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THE EFFECTS OF REPEATED BLOOD SAMPLING ON SURVIVAL IN BROWN-HEADED COWBIRDS[1]

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With the development of microsampling techniques and other manipulative procedures, studies of free-living birds increasingly involve the collection of small amounts of blood and/or body tissue. Removal of a small amount of blood has minimal effect on survival, both in captivity (Stangel 1986) and in the wild (Franks 1967, Raveling 1970, Bigler et al. 1977), although Franks (1967), in the only study to investigate several passerine species, found some indication that small birds (emberizids) suffer increased mortality as a result of blood sampling.

One characteristic of the investigations listed above is that they all involved single sampling events; that is, each bird was bled one time during a given year. However, many studies require repeated sampling of the same bird; for example, those investigating changes in plasma hormone levels over time (e.g., Wingfield and Farner 1978; Dufty and Wingfield 1986a, 1986b). Wingfield and Farner (1976) reported that serially sampled White-crowned Sparrows (*Zonotrichia leucophrys*) do not desert their breeding grounds and have normal reproductive success. However, the effect of such repeated sampling on survival of free-living birds is unknown.

The purpose of the present study was to determine the effect of repeated blood sampling on survival, using data from a 6-year investigation of the Brown-headed Cowbird (*Molothrus ater*). Female cowbirds may be especially sensitive to the effects of reduced blood volume resulting from serial sampling. Female (but not male) cowbirds exhibit a significant decline in hematocrit during the breeding season (Keys et al. 1986), possibly due to the rigors of breeding. Additional reductions in blood volume and hematocrit through repeated blood sampling could adversely affect the survival of female cowbirds more so than males.

METHODS

This study was conducted from 1981–1986 on the grounds of the Rockefeller University Field Research Center in Millbrook, Dutchess County, New York (42°N). Most birds were captured in potter traps and walk-in traps baited with millet seed. On a few occasions Japanese mist nets were used. Trapping occurred between the hours of 06:00–12:30, and traps were checked regularly. The number of trap days varied from year to year and is listed in Table 1. The effect of this variation on the data is presented below.

Each bird captured in 1981 was given a unique combination of four color bands plus a U.S. Fish and Wildlife Service band, measured (wing length, weight, fat score), and released. No blood was collected from these birds. Male cowbirds were aged according to plumage characteristics (Baird 1958, Selander and Giller 1960). From 1982–1986 blood samples (700–800 μl whole blood) were collected from each bird in heparinized capillary tubes by puncturing the wing (alar) vein with a 26-gauge needle. Additional samples were collected throughout the spring and summer at intervals of not less than 1 week. Laparotomies (Wingfield and Farner 1976) were also performed on most birds at their initial capture.

In 1982 nine males were placed in sound chambers for a brief period (24 hr maximum time); their vocalizations were recorded and they were immediately released. These males were included in the analyses.

In 1985 each of 26 male cowbirds was subcutaneously implanted with a 20-mm length of testosterone-filled Silastic tubing. These males are not included in the analyses.

Cowbirds visited the study site from a distance of several kilometers (unpubl. data), and many cowbirds were captured only once (see below). For the following analyses the term 'resident' refers to cowbirds captured two or more times in a given year with at least 1 week intervening. The total number of birds may vary in

TABLE 1. Trapping effort and number of Brown-headed Cowbirds captured, 1981–1986.

Year	No. trap days	Start/end dates	Total no. cowbirds captured: Males	Total no. cowbirds captured: Females
1981	47	28 March–13 July	89	68
1982	78	3 April–2 August	105	70
1983	69	31 March–11 August	92	80
1984	52	29 March–20 July	104	77
1985	40	5 April–17 July	69	46
1986	38	29 March–18 July	63	48
		Total	522	389

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different analyses as some data were inadvertently omitted during data collection (e.g., wing length) or, in a few cases, could not be determined (i.e., age).

A bird is considered to have survived if it was captured in a subsequent year. This is a conservative estimate of survival, as missing birds may have dispersed, or been present but not captured, or may have died. Thus, the term survival actually refers to minimum annual survival.

RESULTS

The number of trap days and the total number of Brown-headed Cowbirds captured from 1981–1986 are shown in Table 1. A total of 911 cowbirds were captured, 522 males and 389 females, giving a male : female ratio of 1.34:1. If only resident cowbirds (i.e., those captured two or more times) are considered, the male : female ratio remains the same (154 males : 115 females = 1.34: 1).

The correlation between trapping effort and the overall number of individuals captured is significant for males (r_s = 0.919, $P < 0.05$, one-tailed), but not for females (r_s = 0.771, ns, one-tailed). However, when only resident birds are considered, there is no correlation in either sex between trapping effort and number of birds captured (males: r_s = 0.457; females: r_s = 0.200, one-tailed tests).

SURVIVAL OF BLED VS. UNBLED RESIDENT COWBIRDS

In 1981 a total of 45 resident male cowbirds were captured and banded. Nineteen (42.2%) of these males were recaptured the following year. From 1982–1985 a total of 81 resident males were identified and bled; 29 (35.8%) of these bled males returned the year after their initial capture. The difference between these two groups is not significant (χ^2 = 0.27, P = ns). Hence, blood sampling does not reduce survivorship from one year to the next in male Brown-headed Cowbirds.

A similar result is seen for female cowbirds. Six of 25 (24.0%) resident females banded in 1981 returned the following year, compared to the return of 23 of 72 (31.9%) females banded and bled from 1982–1985. The difference is not significant (χ^2 = 0.24, P = ns).

It is possible that repeated blood sampling could have an additive negative effect on survival. To examine this, the number of blood samples collected from resident males that returned the following year is compared to the number of blood samples collected from resident males that did not return. As in all of these analyses, birds are included only in the year of their initial capture; i.e., birds that were residents in several years are considered only once. The only exceptions to this are the birds that were banded (but not bled) in 1981. Members of this 1981 cohort that were residents in 1982 (and, therefore, bled for the first time in that year) are included here. A possible bias could occur if this group, which had already returned once to the study site, had a greater return rate than the other birds; however, the return rate of both groups is the same (χ^2 = 1.19, ns) and they are combined. Since only resident birds are considered, the minimum number of captures (and, hence, blood samples) is two.

The mean number (±SE) of blood samples taken from males that returned the following year was 3.80 ± 0.33. The mean for males that did not return was slightly lower than that of the returnees: 3.32 ± 0.27; this difference is not significant.

Male cowbirds can be grouped into two categories by their plumage characteristics. Second-year (SY) males were hatched the previous year and are breeding for the first time. After-second-year (ASY) males are in at least their second breeding season. For resident SY males, 5 of 18 (27.8%) unbled birds and 15 of 48 (31.3%) bled birds returned (χ^2 = 0.001, ns). For resident ASY males, 14 of 26 (53.8%) unbled and 15 of 33 (45.6%) bled birds returned (χ^2 = 0.143, ns). Thus, there is no difference in survival within each of these two age classes as a function of blood sampling.

SY males are smaller than ASY males in this population (Dufty and Wingfield 1986b) and may be more susceptible to any deleterious effects of repeated blood sampling. To examine this I compared the number of blood samples taken from resident SY males that returned with the number taken from resident SY males that did not return. The mean number of blood samples collected was similar in both SY groups (returnees: $\bar{x}$ = 3.73 ± 0.62, n = 15; nonreturnees: $\bar{x}$ = 3.49 ± 0.39, n = 33; t = 0.347, df = 46, ns). A similar analysis shows no difference between returning and nonreturning resident ASY males (returnees: $\bar{x}$ = 3.84 ± 0.38, n = 25; nonreturnees: $\bar{x}$ = 3.12 ± 0.34, n = 26; t = 0.124, df = 48, ns; this analysis includes 1981 males bled for the first time in 1982).

The situation for resident females is somewhat surprising. The mean number of blood samples taken from females that returned the next year (4.64 ± 0.56) was significantly greater than the mean number taken from females that did not return (3.15 ± 0.21). Clearly, the survival of male and female Brown-headed Cowbirds is not adversely affected by repeated blood sampling.

DISCUSSION

The results presented above show that removal of a small amount of blood from cowbirds during the breeding season has no measurable adverse effect on survival in either sex. Franks (1967) also reported that there is no negative effect on survival of the removal of single small (200 μl) blood samples from 15 species of birds (including cowbirds), although when the results from four species of emberizids were grouped, a significant reduction in survival was revealed. His criteria for survival were slightly different from those used here (he included as survivors birds recaptured in the same year they were bled) as were his methods (he collected from the jugular vein, only used mist nets, sampled during migration, and did not distinguish between residents and nonresidents), so the results may not be entirely comparable. In any event, the present data provide strong evidence that blood sampling does not reduce survival to the next breeding season.

Nor is survival reduced if repeated samples are obtained from individual cowbirds. For males, sexual selection theory suggests that large males are at an energetic disadvantage compared to small males (e.g., Searcy 1979), and older, larger male cowbirds may have been expected to exhibit reduced survival with increased removal of blood. Conversely, it could be argued that the accumulated effects of blood removal would be felt more keenly in younger males with their smaller body size and reduced absolute blood volume. However, within each of the two age classes of males there is no difference in survival as a function of the number of blood samples collected.

Female cowbirds in this population are smaller than males (Dufty and Wingfield 1986a); this small size, plus the possible elevated energy demands inherent in their extensive laying season (Keys et al. 1986, but see Ankney and Scott 1980) made females a likely candidate for reduced survival as a result of repeated sampling. However, quite the opposite occurred: female cowbirds that returned to the study site had been sampled significantly more often than females that did not return. It is unlikely that repeated sampling enhanced survival; presumably, it is local birds that were trapped (and bled) most often, and it was these same birds that had the greatest probability of being captured again the following year, given the high level of philopatry in cowbirds (Dufty 1985) and the high recapture rate of female cowbirds (Darley 1971, Burtt and Giltz 1976).

Despite the overall absence of effects of blood sampling during the breeding season on subsequent survival, these data do not preclude possible negative effects if blood is removed under different ecological conditions or at other times of the year. For instance, cowbird populations at high elevations have increased hematocrit levels (Keys et al. 1986) and may be more sensitive to blood loss. Similarly, blood samples taken immediately prior to or during migration could have unfavorable consequences at this time of high oxygen requirement.

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