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THE EFFECTS OF MUSCLE BIOPSY ON SURVIVAL AND CONDITION IN WHITE-THROATED SPARROWS

DAVID F. WESTNEAT[1]

ABSTRACT.—Baker's (1981) muscle biopsy technique has not been used widely for electrophoretic studies of free-living birds because of initial concern over its effect on bird behavior and survivorship. In this study, I report recapture rates, fat deposits, mass, and return rates between years for biopsied and control White-throated Sparrows (*Zonotrichia albicollis*) wintering in North Carolina. Biopsied birds were recaptured significantly more than controls in one of the two seasons. I discuss the possible effect of slight initial differences in weight on recapture rates. Comparison of changes in fat deposits of biopsied and control sparrows revealed no significant differences in either year. Biopsied birds in 1984 lost significantly less body mass than controls (0.00 g vs 0.56 g). There was no significant difference in return rates between years for biopsied and control birds. Biopsies do not appear to affect survival or condition in this migratory songbird. *Received 3 May 1985, accepted 24 Oct. 1985.*

Electrophoresis is a useful technique in long-term studies of behavior as related to genotypes (Sherman 1981), yet its potential has not been realized completely, as most electrophoretic studies have resulted in sacrificing the studied animal. In several recent studies (Gowaty and Karlin 1984, Mumme et al. 1985, Gavin and Bollinger 1985) blood samples from live subjects were analyzed by electrophoresis for comparison with behavioral observations. The results of these studies were limited, however, by the small number of polymorphic loci found in blood. Several other techniques for sampling tissues of living birds have been published. One of these, the pectoralis muscle biopsy of Baker (1981), gives more resolvable genetic loci than samples of blood or feather pulp (Marsden and May 1984) and, unlike the feather pulp method, can be used without recapture throughout the year. The method, however, does result in an open wound in the main flight muscle, and concern over its effect on birds is justified.

Here, I report on a study of the effects of muscle biopsy on survival and condition in wintering White-throated Sparrows (*Zonotrichia albicollis*).

MATERIALS AND METHODS

White-throated Sparrows winter in large numbers at the Mason Farm Biological Reserve of the University of North Carolina in Chapel Hill, North Carolina. During the months of

[1] Dept. Biology, Univ. North Carolina, Chapel Hill, North Carolina 27514.

November to February in 1982–83, 1983–84, and 1984–85, sparrows were captured, individually color banded with plastic split-bands, sexed, aged, measured, and released at the point of capture as part of a study on the relationship between social dominance and genetics. The birds were captured in seed-baited Potter treadle-traps distributed along 300–400 m of rose hedges and field edge. In 1982–83, 10 sites with 3–7 Potter traps at each were spaced roughly 40 m apart along 300 m of hedgerow, and in 1983–84 the trapline was expanded to 16 sites, spaced exactly 25 m apart, with 6 traps at each site.

In March of 1983 and 1984, birds caught along the same 100 m of trapline (3 sites and 18 traps in 1983, 5 sites and 30 traps in 1984) were biopsied and released at point of capture. Observations of dominance were performed at two locations along the trapline. To avoid influencing the dominance relationships at one of these locations, all of the birds I biopsied were captured near the other location. There were no differences in the sex ratios or age distributions at the two locations (W. Piper, pers. comm.). Birds caught and biopsied weighed slightly but significantly less than control birds. This difference should make a test for the effects of biopsy more conservative.

In 1983, birds captured at the remaining traps were used as controls. In 1984, because the trapline was longer, the traps for biopsied birds were located in the interior of the trapline. To calculate recapture rates of controls, I used only birds captured at 36 traps (6 sites) similarly located in the interior of the trapline, and birds captured at several traps at either end of the trapline were not considered in these analyses.

For the biopsy I followed Baker's (1981) procedure, except that I used a suture of black silk thread to close the incision. Approximately 0.02 g of muscle was removed from each bird. Birds were held in the hand with the wings between the first and second fingers and the head pointing toward the wrist. I removed a piece of the surface layer of pectoralis muscle (2 mm wide × 8 mm long × 1 mm deep) with iris dissection scissors. In 1983, the birds were biopsied in the field at the site of capture; biopsies were taken 5–30 min after capture, and birds were released immediately after suturing. In 1984, biopsies were completed at a banding station within one h of capture, and the birds were released 10–50 min later at the site of capture. In both years, control birds were measured at the site of capture and released within one min.

Birds in both groups were scored for deposits of fat in the furcula and on the abdomen. Observers scored fat blindly with respect to the state of the birds except when a biopsy had recently (0–7 days) been performed. In these cases a scab could be seen by the observer when scoring fat. Furcular fat was scored from 0 to 4 using the procedure of Helms and Drury (1960). Abdomen fat was scored as follows: 0 = no fat, 1 = less fat visible than muscle, 2 = fat coverage roughly equal to muscle, 3 = more fat than muscle, and 4 = abdomen completely covered with fat. Individuals were given composite scores of the furcula score plus the abdomen score. In 1984, birds were weighed before biopsy with one of three calibrated Pesola scales.

Trapping and biopsies occurred during a span of four and six weeks in 1983 and 1984, respectively. Birds captured for the first time after two weeks were not considered for the within-season analyses. Recaptured birds were recorded, remeasured, and released. Biopsy wounds were checked and the rate of healing noted.

Birds that returned between years were either trapped or observed at feeding platforms. In all years, the intensity of trapping and observations was uniformly distributed along the trapline, and trapping of control and biopsied birds occurred simultaneously.

I tested for differences in return rates within each year and between years with a Chi-square analysis. Biopsied and nonbiopsied birds were compared with Mann-Whitney *U*-tests for differences in fat scores between initial capture and first recapture. A two-tailed *t*-test was used for the comparison of body mass.

RESULTS

In 1983, 41 White-throated Sparrows were biopsied, and 67 individuals captured and released as controls; in 1984 there were 76 biopsied and 68 control birds. All biopsied birds flew normally on release. Examination of the biopsy wounds of recaptured individuals indicated that the wound formed a scab within a day and was difficult to see in 7–14 days. The suture appeared to facilitate the healing process, primarily by pulling the skin over the wound in the muscle. All birds recaptured after the wound had healed had lost the suture. Birds recaptured up to a year later all had a slight depression in the muscle and a small amount of scar tissue.

The behavior of the birds was not noticeably affected by the biopsy. In 1984, observations of dominance continued after some of the birds had been biopsied, and in no case did the biopsy affect a bird's rank (W. Piper, pers. comm.). Additionally, although some individuals began migrating during this time, both control and biopsied sparrows were still present into May (W. Piper, pers. comm.).

I analyzed the major effects of muscle biopsy by comparing recapture rates of biopsied and control birds under the assumption that biopsy would affect the birds within seven days. In 1983, 66% of the biopsied birds and 52% of the control birds were recaptured after six days ($\chi^2 = 1.92$, df = 1, $P > 0.20$). In 1984, however, significantly more biopsied than control birds were recaptured after six days (59% vs 34%, $\chi^2 = 5.48$, df = 1, $P < 0.02$). No differences between the two groups were found in the number of days elapsing until recapture in 1983 ($\chi^2 = 2.44$, df = 2, $P > 0.25$) or in 1984 ($\chi^2 = 0.57$, df = 3, $P > 0.90$). In 1983, no birds were recaptured in the two days after initial capture, and in 1984 less than a third were recaught on the day after initial capture.

Birds in both groups in both years lost fat during the interim between initial capture and recapture. A Mann-Whitney U-test of the differences in changes in fat between initial capture and first recapture for biopsied and control birds was not significant in either year (1983, $z = 0.10$, $P > 0.45$; 1984, $z = 0.93$, $P > 0.17$) (Table 1). The power of this test against the alternative that biopsy actually caused the loss of one unit of fat was 0.97 in 1983 and was 0.84 in 1984.

Control birds lost significantly more mass than did biopsied birds (0.56 ± 0.84 [about 2% of total body mass] vs 0.00 ± 1.17, two-tailed t-test, $t = 2.06$, df = 77, $P < 0.05$).

In both years biopsied birds returned to the study area the following year at the same rate as did control birds (1983, 40.4% vs 31.2%, $\chi^2 = 1.26$, df = 1, $P > 0.25$; 1984, 41.0% vs 47.0%, $\chi^2 = 0.35$, df = 1, $P > 0.70$).

TABLE 1
CUMULATIVE (FURCULA PLUS ABDOMEN) FAT SCORES FOR BIOPSIED AND CONTROL WHITE-THROATED SPARROWS

	1983		1984	
	Initial capture	Recapture	Initial capture	Recapture
Biopsied	4.3[a] (41)[b]	2.9 (26)	3.5 (76)	2.7 (63)
Control	4.5 (66)	3.0 (32)	3.1 (69)	3.0 (35)

[a] See text for explanation of fat scores.
[b] Number of birds.

DISCUSSION

One possible explanation for why more biopsied birds than controls were recaptured is that biopsies might have prevented birds from initiating migration. If control birds in good condition left the study area while those in poor condition stayed, then the remaining birds would be biased toward birds in poor condition. In 1984, however, those control birds that stayed did not have significantly greater mass on initial capture than those birds that were not recaptured (two-tailed t-test, $t = 1.72$, df $= 28$, $P > 0.05$). In addition, the return rates between years suggest that biopsied birds had no more difficulty in preparing for and completing a migration of over 1000 miles round-trip (Chapel Hill, North Carolina, to southern Canada) than the controls.

A possible explanation as to why biopsied birds lost less weight than controls is a difference in time elapsing before first recapture between biopsied and control birds (cf. Mueller and Berger 1966). In the present study, however, there was no difference between the biopsied and control groups in days elapsed until recapture in either year.

Differences between biopsied and control birds in recapture rate and weight loss might have resulted from systematic initial differences between controls and biopsied birds. The recaptures in the two months preceding the 1984 biopsies showed a trend for birds caught in the traps designated for biopsies to be recaptured more frequently in the short run. Also, comparison of masses of all biopsied and control birds on initial capture revealed that biopsied birds averaged 1.3 g less than controls, a highly significant difference (two-tailed t-test, $t = 3.25$, df $= 103$, $P < 0.002$). This result suggests that differences existing before biopsy might have caused the higher short-term recapture rate of the biopsied birds.

My results indicate that biopsies do not affect condition or survival of wintering White-throated Sparrows. If biopsied birds were in poorer condition initially, then the biopsy should have had an even greater effect on these individuals. Biopsies, however, did not impair these birds' ability to maintain fat deposits and mass or to survive the rigors of migration.

Biopsies may affect birds of other species and at other times of year more severely. In a study of breeding Indigo Buntings (*Passerina cyanea*), in collaboration with R. B. Payne and S. Doehlert (Westneat et al. 1986), I found no adverse effects on the condition of breeding males, nesting females, and nestlings. Resighting rates and return rates between seasons did not differ between biopsied and control birds except for nestlings. However, when Frederick (1985) used this technique on 3 White Ibises (*Eudocimus albus*) (2 females, 1 male) that were rearing young nestlings, none of the birds was seen again. Ibises have a completely feathered breast, and incisions resulted in far more bleeding than in passerines.

Recently, Mumme et al. (1985) remarked that the low degree of variability in blood samples from Acorn Woodpeckers (*Melanerpes formicivorus*) severely limited the results of their study. Considerably more loci can be resolved from muscle tissue than blood or feather pulp (Marsden and May 1984). Additional loci will not make parental assignment substantially easier, but they will increase the probability of excluding a putative parent if it is not the actual parent.

Although caution is required in the study of details of population dynamics, the present study shows that long-term study of genetics and behavior is possible without measurable harm to the subjects.

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