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SAMPLING BLOOD FROM BIRDS: A TECHNIQUE AND AN ASSESSMENT OF ITS EFFECT[1]

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Abstract. We describe a technique and apparatus for extracting blood samples from birds in the field. We tested the effect of our technique on the health and behavior of captive Brown-headed Cowbirds (*Molothrus ater*) and free-living Red-winged Blackbirds (*Agelaius phoeniceus*). We found that captive Brown-headed Cowbirds that had been bled did not lose any more weight than birds that had not been bled. Territory loss by male Red-winged Blackbirds was not affected by taking blood samples, nor was annual return rate. Female Red-winged Blackbirds that were bled did not differ significantly in their rate of nest abandonment, nest success, fledging rate or annual return relative to females that were not bled. We conclude that blood sampling is not obviously harmful to wild birds as long as proper precautions are taken. Given the ease of the field technique and the vast potential for information to be gained, field ornithologists should not preclude adding laboratory blood analyses to their research program because of concerns about the technique negatively affecting the birds' health or behavior.

Key words: Blood sampling; Red-winged Blackbird; Brown-headed Cowbird; Agelaius phoeniceus; Molothrus ater.

INTRODUCTION

Many insights into avian biology have been gained with techniques involving laboratory analysis of blood samples taken from wild birds. Avian blood can yield information for a wide variety of disciplines, including endocrinology (Oring et al. 1988), energetics (Utter and LeFebvre 1970), genealogy (Sherman 1981, Burke 1989), hematology (Puerta et al. 1990), pathology (Seegar 1979), population genetics (Evans 1980, Barrowclough et al. 1985, Burson 1990), and taxonomy (Sibley and Ahlquist 1983). However, field ornithologists may be reticent about extracting blood from their study animals because of a lack of clearly defined and readily available accounts of field techniques and a fear that birds will be adversely affected by the process.

A wide variety of techniques and apparatus for blood sampling have been described (American Ornithologists' Union 1988). They involve either extracting blood directly from a vein with a hypodermic needle and syringe or puncturing a vein with a needle and then drawing blood off of the skin surface with capillary tubes or syringes. However, methods have rarely been illustrated (but see Kerlin 1964 and Arctander 1988) and descriptions have not been sufficiently explicit to allow new researchers to adopt the techniques without extensive trial-and-error learning. Therefore, our first objective in this paper is to describe a method we have developed for use in the field.

In addition to overcoming methodological problems, researchers who wish to collect blood samples from wild birds must also satisfy themselves that the collection technique does not adversely affect the study animals. Obviously, interpretation of behavioral or ecological data must take into account any effects that research activity has on the study animal. In general, birds are known to be fairly resilient to blood loss since they do not exhibit acidosis and thus do not go into shock when blood is lost (Sturkie 1986). Therefore, they can survive relatively greater blood loss than mammals (Kovach et al. 1969). A lack of effect of blood sampling on survival in the wild has been documented for a number of species (Franks 1967, Raveling 1970, Wingfield and Farner 1976, Bigler et al. 1977, Evans 1980, Colwell et al. 1988, Dufty 1988). Other studies have suggested that blood sampling does not affect mass changes of birds in captivity (Evans 1980, Stangel 1986). Although survival and mass changes are not obviously affected by blood sampling, very few studies have examined shorter term effects, particularly on behavior. Anecdotal

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evidence suggests that bird behavior is not strongly affected by blood sampling (LeFebvre 1964, Utter and LeFebvre 1970, Utter et al. 1971, Wingfield and Farner 1976, Frederick 1986). However, the only experimental study of effects on behavior yielded mixed results (Colwell et al. 1988). In their study of blood sampling effects on nest desertion for three shorebird species, Colwell et al. (1988) found that the rate of nest desertion by the two uniparental species was not affected by bleeding the attending parent. However, incubating biparental Semipalmated Sandpipers (*Calidris pusilla*) were more likely to desert nests if blood was taken from both parents than if neither parent was bled.

Collectively, the studies done to date suggest that blood sampling does not have a strong detrimental effect on birds, but the evidence is far from comprehensive. Therefore, our second aim in this paper is to present the results of two studies we undertook to determine whether our blood sampling technique is harmful to birds. In one study we examine the short term effects of blood sampling on mass changes in Brown-headed Cowbirds (*Molothrus ater*) maintained in an aviary. In the other study we look at both short and long term effects on reproductive performance and survival of free-living Red-winged Blackbirds (*Agelaius phoeniceus*) over a six-year breeding study.

METHODS

BLOOD SAMPLING TECHNIQUE

Below we describe both the method and some of the considerations that led us to it, with the intention that this information will help others wanting to sample blood in the field. However, we recognize that for different researchers and different bird species, modifications of the technique may be required to make it more effective.

In establishing a blood sampling technique, we sought a method that would allow one person to take relatively large samples (0.5 ml) in the field with minimum disturbance to the birds being sampled. Preliminary testing was done on a group of captive male Red-winged Blackbirds. We first tried to collect blood using the wing vein technique (Arctander 1988). This method did not work well because most needles were too large to be easily inserted into the vein. Puncturing the vein and drawing blood off the surface also met with little success because the blood coagulated too quickly. These problems were overcome by extracting blood directly from the jugular vein (Kerlin 1964).

The optimal syringe-needle combination for this technique depends on the size of the bird (or vein size), the amount of blood required and the researcher's personal preference. Larger volume syringes can be easier to work with since less motion of the plunger is required for a given volume of blood. Larger needle diameter also makes blood collection faster and easier, but a small needle is less likely to cause injury to the bird. We used a 3 ml Becton-Dickinson syringe with a 19 mm 27.5 g or 30 g needle.

Our technique for holding a bird and extracting blood (Fig. 1) allows one person to perform the blood extraction while simultaneously limiting movement of the bird. The bird is held firmly in the left hand with the head between the index and middle fingers and the legs between the ring and small fingers. The jugular vein is exposed by pulling down on the right wing with the ring and small fingers and blowing on the feathers just above the bird's right shoulder. It may be necessary to turn the head to the left or right in order to make the vein stand out as much as possible.

Once a firm hold on the bird has been established, the area around the vein is sterilized by swabbing with alcohol. This also moistens the surrounding feathers, which then can be groomed away from the vein, allowing even clearer access. The syringe is held in the sampler's right hand with the needle approaching the vein at an acute angle (about 15 degrees) to the skin and the surface of the needle tip facing upwards. The needle can be steadied by resting the hand on the bird's body. The needle should be inserted into the skin by moving the needle forward slowly and adjusting its angle if necessary. The vein is penetrated in a similar fashion by slowly moving the needle forward and adjusting its angle. Both the skin and vein usually offer some resistance to puncture and will be pushed forward by the needle to some extent, but will spring back to the original position once the needle has penetrated. Thus, if the needle is pushed forward too quickly, it may be difficult to control the depth of penetration. This is especially important when piercing the vein, because too much force may result in the needle piercing right through the vein.

Once in the vein, the needle should be inserted to a depth of 0.5 to 1.0 cm. Blood is then ex-

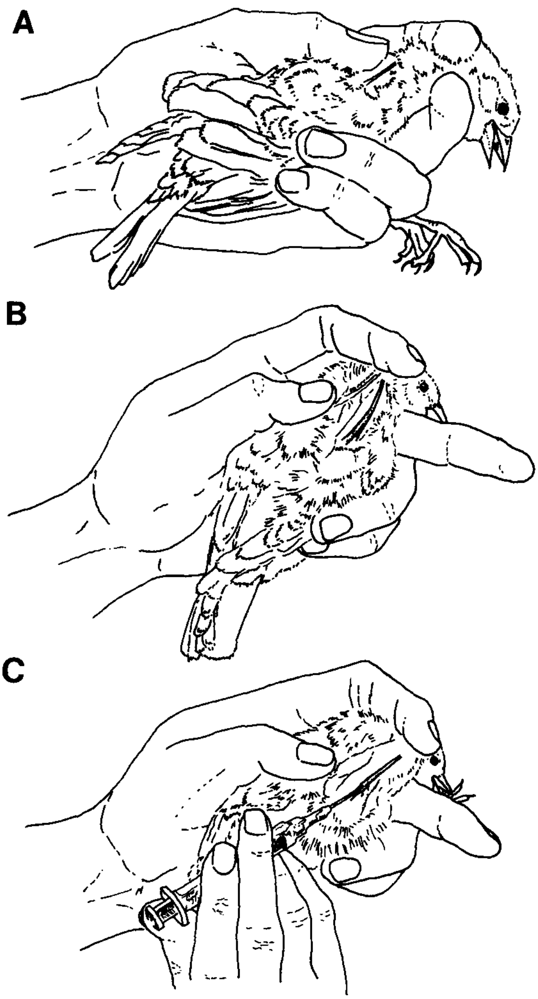

FIGURE 1. Technique for holding a bird while extracting blood, showing: (A) side view, (B) top view with jugular vein exposed, and (C) top view with syringe in place and thumb tip under the end of the plunger.

tracted by extending the thumb backwards, thus pushing outward on the plunger with the tip of the thumb. For some syringes and some samplers, it may be easier to draw back on the plunger with the mouth. If blood does not immediately start flowing into the needle, the vein wall has probably blocked the opening of the needle. In this case, suction should be stopped and the needle moved backwards slightly, or rotated so that the needle opening no longer faces upwards. Once blood is flowing into the syringe, suction is maintained until the desired amount has been extracted. Then pressure is eased off on the plunger and the needle is slowly withdrawn.

The technique for extracting blood samples from nestlings is generally the same as described above. Nestlings are actually easier to work with because they have fewer feathers and they move less than adults. The age at which blood can be sampled from nestlings will depend on the species being studied. We bled Red-winged Blackbird nestlings two or three days before fledging (i.e., seven or eight days after hatching). At this point they are large enough to provide a good sample and they still have time to recover before they leave the nest.

The volume of blood extracted will depend on the species being sampled and the purpose for which blood is required. The American Ornithologists' Union (1988) recommends that no more than 10–20% of the total blood volume be collected. Kovach et al. (1969) found that no mortality occurred before about 35–50% of the total blood volume was extracted from various domestic species. Total blood volume for birds is usually about 6–8 ml per 100 g body mass (Sturkie 1986). We extracted 0.5 ml of blood from Red-winged Blackbirds, which would be about 10–20% of the total blood volume.

BLOOD SAMPLING EFFECTS ON CAPTIVE BIRDS

We conducted this experiment at the Queen's University Biological Station from 5–28 July, 1990. We caught hatching-year Brown-headed Cowbirds in a baited walk-in decoy trap and then moved them to an outdoor aviary where the experiment took place. The experimental protocol involved monitoring weight changes of individuals kept in small groups, within which half the birds were bled and half were not. Each group consisted of either four or six birds captured on the same day. On the day of capture, we placed individuals in the aviary and within each group we paired birds according to size (pairs were chosen so as to minimize differences in mass and wing length—most pairs did not differ by more than 3 mm in wing length and 5 g in mass). We took a 0.5 ml blood sample from one randomly selected member of each pair. Thus, we could perform paired comparisons in which differences due to capture date, size or differences between trials were controlled. We housed each trial group separately in 3 × 2 × 1.5 m sections of the aviary with an ad libitum supply of food and water. We weighed individuals every day at dusk for one week. We used 11 groups with a total of 56 birds.

We did not know in advance what the dynamics of weight change would be, though from other studies it was evident that captive birds often lose weight (Evans 1980, Krebs 1982, Stangel 1986, Beletsky and Orians 1987). Therefore, we compared three different measures of mass change between birds that were bled and those that were not bled: one day mass loss (initial weight minus weight after one day), one week mass loss (initial mass minus mass after one week) and maximum mass loss (initial mass minus lowest mass during the week). If blood sampling is deleterious, we predicted that one or some combination of these measures would be greater for birds that were bled than for those that were not bled.

BLOOD SAMPLING EFFECTS ON FREE-LIVING BIRDS

Data for this study were collected as part of a long term (1985–1990) breeding study of Red-winged Blackbirds at the Queen's University Biological Station. The study area consisted of 10 marshes located within 10 km of the biological station (not all marshes were used in all years). In each year we began banding males with unique four-band combinations of anodized aluminum bands as they became territorial in April. We banded females during the breeding season (May–July). Most females were captured by placing mist nets near their nests, although some were captured before they began nesting when they flew into mist nets being used to capture territorial males. We collected blood samples from all birds we banded from the middle of the 1986 season through to the completion of the study. Blood samples are used for DNA fingerprinting analyses of parentage (Gibbs et al. 1990).

We monitored territories at least twice a week throughout each breeding season. We assumed a male had lost his territory if he disappeared from his territory and was replaced by another male before the end of the breeding season. We searched marshes every second day for new nests and to check the progress of nests discovered previously. We assumed that a female had abandoned her nest if we ceased to see her at the nest and either the eggs became cold and did not hatch or all the nestlings died in the nest. Because males provide little parental care other than nest defense (Weatherhead 1990), if a female abandons her nest, the eggs or nestlings perish. This definition of abandonment would cause us to interpret predation of a nesting female as nest abandonment. However, this is not inconsistent with our purpose since a negative effect of blood sampling could also be an increased susceptibility to predation. We considered a nest successful if at least one young was still in the nest nine days after the first egg hatched. We assumed that nest predation had occurred when all the eggs or nestlings disappeared from a nest before day nine.

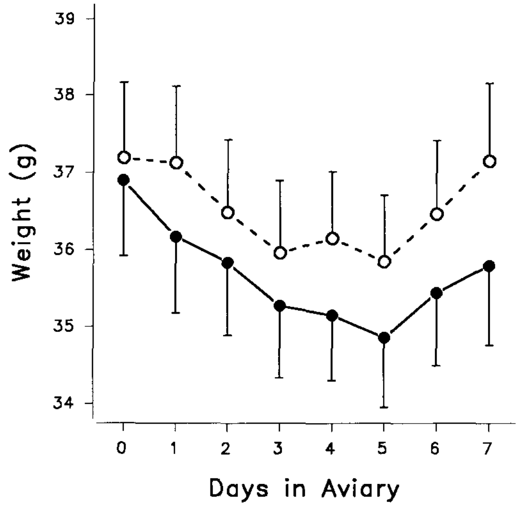

FIGURE 2. Mean daily mass (±SE) of Brown-headed Cowbirds that were bled (filled circles) and Brown-headed Cowbirds that were not bled (open circles) in the aviary trials. $n = 24$ for all cases.

RESULTS

Between 1986 and 1990 we extracted blood samples from 203 male, 292 female, and 1,156 nestling Red-winged Blackbirds. We never saw obvious injuries due to blood sampling, but mortality immediately after sampling did occur for one male, five females and two nestlings. We do not know whether all of these deaths occurred as a direct result of blood sampling or whether there were other elements of stress involved. The male that died had been kept in a bag before sampling on a very hot day. Two of the females that died had been caught in the bottom tiers of the mist nets and got wet before being removed.

BLOOD SAMPLING EFFECTS ON CAPTIVE BIRDS

Captive Brown-headed Cowbirds tended to lose weight for the first several days and then started

TABLE 1. Mean (±SD) mass (g) and mass losses of Brown-headed Cowbirds that were bled versus those that were not bled (t-values based on paired t-tests, P-values are one-tailed).

	Bled	Not bled	t	P
Initial mass	36.9 ± 4.8	37.2 ± 4.8	−0.44	0.331
One day loss	0.7 ± 1.8	0.1 ± 1.9	1.29	0.105
Maximum loss	2.8 ± 2.0	2.2 ± 2.7	0.93	0.182
One week loss	1.1 ± 2.5	0.1 ± 2.9	1.22	0.118

to regain weight, regardless of whether or not they had been bled (Fig. 2). During the course of the experiment, four birds died of unknown causes. None of these were birds from which blood samples had been taken. When a bird died we removed it from the aviary immediately, along with the other member of the pair. We excluded these four pairs of birds from the analysis. Birds that were bled and birds that were not bled did not differ in initial mass, one day mass loss, maximum mass loss, or one week mass loss (Table 1).

BLOOD SAMPLING EFFECTS ON FREE-LIVING BIRDS

To determine whether blood sampling caused males to lose their territories, we compared males that were banded and bled with those that were only banded (i.e., males caught in 1985 and early 1986). We restricted the analysis to the year that males were banded, so each male was only used once in the analysis. We also only considered males banded and bled or banded only before June. The latter restriction reduced possible confounding seasonal effects on parental investment decisions (Montgomerie and Weatherhead 1988). The rate of territory loss in male Red-winged Blackbirds did not differ between birds that were bled and birds that were not bled (Table 2). The relatively high rate of territory loss in birds that were not bled may have been the result of when birds were captured. In both bled and unbled groups, capture dates were earlier for territory losers than for males that held their territories (Mann-Whitney U-tests, P's < 0.05). Thus, it appears that there is a higher rate of territory turnover early in the season. In 1985, when we did not take blood samples, capture dates tended to be earlier than other years, when blood samples were taken (Table 3). Therefore, for males that lost their territories, capture dates tended to be earlier for unbled birds than for birds that were bled (Mann-Whitney U-test, $P_{2\text{-tail}} = 0.01$), and this is reflected in the high rate of territory loss for males that were not bled.

To determine whether blood sampling caused breeding females to abandon their nests we compared the rate of nest abandonment by females caught at their nests and bled, with that of females caught at their nest and not bled (i.e., caught in 1985 or early 1986 before we began blood sampling) plus females banded and bled early in the spring before they began nesting. We restricted the analysis to females that initiated nests before June and excluded any known renesting females that initiated nests in May. Thus, nearly all females were on their first nesting attempt of the season. We found no difference in the rate of

TABLE 2. Effects of blood sampling on Red-winged Blackbirds (sample sizes in parentheses).

	Bled	Not bled	Statistical test
Males			
% territories lost	25.6 (90)	34.5 (29)	$\chi^2 = 0.48, P = 0.49$
% returned	43.8 (80)	57.7 (15)	$\chi^2 = 1.02, P = 0.31$
Females			
% nests abandoned	7.8 (90)	9.6 (73)	$\chi^2 = 0.02, P = 0.90$
% nests successful	57.0 (114)	50.0 (32)	$\chi^2 = 0.25, P = 0.61$
% returned	35.5 (183)	43.6 (39)	$\chi^2 = 0.59, P = 0.44$
No. fledged per nest[a]	1.59 ± 1.53 (110)	1.41 ± 1.60 (32)	$Z = 0.64, P = 0.26$[b]
No. fledged per successful nest[a]	2.69 ± 1.00 (65)	2.81 ± 1.05 (16)	$Z = 0.44, P = 0.33$[b]

[a] Mean ± SD.
[b] Mann-Whitney U-test, P-value is one-tailed.

nest abandonment attributable to blood sampling (Table 2).

To assess potential effects of blood sampling on nesting success we used the same group of females used in the abandonment analysis, with the addition of females with nests that were preyed on. There was no difference in overall nest success between females that were bled and those not bled (Table 2). Furthermore, there were no significant differences in the number of young fledged per nest or in the number of young fledged per successful nest between females that were bled and females that were not bled (Table 2).

We can determine whether blood sampling had any detrimental long-term effects by comparing return rates between years of birds that were bled with those not bled. We again restricted this analysis to birds caught for the first time. There was no significant difference in return rates the year following banding between birds that were bled and those that were not bled for either males or females (Table 2).

TABLE 3. Mean Julian date of capture by year for territorial male Red-winged Blackbirds.

Year	$\bar{x}$	SD	n
1985	115.5	14.8	25
1986	135.2	17.1	9
1987	121.3	12.3	26
1988	124.1	17.8	23
1989	122.5	17.2	21
1990	120.7	12.6	15

DISCUSSION

Our studies of both captive Brown-headed Cowbirds and of free-living Red-winged Blackbirds failed to provide any evidence that blood sampling had detrimental short or long term effects. Previous studies of blood sampling on captive birds did not find negative effects over periods of a week (Stangel 1986) or a month (Evans 1980). Our data for Brown-headed Cowbirds indicated no effect even over the first 24 hr. However, we only considered patterns of mass change, so we cannot rule out the possibility that blood sampling might affect behavioral traits such as dominance rank in captive birds.

Our field study of Red-winged Blackbirds used *a posteriori* analyses of data collected for other purposes. However, our criteria for including birds in the analyses resulted in control and experimental groups similar to those one would use in an experiment explicitly designed to assess blood sampling effects in the field. In fact, our conclusion that blood sampling did not increase nest abandonment is strengthened by a bias in the control group. Some control females were banded before they began nesting and therefore suffered no disruption during nesting. By contrast, all experimental females were banded and bled while they had an active nest. Since handling alone can cause parents to abandon nests (Colwell et al. 1988, Lombardo 1989), we could have found more nest abandonment by our experimental females just due to handling differences. That we did not indicates that female Red-winged Blackbirds are very tolerant of disturbance (both handling and bleeding) while nesting. Other species may be much less tolerant, although from our results we can hypothesize that species that tolerate handling will also tolerate being bled.

Our studies, as well as those previously published, collectively suggest that sampling blood is not obviously detrimental to birds. Furthermore, one can also minimize any potentially detrimental effect of blood sampling if other sources of disturbances (e.g., capturing females early in the nest cycle, extended holding time in mist nests or holding bags) are also kept to a minimum (Arctander 1988). It would also be prudent to look for any effects in species sampled for the first time. However, we agree with Colwell et al. (1988) that blood sampling appears to be compatible with most behavioral and ecological research and that the costs are far outweighed by the potential benefits of the knowledge we gain.

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