic fuels (e.g. Guglielmo et al. 1998), (4) measure stable isotopes for migratory tracking (e.g. Norris et al. 2005), (5) examine immune function (e.g. Casto et al. 2001), and (6) track emerging infectious diseases such as malaria, West Nile virus, avian influenza (e.g. Gancz et al. 2006). Such data are important for basic research, as well as avian conservation and human health (Romero 2004, Cockrem 2005, Walker et al. 2005, Wikelski and Cooke 2006).

It is important to determine the impacts, if any, of blood collection on wild birds. Previous studies of wild birds, predominantly adults in the temperate zone, have shown no serious long-term negative effects of blood collection (e.g. Franks 1967, Dufty 1988, Hoysak and Weatherhead 1991). Few studies have examined the effects of blood collection on developing birds, but no serious long-term negative effects have been reported (e.g. Lanctot 1994, Schmoll et al. 2004). Additionally, very little is known about the effects of blood sampling on tropical birds (Goymann and Wingfield 2004), with no reports on neotropical birds. Both Oring et al. (1988) and its subsequent revision (Gaunt et al. 1997) provided useful guidelines for researchers performing blood collection on avian species. However, it is important that after a decade of both advances in techniques and further research, that this information is updated and reviewed.

An important, but often overlooked, consideration is the site from which the blood is collected. Avian researchers collect blood from a variety of sites, including the brachial (Bigler et al. 1977), femoral (Gaunt et al. 1997), and jugular (Kerlin 1964, Hoysak and Weatherhead 1991) veins. The site of blood collection is important because it may influence the biological endpoint being measured. For example, jugular blood exiting the brain can be enriched with neurally-synthesized steroids (Schlinger and Arnold 1993), and venous blood yields higher hematocrit and calcium concentration than cardiac blood (Kern and De Graw 1978). Additionally, different sampling sites require different handling techniques and may have different impacts on birds.

Here, we examined the effects of blood sampling on free-living adults and nestlings from three phylogenetically diverse avian species. First, we examined the effects of brachial blood sampling on survival and reproductive success in adult buff-breasted wrens *Thryothorus leucotis*, a neotropical species, in Panama. Second, we examined the

effects of femoral and jugular blood sampling on mass gain in nestling smooth-billed anis *Crotophaga ani* in Puerto Rico. Third, we examined the effects of brachial and jugular blood sampling on body condition in nestling European starlings *Sturnus vulgaris* in Canada. Finally, we present a comprehensive review of the effects of blood sampling on development, behavior, reproductive success, and survival in free-living adult and developing birds.

Materials and methods

Experiment 1: adult buff-breasted wrens

Subjects

S.A.G. studied buff-breasted wrens in Gamboa, Panama (9°7′N, 79°42′W) between 1997–1999 to investigate genetic monogamy (Gill et al. 2005, Gill and Stutchbury 2006) and between 2004–2005 to investigate the hormonal regulation of aggression (Gill et al. 2007). The breeding season of buff-breasted wrens lasts ca. six months, with clutches initiated in April. Most females lay clutches of three eggs, and pairs fledge less than 2 young on average (Gill et al. 2007). Offspring remain on their natal territories for extended periods (mean = 10 months, S. Gill and B. Stutchbury unpubl. data), although they typically disperse prior to their parents' next reproductive attempt (Gill 2004).

Sampling protocol

We collected one to three blood samples from adult buff-breasted wrens during pre-breeding and breeding (incubation to fledging) periods. Within a period, we separated sequential sampling of individuals by 10 days. We captured adult wrens in mist-nets. In 2004–2005, we collected ~200 µl blood samples by puncturing the brachial vein with a sterile 26-gauge needle (Becton Dickenson and Co.) (time between capture and completion of bleeding = 6.07 ± 0.003 min, n = 103). Following bleeding, a sterile cotton pad was applied with gentle pressure to the site to stop the blood flow. Blood samples were collected from 06.00–13.00 h, and 16.00–18.00 h CST. We then weighed (females: 17.9 ± 0.8 g, n = 35; males: 20.2 ± 1.0 g, n = 31) and measured birds. These data were used to sex territory holders; within pairs, males are larger than their mates in all body measures (Gill and Vonhof 2006). Finally, we banded all birds with a unique combination of one numbered aluminum, and three colored bands. We also captured and banded offspring that were living on their parents' territory. In 1997–1999, we banded birds and then collected ~30 µl blood samples. In 1997–1999, we did not record the exact time between capture and bleeding.

We examined the effect of the blood volume sampled on annual survival of adult male and female buff-breasted Wrens by comparing the number of banded territory holders from which small (~30 µl) or large blood samples (~200 µl) were drawn that survived to the following year. We compared annual survival of banded adult territory holders between 2004–2005, during which time we collected from one to three 200 µl blood samples, with estimates from 1997–1999, during which time we collected single 30 µl blood samples for paternity analysis (Gill 2003).

The delayed dispersal of offspring from natal territories permitted us to conservatively estimate reproductive success (RS) in the previous year, as independent offspring present on territories in year t had fledged in year t − 1. Thus, we estimated RS by quantifying the number of pairs with at least one independent juvenile on their territory in March of 2004 (which provided an estimate of RS for 2003, n = 13) and 2005 (which provided an estimate of RS for 2004, n = 17). We had not drawn blood from any birds breeding in 2003 in the previous five years, whereas we sampled at least once all pairs breeding in 2004. Thus, if blood sampling negatively affected reproduction in Buff-breasted Wrens, estimated reproductive success should be lower in 2004 than 2003. These values underestimate true RS (some offspring may have dispersed prior to our surveys), but we assumed that any dispersal differences would be minimal between years, as patterns of delayed dispersal do not show significant annual differences (S. Gill and B. Stutchbury unpubl. data).

Statistics

Comparisons of survival were made using a χ^2 test between survival of males and females sampled in 2004 and survival of birds in 1997–1999. Comparisons of reproductive success were made using a χ^2 test between pairs sampled in 2004 versus 2005.

Experiment 2: developing smooth-billed anis

Subjects

Smooth-billed anis were studied by G.S. at the Laguna Cartagena and Cabo Rojo National Wildlife refuges in Puerto Rico (18°01′N, 67°06′W, and 17°59′N, 67°10′W, respectively) during the 2003–2004 breeding seasons (mid-September to mid-December). Blood samples were collected to study the hormonal control of begging behavior and to perform paternity analysis. Female anis at our two field sites generally lay 4–7 eggs per clutch, and nests contain up to 54 eggs in multi-female groups (G. Schmaltz unpubl. data). Nests were checked daily from the day the first chick hatched until seven days post-hatch (P7) and the mass of every chick present in the nest was recorded daily (brood size = 6.5 ± 0.6 chicks).

Sampling protocol

All blood samples were collected between 07.00–11.00 h, and 16.00–18.00 h EST using two techniques. Newly hatched chicks (P0–P1) were bled via the femoral vein. The femoral vein was punctured at the level of the knee using a sterile 30-gauge needle and the knee was then bent back and forth toward the chick's body. Each bending movement resulted in a drop of blood. Two to three drops of blood (~50 µl total, 0.37% of body mass) were collected in a heparinized capillary tube. With the second technique, 200 µl (0.73% of body mass) of blood was drawn from the jugular vein of P4–P5 chicks, using a 1 ml syringe with a sterile 30-gauge needle. For both techniques, chicks were bled immediately, then gentle pressure was applied to the wound for 30 sec, and the chick was returned to its nest (mean time between capture and completion of

bleeding = 4.8 ± 0.5 min, n = 76). All chicks were marked for identification by clipping the end of one toenail and returned to their nests.

Statistics

Paired *t*-tests (two-tailed, SPSS 10.0) were used to evaluate the effect of bleeding on chick growth, with daily mass used as a measure of chick growth. Paired chicks came from the same nest and were the same age (± 6 h), but one chick was bled and the other chick was not bled. Each chick was only bled once, and unbled chicks were processed in the same manner as their unbled counterparts.

Experiment 3: developing European starlings

Subjects

Blood samples from wild European starling nestlings were collected for hormonal studies of sexual differentiation. Nestling samples were obtained from a breeding population from April though July 2005 at the Davistead Farm in Langley, B.C., Canada, (49°8′N, 122°37′W). Starlings at this field site generally lay 4–6 eggs per clutch, and fledge chicks approximately 20–24 d following hatching. Nest boxes were checked daily to monitor clutch initiation, completion, hatch date, and brood survival. Nestlings were marked with food coloring at hatch for identification purposes, and banded with a numbered metal band at 10 d of age.

Sampling protocol

Nestlings were randomly assigned to be sampled at post-hatching d 0 (P0), P8, P16, or P20. Individual birds were re-sampled after a minimum of one week. Broods were randomly assigned to be sampled from either the jugular or brachial vein. For the brachial vein, after venipuncture with a sterile 26-gauge needle, blood was collected into either heparinized micro-hematocrit capillary tubes or heparinized Natelson blood collecting tubes. For the jugular vein, blood was collected using sterile 26-gauge needle, into heparinized 0.5 ml syringes (Becton Dickenson and Co.). Maximum blood volume to be collected was calculated as 1% of body mass. All samples were taken within 5 min of opening nest boxes and between the hours of 1200 and 1600 Pacific Daylight Time to minimize effects of stress and potential diel variation in hormones.

Following blood collection, body mass and length of exposed culmen, metatarsus, antibrachium, and wing chord were recorded. Prior to P10, the wing chord measurement did not include the primary and secondary flight feathers. Body measurements (culmen, metatarsus, antibrachium, wing chord) were analyzed using factor analysis (as in Lorentsen 1996). A single factor was extracted using principal components analysis, which was then regressed linearly against body mass, from which residuals were calculated as an index of body condition (IBC), a measure of body mass corrected for body size (Lorentsen 1996).

Molecular sexing

Starling chicks were genotyped using polymerase chain reaction (PCR; Chin et al. 2005, Love et al. 2005). DNA was isolated from red blood cells using Insta-gene matrix (Bio-Rad Laboratories, Hercules, CA). PCR amplification was run using the P2 (5′-TCTGCATCGCTAAAT CCTTT) and CW (5′-AGAAATCATTCCAGAAGTT CA) primer set.

Statistics

Data were analyzed using SPSS 10.0. IBC was examined at P8, P16 and P20. At each age, we compared unbled chicks (control) with previously bled chicks. At P8, the bled chicks had been sampled at P0 only. At P16 and P20, the bled chicks had been sampled once or twice previously (at least one week before); with bled chicks being pooled if no difference between those bled once or twice previously was found. Comparisons of IBC between bled and unbled groups were performed using ANOVA. To test whether blood sampling affected chick survival, we examined nest mortality at P16 between unbled and bled birds using ANOVA. Assessments of nest mortality after P16 were not possible due to some instances of early fledging in certain broods. We corrected for multiple comparisons using the Bonferroni correction. All values presented are mean ± SEM.

Results

Experiment 1: adult buff-breasted wrens

From 1997–1999, when small blood samples (~30 µl) were collected for paternity analysis, male and female survival was 75.6 ± 6.8% (n = 61) and 68.1 ± 9.0% (n = 66), respectively, and did not differ among years. In 2004–2005, when larger blood samples (~200 µl) were collected for hormone analyses, survival of males and females was 72.7% (n = 11) and 81.8% (n = 11), respectively. Adult survival did not differ between these two periods (males: $\chi^2 = 0.04$, P = 0.87, power = 0.088, effect size = 0.067; females: $\chi^2 = 0.31$, P = 0.60, power = 0.876, effect size = 0.355; Fig. 1), although for males, the power of this analysis is low.

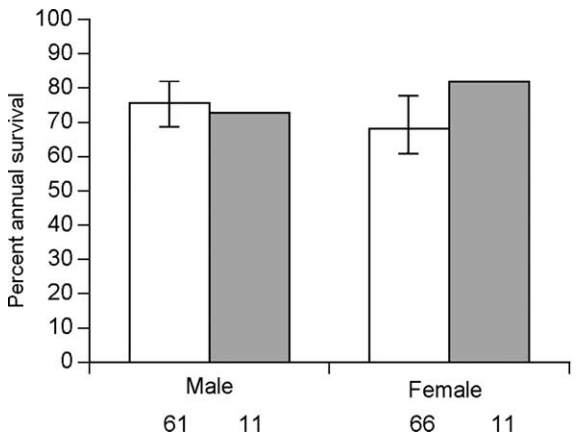

Figure 1. Effect of collecting a 30 µl or 200 µl blood sample on annual survival of adult buff-breasted wrens. Open bars indicate mean annual survival when a 30 µl sample was taken. Shaded bars indicate mean annual survival when a 200 µl blood sample was taken. Numbers below bars indicate sample sizes.

Reproductive success of buff-breasted wren pairs that had not been bled within the previous five years did not differ from the reproductive success of wrens that had been bled one to three times during their breeding season. In unbled pairs, 39% (n = 13) had retained offspring present on their territories in March of the following year. In bled pairs, 53% (n = 17) had retained offspring present on their territories in March of the following year. Estimated reproductive success was not significantly different between unbled and bled pairs ($\chi^2 = 0.175$, P = 0.7, power = 0.066, effect size = 0.28), although the power of this analysis is low.

Experiment 2: developing smooth-billed anis

Femoral bleeding did not significantly affect the growth of newly hatched chicks (n = 18; $t = 0.72$; P = 0.48), as both control and bled chicks gained 4.5 ± 0.5 g between nest visits (Fig. 2). Similarly, jugular bleeding did not affect P4–P5 chick growth significantly (n = 58; $t = -0.91$; P = 0.37), as both control and bled chicks gained 7.6 ± 0.6 g between nest visits (Fig. 2).

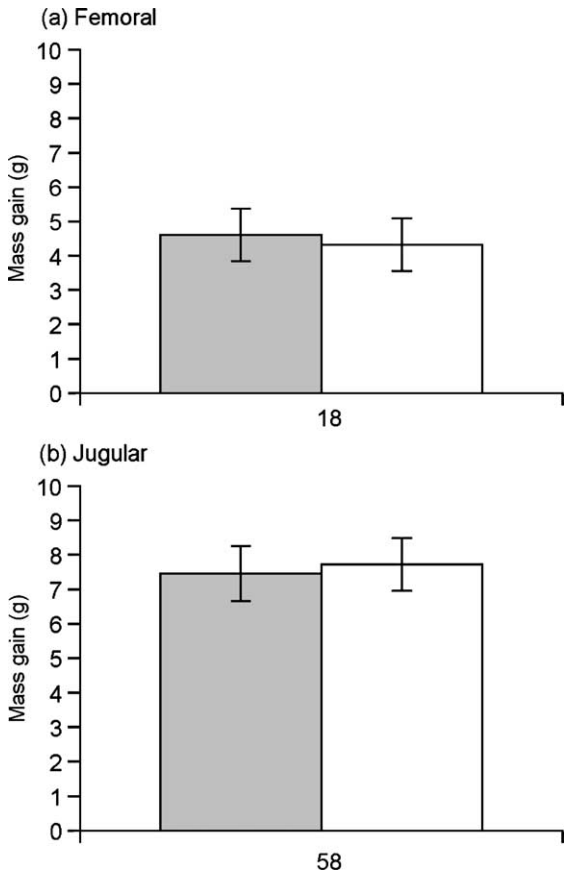

Figure 2. Smooth-billed ani nestling weight gain (in grams) between successive nest visits for chicks that were either bled (solid bar) or not bled (open bar) with two different bleeding methods (a) femoral and (b) jugular bleeding. Chicks sampled from the femoral vein were ages P0–P1, and those sampled from the jugular vein were ages P4–P5. Numbers below bars represent the number of pairs of nestlings.

Experiment 3: developing European starlings

Effects of blood sampling on an index of body condition (IBC) were assessed at P8, P16 and P20. At P8, we compared IBC between unbled nestlings and bled nestlings (bled previously at P0). At P8, there was no effect of sex on IBC ($F = 0.08$, df = 1, P = 0.78), nor was there an interaction between sex and sampling site on IBC ($F = 1.16$, df = 2, P = 0.33). However, sampling site had a significant effect on IBC at P8 ($F = 3.81$, df = 2, P = 0.03; Fig. 3a); meaning there was a significant difference between the effects of jugular and brachial bleeding at P0, on IBC at P8. Using pairwise comparisons, IBC was similar in controls and nestlings bled from the brachial vein (P = 0.12). IBC was significantly higher in controls than nestlings bled from the jugular vein (P = 0.04).

At P16, there was no effect of sampling on IBC ($F = 1.54$, df = 2, P = 0.22, Fig. 3b), nor an interaction between sex and sampling site on IBC ($F = 1.35$, df = 2, P = 0.27). However, there was a significant sex difference in IBC, with males having a higher IBC than females ($F = 7.24$, df = 1, P = 0.01). Birds bled once (at P0 or P8) and twice (at P0 and P8), were pooled as there was no significant difference between the groups (all P values > 0.05). Within bled birds, there was no significant difference between the effects of previous jugular and brachial bleeding, on IBC at P16 (all P values > 0.05). Using pairwise comparisons, IBC at P16 was similar in controls and nestlings bled from the brachial vein (P = 0.34). IBC at P16 was also similar in controls and nestlings bled previously from the jugular vein (P = 0.37). Thus, the effect of jugular sampling seen at P8 was transient and absent by P16.

At P20, there was no effect of blood sampling ($F = 1.99$, df = 2, P = 0.15, Fig. 3c), sex ($F = 0.44$, df = 1, P = 0.51; Fig. 3C), or an interaction between sex and blood sampling on IBC ($F = 1.01$, df = 2, P = 0.37). Within bled birds, there was no significant difference between the effects of previous jugular and brachial bleeding, on IBC at P20 (P = 0.62). Using pairwise comparisons, IBC at P20 was similar in controls and nestlings bled previously from the brachial vein (P = 0.89). IBC at P20 was also similar in controls and nestlings bled previously from the jugular vein (P = 0.19).

Lastly, there was no effect of blood sampling on nest mortality up to P16 ($F = 0.10$, df = 2, P = 0.91).

Discussion

Blood collection from adults or nestlings in three species of free-living birds had little effect on the variables measured. These results illustrate that the sampling of blood from breeding adults or developing chicks can provide important information without significant adverse effects on nestling development, annual reproductive success, or adult survival.

Effects of blood collection on adult birds

Although previous studies have examined the effects of blood collection in a wide variety of species (Table 1), no studies have reported the effects of blood collection on free-living neotropical birds. Sampling tropical birds could

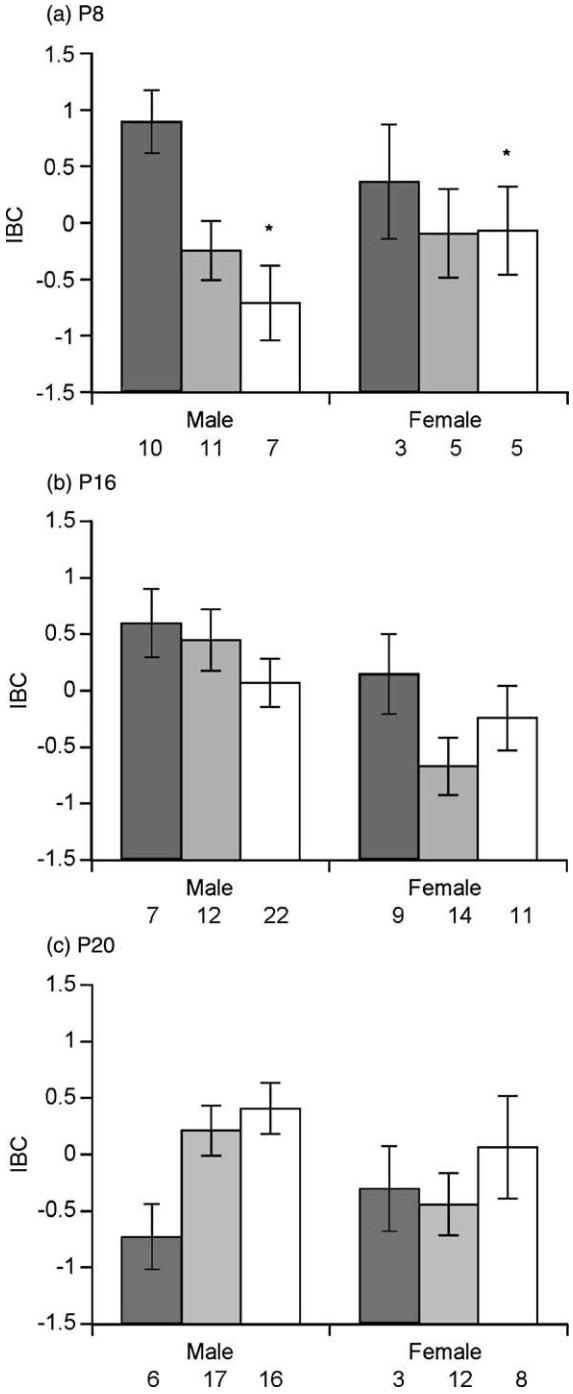

Figure 3. Effect of blood sampling on index of body condition (IBC) of European starling nestlings. Comparisons were made at (a) P8, (b) P16, and (c) P20. Dark solid bars = control, previously unsampled; light solid bars = previously sampled from the brachial vein; open bars indicate those previously sampled from the jugular vein. Numbers below bars represent sample size of nestlings in each group. An asterisk indicates a significant difference from CON nestlings of the same sex ($P < 0.05$).

theoretically present unique challenges, such as increased risk of wound infection or nest desertion. In adult buff-breasted wrens, annual survival did not differ between individuals from which small (~30 µl), or large (~200 µl) blood samples were collected. Although these tests had adequate power for female analyses, there was low power for male analyses, because the effect size was small in males. An important caveat is that small and large blood samples were collected in different years, so we cannot rule out an effect of year. Similarly, in temperate species, blood collection had no effect on short-term or long-term adult survival, measured via same-site recapture or return (Table 1, e.g. Franks 1967, Utter et al. 1971, Dufty 1988, Bigler et al. 1977).

In adult buff-breasted wrens, apparent reproductive success (RS) was not affected by blood collection during the breeding season. Apparent RS did not differ between years with blood sampling and years without sampling. The power for this analysis was low, but note that the trend was for higher RS in years with blood sampling. We cannot rule out an effect of year, and it is possible that we may have detected a difference in RS if we had measured the number of young fledged. Blood sampling does not affect RS in most previous studies of wild birds (Table 1; e.g. Wingfield and Farner 1976, Frederick 1986, Hoysak and Weatherhead 1991, Goymann and Wingfield 2004). The sole exception is a study of semipalmated sandpipers *Calidris pusilla*; breeding pairs are more likely to desert their clutches if both incubating adults are sampled, relative to unbled controls (Colwell et al. 1988). If only one adult of the pair is sampled, then there is no effect on clutch desertion (Colwell et al. 1988).

Furthermore, in adult birds, there is no evidence for significant long-term effects of blood sampling on body condition (Table 1; e.g. Hoysak and Weatherhead 1991) or behavior (Table 1; e.g. Ardern et al. 1994). To our knowledge, the effects of blood sampling during molt have not been examined. Existing data indicate that blood collection has no significant long-term effects on adult survival, reproductive success, body condition, or behavior in wild birds.

Effects of blood collection on developing birds

In developing smooth-billed anis, neither femoral nor jugular blood collection had an effect on nestling mass gain. Femoral bleeding is a useful method to collect blood from young chicks when small quantities are required.

In developing European starlings, blood collection from newly hatched birds via the jugular vein, but not brachial vein, decreased body condition at P8. It is possible that jugular sampling induces a larger hematoma than brachial sampling. However, the effect of jugular sampling on body condition was transient. The body condition of P16 and P20 chicks that had been bled from the jugular or brachial vein one or two times previously did not differ from the body condition of control chicks. There was also no effect of sampling at either site on survival to 16 d of age. These results suggest that it is important to assess the effects of blood sampling at multiple ages in developing birds and that blood collection from developing birds does not have significant long-term effects on growth or survival to fledging. In contrast to European starlings, in precocial buff-breasted sandpipers *Tryngites subruficollis*, Lanctot (1994) bled P1 chicks from the jugular vein and found no effect of blood sampling on mass gain.

Table 1. Effects of blood sampling on adult birds.

Order	Species	Blood sampled from	Percent body mass	n	Effects analysis	Reference
Anseriformes	Canada goose *Branta canadensis*	Brachial vein	0.07	63	No effect on survival as indicated by observation of presence in 2–5 wks	Raveling 1970
Piciformes	Downy woodpecker *Dendrocopos pubescens*	Jugular vein	0.82	50	No effect on survival as indicated by annual recapture	Franks 1967
Cuculiformes	African black coucal *Centropus grillii* (male)	Brachial vein	0.21	47	No effect on nestling-feeding rate by males	Goymann and Wingfield 2004
	African black coucal *Centropus grillii* (female)	Brachial vein	0.12	34	No effect on parental care, territory or mate acquisition	Goymann and Wingfield 2004
	Southern fulmar *Fulmarus galcialoides*	Footweb	<0.5	45	No effect on breeding success	Van den Brink and Pigot 1996
Columbiformes	Common pigeon *Columbia livia*	Heart	0.07	–	No effect on health or body mass after 2–3 days	Utter et al. 1971
	Mourning dove *Zenaida macroura*	Brachial vein	0.39	2,56	No effect on survival	Bigler et al. 1977
Charadriiformes	Spotted Sandpiper *Actitis macularia*	Brachial vein	0.88	207	No effect on nest attendance or following year return rates	Colwell et al. 1988
	Semipalmated sandpiper *Calidris pusilla*	Brachial vein	1.31	343	No effect on return rates; but clutch desertion more likely if both adults sampled	Colwell et al. 1988
	Red-necked phalarope *Phalaropus lobatus*	Brachial vein	0.98	131	No effect on male nest attendance or return rates following year	Colwell et al. 1988
	Wilson's phalarope *Phalaropus tricolour*	Brachial vein	0.80	338	No effect on male nest attendance or return rates following year	Colwell et al. 1988
Ciconiiformes	White ibis *Eudocimus albus*	Brachial vein	0.13	57	No effect on parental care or resighting at wintering grounds	Frederick 1986
Passeriformes	Chatham Isl. black robin *Petroica traverse*	Brachial vein	<1	20	No effect on resting, foraging, self-maintenance and interaction behaviors or survival	Arden et al. 1994
	Blue jay *Cyanocitta cristata*	Jugular vein	0.24	45	No effect on survival indicated by annual recapture	Franks 1967
	Red-eyed vireo *Vireo olivaceus*	Jugular vein	1.05	88	No effect on survival as indicated by annual recapture	Franks 1967
	House sparrow *Passer domesticus*	Brachial vein	0.78	17	No effect on mass or survival	Stangel 1986
	Ovenbird *Seiurus aurocapillus*	Jugular vein	0.91	161	No effect on survival as indicated by annual recapture	Franks 1967
	Scarlet tanager *Piranga olivacea*	Jugular vein	0.66	63	No effect on survival as indicated by annual recapture	Franks 1967
	Red-winged blackbird *Agelaius phoeniceus*	Jugular vein	0.92	1,651	No effect on male territory maintenance or female nest attendance No effect on nest success, number of young fledged per nest, or return rates	Hoysak and Weatherhead 1991
	Brown-headed cowbird *Molothrus ater*	Brachial vein	1.70	911	No effect on survival	Dufty 1988
		Jugular vein	0.45	38	No effect on survival as indicated by annual recapture	Franks 1967
		Jugular vein	1.14	56	No effect on mass change	Hoysak and Weatherhead 1991
	Common grackle *Quiscalus quisqula*	Heart	0.23	–	No effect on survival	Utter et al. 1971
	Common amakihi *Hemignathus virens*	Brachial vein	0.19	–	No effect on recapture rate	Wingfield et al. 1997
	Apapane *Himatione snaguinea*	Brachial vein	0.17	–	No effect on recapture rate	Wingfield et al. 1997
	Iiwi *Vestiaria coccinea*	Brachial vein	0.13	–	No effect on recapture rates	Wingfield et al. 1997
	Grass-hopper sparrow *Ammodramus savannadrum*	Heart	1.47	–	No effect on survival	Utter et al. 1971
	Dark-eyed junco *Junco hyemalis*	Heart	1.04	–	No effect on survival	Utter et al. 1971
	Song sparrow *Melospiza melodia*	Heart	0.77	–	No effect on survival	Utter et al. 1971
		Jugular vein	0.62	47	No effect on survival as indicated by annual recapture	Franks 1967

Table 1 (*Continued*)

Order	Species	Blood sampled from	Percent body mass	n	Effects analysis	Reference
	Rufous-sided towhee *Pipilo erythrophthalmus*	Jugular vein	0.48	119	No effect on survival as indicated by annual recapture	Franks 1967
		Heart	0.60	–	No effect on survival	Utter et al. 1971
	Chipping Sparrow *Spizella passerina*	Jugular vein	1.54	58	No effect on survival as indicated by annual recapture	Franks 1967
	Field sparrow *Spizella pusilla*	Jugular vein	1.54	115	No effect on survival as indicated by annual recapture	Franks 1967
	White-throated sparrow *Zonotrichia albicollis*	Heart	0.93	–	No effect on survival	Utter et al. 1971
	White-crowned sparrow *Zonotrichia leucophrys*	Brachial vein	2.72	47	No effect on clutch desertion or brood fledging	Wingfield and Farner 1976
					No effect on ability to start a second brood- no disruption in breeding cycle	
	Gray catbird Dumetella carolinensis	Heart	0.62	–	No effect on survival	Utter et al. 1971
		Jugular vein	0.49	186	No effect on survival indicated by annual recapture	Franks 1967
	Northern mockingbird *Mimus polygottus*	Heart	0.49	–	No effect on survival	Utter et al.1971
	Brown thrasher *Toxostoma rufum*	Heart	0.33	–	No effect on survival	Utter et al. 1971
	Buff-breasted wren *Thryothorus leucotis*	Brachial vein	1.12	30	No effect on reproductive success	Current study
		"	"	72 M 77 F	No effect on survival	"
	Tufted titmouse *Parus bicolour*	Jugular vein	0.91	56	No effect on survival as indicated by annual recapture	Franks 1967
	Black-capped chickadee *Parus atricapillus*	Jugular vein	1.741	141	No effect on survival as indicated by annual recapture	Franks 1967
	Great tit *Parus major*	Ulnar vein	0.28	91	No effect on parental ability to fledge offspring	Lubjuhn et al. 1998
	Wood thrush *Hylocichlia mustelina*	Jugular vein	0.44	185	No effect on survival indicated by annual recapture	Franks 1967
		Jugular or Brachial vein	–	221	No effect on return rates	Perkins et al. 2004
	American Robin *Turdus migratorius*	Heart	0.32	–	No effect on survival	Utter et al. 1971
		Jugular vein	0.26	217	No effect on survival as indicated by annual recapture	Franks 1967

–=data not available.

Table 2. Effects of blood sampling on developing birds.

Order	Species	Blood sampled from	Percent body mass	n	Age	Effects analysis	Reference
Anseriformes	Canada goose *Branta canadensis*	Brachial vein	0.12	301	7 weeks	No effect on survival as indicated by observation of presence in 2–5 wks	Raveling 1970
	Greater snow goose *Chen caerulescens atlantica*	Chorioallantois membrane	0.56	882	Before hatch	No effect on gosling survival at 3 days and 5 weeks as indicated by resighting	LeComte et al. 2006
Piciformes	Red-cockaded woodpecker *Picoides borealis*	Brachial vein	0.68	25	P9–P12	No effect on survival to fledging	Stangel and ennartz 1988
Cuculiforms	Smooth-billed ani *Crotophaga ani*	Femoral vein	0.37	18	P0–P1	No effect on mass gain	Current study
		Jugular vein	0.73	58	P4–P5	No effect on mass gain	
Ciconiiformes	Ring-billed gull *Larus delawarensis*	Jugular vein	–	78	–	No effect on fledging success or chick survival	Brown 1995
Charadriiformes	Buff-breasted sandpiper *Tryngites subruficollis*	Jugular vein	0.55	68	P1	Effect on 1 day movement from nest No effect on rate of mass gain in first 5 days, on long-term movement/distance from nest or fledging success	Lanctot 1994
Passeriformes	Great tit *Parus major*	Ulnar vein	0.36	675	P10	No effect on reproductive success the following year, immediate survival or survival to P10, or survival to next breeding season	Lubjuhn et al. 1998
	Coal tit *Parus ater*	Brachial vein	0.5	487	P9–P13	No effect on fledging, dispersal distances or local recruitment	Schmoll et al. 2004
	European starling *Sturnus vulgaris*	Jugular vein	1.00	88	P0–P20	No effect on survival No effect on body condition at P16 or P20 Effect on body condition at P8	Current study
		Brachial vein	1.00	90	P0–P20	No effect on survival No effect on development	Current study

–=data not available.

Previous studies in developing birds support the present results (Table 2; e.g. Raveling 1970, Lubjuhn et al. 1998, Lecomte et al. 2006). Importantly, in one study Lanctot (1994) found that in precocial Buff-breasted Sandpiper chicks, birds bled at P1 were found farther from the nest than control birds, which could be attributed to short-term stress effects. However, at fledging, bled birds showed no difference in distance from nest or likelihood of being resighted (Lanctot 1994). Taken together, these data suggest that short-term and long-term survival of developing birds are not significantly affected by blood sampling.

Conclusions

Our new data on three species of wild birds and our review of the literature indicate that blood sampling has no major long-term adverse effects on wild adult or developing birds. It is critical that researchers follow established guidelines (Gaunt et al. 1997, C.C.A.C. 2003) when collecting blood samples. Under such conditions, blood samples can provide critical information for studies of avian physiology, behavior, ecology and conservation.

Acknowledgements – We thank T.D. Williams and O. Love for their assistance on the European starling study. We thank J.S. Quinn and the US Fish and Wildlife Service for support on the smooth-billed ani project. Autoridad Nacional de Ambiente granted permission and the Smithsonian Tropical Research Institute provided support for the wren project. This research was funded by Natural Sciences and Engineering Council of Canada (NSERC) (K.K.S., J.S.Q., S.A.G., E.H.C., A.E.M.N., L.D.S.) and National Science Foundation (NSF; M. Hau). NSERC and NSF grants to T.D. Williams and J.C. Wingfield supported the Canadian and US E-bird networks, respectively.

References

Ardern, S. L., Mclean, I. G., Anderson, S., Maloney, R. and Lambert, D. M. 1994. The effects of blood sampling on the behaviour and survival of the endangered Chatham Island black robin (*Petroica traversi*). – J. Conserv. Biol. 8: 857–862.

Bigler, W. J., Hoff, G. L. and Scribner, L. A. 1977. Survival of mourning doves unaffected by withdrawing blood samples. – J. Field Ornithol. 48: 168.

Brown, K. M. 1995. Does blood sampling ring-billed gulls increase parental desertion and chick mortality? – Col. Waterbirds 18: 102–104.

Casto, J. M., Nolan Jr., V. and Ketterson, E. D. 2001. Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). – Am. Nat. 157: 408–420.

C.C.A.C. 2003. Guidelines on: the care and use of wildlife. – Canadian Council on Animal Care.

Chin, E. H., Love, O. P., Clark, A. M. and Williams, T. D. 2005. Brood size and environmental conditions sex-specifically affect nestling immune response in the European starling *Sturnus vulgaris*. – J. Avian Biol. 36: 549–554.

Cockrem, J. F. 2005. Conservation and behavioral neuroendocrinology. – Horm. Behav. 48: 492–501.

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

Dufty, A. M. 1988. The effects of repeated blood sampling on survival in brown-headed cowbirds. – Condor 90: 939–941.

Dufty, A. M. and Crandall, M. B. 2005. Corticosterone secretion in response to adult alarm calls in American kestrels. – J. Field Ornithol. 76: 319–325.

Franks, E. C. 1967. Mortality of bled birds as indicated by recapture rate. – J. Field Ornithol. 38: 125–130.

Frederick, P. C. 1986. Parental desertion of nestlings by white ibis (*Eudocimus albus*) in response to muscle biopsy. – J. Field Ornithol. 52: 168–170.

Gancz, A., Smith, D. A., Barker, I. K., Lindsay, R. and Hunter, B. 2006. Pathology and tissue distribution of West Nile virus in North American owls (family: Strigidae). – Avian Pathol. 35: 17–29.

Gaunt, A. S., Oring, L. W., Able, K. P., Anderson, D. W., Baptista, L. F., Barlow, J. C. and Wingfield, J. C. (eds). 1997. Guidelines to the use of wild birds in research. – The Ornithological Council, Washington, D.C.

Gill, S. A. 2003. Timing and duration of egg laying in duetting buff-breasted wrens. – J. Field Ornithol. 74: 31–36.

Gill, S. A. 2004. First record of cooperative breeding in a *Thryothorus* wren. – Wilson Bull. 116: 337–341.

Gill, S. A. and Stutchbury, B. J. M. 2006. Long-term mate and territory fidelity in neotropical buff-breasted wrens (*Thryothorus leucotis*). – Behav. Ecol. Sociobiol. 61: 245–253.

Gill, S. A. and Vonhof, M. J. 2006. Sexing monochromatic birds in the field: cryptic sexual size dimorphism in buff-breasted wrens (*Thryothorus leucotis*). – Ornitol. Neotrop. 17: 409–418.

Gill, S. A., Alfson, E. D. and Hau, M. 2007. Context matters: female aggression and testosterone in a year-round territorial, neotropical songbird (*Thryothorus leucotis*). – Proc. R. Soc. B 2674: 2187–2194.

Gill, S. A., Vonhof, M. J., Stutchbury, B. J. M., Morton, E. S. and Quinn, J. S. 2005. No evidence for acoustic mate guarding in duetting buff-breasted wrens (*Thryothorus leucotis*). – Behav. Ecol. Sociobiol. 57: 557–565.

Griffiths, R., Daan, S. and Dijkstra, C. 1996. Sex identification in birds using two CHD genes. – Proc. R. Soc. B 1374: 1251–1256.

Goymann, W. and Wingfield, J. C. 2004. Competing females and caring males. Sex steroids in African black coucals, *Centropus grilli*. – Anim. Behav. 68: 733–740.

Guglielmo, C. G., Haunerland, N. H. and Williams, T. D. 1998. Fatty acid binding protein, a major protein in the flight muscle of migrating wstern sndpipers. – Comp. Biochem. Physiol. B Biochemistry and molecular biology 119: 549–555.

Hoysak, D. J. and Weatherhead, P. J. 1991. Blood sampling from birds: a technique and assessment of its effect. – Condor 93: 746–752.

Irwin, D. E., Bensch, S., Irwin, J. H. and Price, T. 2005. Speciation by distance in a ring species. – Science 307: 414–416.

Kerlin, R. E. 1964. Venipuncture of small birds. – J. Am. Vet. Med. Assoc. 144: 870–874.

Kern, M. D. and De Graw, W. A. 1978. Differences in the composition of venous and cardiac blood from white-crowned sparrows. – Condor 80: 230–234.

Lanctot, R. B. 1994. Blood sampling in juvenile buff-breasted sandpipers: movement, mass change and survival. – J. Field Ornithol. 65: 534–542.

Lecomte, N., Gauthier, G., Bernatchez, L. and Giroux, J.-F. 2006. A non-damaging blood sampling technique for waterfowl embryos. – J. Field Ornithol. 77: 67–70.

Lorentsen, S-H. 1996. Regulation of food provisioning in the Antarctic petrel *Thallassoica anarctica*. – J. Anim. Ecol. 65: 381–388.

Love, O. P., Chin, E. H., Wynne-Edwards, K. E. and Williams, T. D. 2005. Stress hormones: a link between maternal condition

and sex-biased reproductive investment. – Am. Nat. 166: 751–766.
Lubjuhn, T., Brun, J., Winkel, W. and Muth, S. 1998. Effects of blood sampling in great tits. – J. Field Ornithol. 69: 595–602.
Norris, R. D., Marra, P. P., Kyser, K. T. and Ratcliff, L. M. 2005. Tracking habitat use of a long-distance migratory bird, the American redstart *Setophaga ruticilla*, using stable-carbon isotopes in cellular blood. – J. Avian Biol. 36: 164–170.
Oring, L. W., Able, K. P., Anderson, D. W., Baptista, L. F., Barlow, J. C., Gaunt, A. S., Gill, F. B. and Wingfield, J. C. 1988. Guidelines for use of wild birds in research. – Auk 105 (Suppl.): 23a–24a.
Perkins, K. A., Roth, R. R., Bowman, J. L., Gree, J. and Brittingham, M. 2004. Flushing, capture, and bleeding do not affect return rate of female wood thrushes (*Hylocichla mustelina*) in Delaware. – Auk 121: 354–360.
Raveling, D. G. 1970. Survival of canada geese unaffected by withdrawing blood samples. – J. Wildl. Manage 34: 941–943.
Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. – Trends Ecol. Evol. 19: 249–255.
Saldanha, C. J. and Schlinger, B. A. 1997. Estrogen sythesis and secretion in the brown-headed cowbird (*Molothrus ater*). – Gen. Comp. Endocrinol. 105: 390–401.
Schew, W. A. and Ricklefs, R. E. 1998. Developmental plasticity. – In: Starck, J. M. and Ricklefs, R. E. (eds). Avian growth and development. Oxford University Press, Oxford.
Schlinger, B. A. and Arnold, A. P. 1993. Estrogen synthesis in vivo in the adult zebra finch: additional evidence that circulating estrogens originate in the brain. – Endocrinol. 133: 2610–2616.
Schmoll, T., Dietrich, V., Winkel, W. and Lubjuhn, T. 2004. Blood sampling does not affect fledging success and fledgling local recruitment in coal tits (*Parus ater*). – J. Ornithol. 145: 79–80.
Soma, K. K. 2006. Testosterone and aggression: berthold, birds and beyond. – J. Neuroendocrinol. 18: 543–551.
Soma, K. K. and Wingfield, J. C. 2001. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. – Gen. Comp. Endocrinol. 123: 144–155.
Stangel, P. W. 1986. Lack of effects from sampling blood from small birds. – Condor 88: 244–245.
Stangel, P. W. and Lennartz, M. R. 1988. Survival of red-cockaded woodpecker nestlings unaffected by sampling blood and feather pulp for genetic studies. – J. Field Ornithol. 59: 389–394.
Utter, J. M., LeFebvre, E. A. and Greenlaw, J. S. 1971. A technique for sampling blood from small passerines. – Auk 88: 169–171.
Van den Brink, N. W. and Pigott, K. 1996. Effects of sampling blood and uropygial oil on breeding success of Antarctic birds. – J. Field Ornithol. 67: 623–629.
Walker, B. G., Boersma, P. D. and Wingfield, J. C. 2005. Field endocrinology and conservation biology. – Integr. Comp. Biol. 45: 12–18.
Wikelski, M. and Cooke, S. J. 2006. Conservation physiology. – Trends Ecol. Evol. 21: 38–46.
Wingfield, J. C. and Farner, D. S. 1976. Avian endocrinology-field investigations and methods. – Condor 78: 570–573.
Wingfield, J. C., Hunt, K., Breuner, C., Dunlap, K., Fowler, G. S., Freed, L. and Lepson, J. 1997. Environmental stress, field endocrinology and conservation biology. – In: Clemmons, J. R. and Buchholz, R. (eds). Behavioural approaches to conservation in the wild. Cambridge University Press, Cambridge.