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EFFECTS OF MUSCLE BIOPSY ON SURVIVAL AND BREEDING SUCCESS IN INDIGO BUNTINGS[1]

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Abstract. Breeding Indigo Buntings (*Passerina cyanea*) were sampled by pectoral muscle biopsy for electrophoretic analysis, and their breeding success and survival were compared with buntings that were only handled and color-banded and with buntings banded in an earlier year. Behavioral and reproductive comparisons showed little difference in males or in females among the three groups. First-year males were more likely to disappear (and presumably to disperse to a new breeding site) after being caught and biopsied before breeding than after being caught and only marked. Mating and fledging success of breeding buntings was not affected by biopsy or by capture and marking. Annual survival of adults was independent of biopsy. Nestling buntings survived to fledging equally well in biopsied and in banded broods, but were less likely to survive when their mother was netted near the nest than when she was not, due to predation. Buntings biopsied as nestlings returned and bred in the study area but at a lower incidence than control buntings banded as nestlings. Muscle biopsy appears to be a nondestructive technique that is compatible with most aspects of long-term population studies of small songbirds.

Key words: *Muscle biopsy; Indigo Buntings;* Passerina cyanea; *electrophoretic analysis.*

INTRODUCTION

The usefulness of electrophoretic techniques to study population structure, kinship, paternity, and intraspecific and interspecific brood parasitism in birds (Sherman 1981; Barrowclough 1983; Mock 1983; Gowaty and Karlin 1984; Fleischer 1983, 1985; Mumme et al. 1985) leads us to ask whether genetic sampling is compatible with long-term population studies. While human geneticists routinely sample isozymes in blood and other tissues with no demographic impact of their technique on their subjects, avian biologists usually sacrifice their subjects as specimens to obtain tissues for genetic analysis (Johnson and Brown 1980, Barrowclough 1983) or rely on blood or feather pulp which have few genetically variable loci (Marsden and May 1984, Gowaty and Karlin 1984, Mumme et al. 1985). Muscle biopsy (Baker and Fox 1978, Baker 1981) yields more loci usable for testing biological questions of kinship, but the effects of the biopsy procedure on survival and breeding success have not been determined in detail in a natural breeding population. We present an analysis of the survival and breeding success of Indigo Buntings (*Passerina cyanea*) that were biopsied, and we compare the results with those of unbiopsied birds in the same population.

METHODS

Indigo Buntings were captured, color-banded and biopsied in the field in a population living in an area of about 140 ha located 5 km northeast of Niles in Cass County, Michigan. The population has been observed each year from 1978 through 1985 to determine seasonal and lifetime individual breeding success and survival. Our observations of extra-pair matings in buntings (Payne 1982, 1983a, b) led us to test whether the genetic consequences of these matings could be detected without disrupting the population study.

Three groups of buntings were used for a comparison of the effects of biopsy and of the handling associated with capture and color-banding on behavior, breeding success and survival. First, some adults that had been color-marked in an earlier year were observed with no further capture. Second, birds that had not previously been captured and color-banded were caught, marked, and released. Third, other birds that had not previously been captured, or returning adults that had been captured and marked in an earlier year, were caught, the pectoral muscle was sampled in the live bird with biopsy, and the birds were marked and released. The times of capture var-

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ied for the biopsied group and the new color-banded group insofar as males to be biopsied were not captured during mating; the design was to avoid altering male behavior during the period when the mate was sexually receptive. Males were captured in a mist net by broadcasting a recorded bunting song. Females were captured in mist nets near the nest. Nearly all females were caught when they had nestlings. Nestlings were biopsied at five to seven days, when they were large enough to have developed the pectoral muscle but small enough not to fledge prematurely upon being handled. The control nestlings were banded but not biopsied at four to seven days. Indigo Buntings in the Niles population fledge at variable times depending on the weather and season from 8 to 12 days after hatching. Cases were selected for biopsy or for a control group according to prior determination of sections of the study area.

The biopsy procedure followed Baker (1981). Birds were biopsied in the field at the site of capture. The wings were secured between the investigator's first and second fingers, the head was directed towards the investigator, and the legs were restrained by the second and third fingers. The feathers along the left side of the breast were pushed aside, and the skin was wetted with propanol. A longitudinal incision of about 12 mm was made through the skin from the distal end of the sternum across the breast, parallel to the breast muscle fibers. A strip of muscle approximately 8 × 2.5 × 1.5 mm was removed by two long cuts along the muscle fibers and two short cuts across the ends. The muscle strip was lifted from the muscle body with sharp forceps. The incision was closed and the skin sutured with silk thread and a curved needle (Ethicon, Inc.). Nearly all biopsies were carried out by Westneat. All birds were released immediately (total handling time, 15 to 25 min) and nestlings were returned to the nest after biopsy was completed.

Buntings were observed throughout the season on all territories. From frequent (one- to seven-day) censuses we compared the seasonal activity of buntings in relation to their history of banding and biopsy. From the success of the nest of a bunting when captured and biopsied, as well as from any subsequent nestings in the season, we compared the effect of the procedures on breeding success of the adults and survival of the young. Observations were conducted from the first buntings to arrive in early May until the last breeding females left their nests on the study area in early September in 1983 and 1984, so the complete seasonal breeding success was determined for all buntings in the area. Survival of biopsied and unbiopsied adults was compared from the proportion of birds in each class that returned to the study area in the following year (1984 and 1985). Males were aged at capture by noting whether the greater primary coverts were blue (adult) or brown (first-year). As breeding success and annual survival (= local returns) differ between first-year and adult male buntings (Payne 1982, unpubl.), the age classes were analyzed separately. Female buntings cannot be aged with certainty, so no age groups were used in the analyses.

RESULTS

Muscle biopsy had no immediate effect on behavior. Indigo Buntings flew well upon release in almost all cases, and some males sang within 2 min of release. Several males had been chasing in territorial conflicts with neighboring males or intruders when they were captured and biopsied. These males all held their territories after they were released. Females were observed feeding their nestlings within an hour of release. In the cases where the biopsied young were observed shortly after the operation, they begged actively and were fed within an hour of being replaced in the nest. Biopsy had no obvious long-term effects on those individuals that were recaptured in a later year except for a slight depression in the muscle mass where the tissue had been removed, both in birds biopsied as nestlings and in birds biopsied as adults.

Most birds remained on their old territory and were resighted. Not all birds were seen alive after capture, and most of these may have deserted the area, as they were in apparent good condition upon release. However a few birds did not fly upon release. Of these 11 birds, 8 had been biopsied and 3 were captured controls. Five of these biopsied birds were seen later in the season, including two females escorting their fledglings. Three biopsied birds that did not fly appeared to be injured from struggling when their wings were restrained in biopsy; the muscles or tendons may have been strained. Each hopped away; one was later seen flying, and it returned the next breeding season. The risk of injury in biopsy appears to be associated with handling, as much as with loss of the excised muscle or with bleeding. Bleeding was generally less pronounced than in exploratory laparotomy of other small birds. No bird bled excessively. One of the most profuse bleeders sang within 2 min, stayed on his territory, and bred. He returned and bred in the following year as well, and he fathered fledglings in both years. In other years we had occasional birds that did not fly after capture and banding. Several were seen later and bred successfully; they had recovered from handling.

TABLE 1. Effect of capture and biopsy on the proportion of male Indigo Buntings (*Passerina cyanea*) that remained on the study area for at least seven days after capture or initial sighting.

Age	Group	No. of birds resighted	
		Yes	No
Adult	Not recaptured	101	3
	Marked	25	4
	Biopsied	53	4
First-year	Marked	44	11
	Biopsied	26	17

In 1983 and 1984 a total of 173 adult buntings were captured and biopsied. The apparent loss of 3 birds that were biopsied but did not fly and were not seen again out of 173 leads us to caution that handling and biopsy may carry a 2% risk to the bird.

MALE ACTIVITY AND SEASONAL TERRITORIALITY

The sample of male buntings for 1983 and 1984 included 295 cases to test the effects of capture and biopsy on behavior, survival, and breeding success. Group 1 included 104 adults that had been color-marked in an earlier year and were not recaptured in the year of observation. Group 2 included 29 adults and 57 first-year males that were captured and color-marked but were not biopsied. Group 3 included 57 adults and 43 first-year males that were biopsied.

In the analyses of the effects of biopsy on survival and breeding activity of males in the season of capture, we selected certain cases for comparison, because birds were captured and biopsied at varying times through the breeding season as they arrived on the study area throughout the period from early May through early August. All males that were captured and marked, whether or not they were biopsied, were selected to test whether they were seen at least seven days after capture. For the analysis of the total season of activity after capture, we selected only the males captured before 1 July (new males appear on the study area as late as 10 August and sometimes breed after they arrive, but late-arriving males have a shorter local season). The adults banded in an earlier year were used in comparison of total days only if they were first seen before 1 July in the current season. For the total time of residency after "capture" of returning adults (Group 1), we used the total number of days that the male was present after 21 May, which was the median date of capture of the males that were captured before 1 July.

The likelihood that a male would desert the area as a result of capture or biopsy was determined by comparing the proportion of males in each group that were sighted again at least seven days after initial sighting (Group 1) or capture (Groups 2 and 3). Nearly all adult males that were captured or resighted were sighted again at least seven days later. The proportion seen after capture was similar for biopsied birds, captured birds, and nonrecaptured banded birds that returned from an earlier year (Table 1). In adults the capture and biopsy had no significant effect on desertion ($\chi^2 = 3.10$, 2 df, $P > 0.05$, ns). However, in first-year males the biopsied birds were less likely to remain than were birds that were only captured ($\chi^2 = 4.51$, 1 df, $P < 0.05$).

TABLE 2. Effect of capture and biopsy on the proportion of male Indigo Buntings that remained at least seven days after capture: comparison of birds captured before any nesting and captured after nesting had begun for the season (within eight days of the first egg of the first nest).

Nesting when captured	Age	Group	No. of birds resighted	
			Yes	No
Yes	Adult	Not recaptured	20	1
		Marked	13	2
		Biopsied	28	2
	First-year	Marked	24	3
		Biopsied	13	1
No	Adult	Not recaptured	83	5
		Marked	12	2
		Biopsied	25	2
	First-year	Marked	20	8
		Biopsied	13	16

In males that were captured after they had bred at least once in the season (including the eight days prior to the laying of the first egg, a period in which the males were seen to copulate with their mate), nearly all were seen again at least seven days later (Table 2). The proportion sighted again was not significantly different among the three groups of adult males ($\chi^2 = 0.98$, 2 df, $P > 0.05$, ns) or between groups of first-year males ($\chi^2 = 0.16$, 1 df, $P > 0.05$, ns). Neither in adults nor in first-year males did biopsy have an effect on birds deserting the territory, mate and nest, or the study area. The experience of being captured and marked also had no effect on the probability that a male would disappear, as the adults that had been marked in an earlier year were no more likely to remain than were the adults captured in the current year.

In the males that were captured before they had bred (at least nine days before any eggs were laid), capture and biopsy had no apparent effect on the probability that an adult would remain (Table 2); nearly all adult buntings remained in all treatment groups. First-year males were more likely to remain if they were

TABLE 3. Total number of days a male Indigo Bunting remained on the study area after capture: ANOVA comparison of control, captured and marked, and biopsied birds.[1]

Age	Group	*n*	Mean	SD	*F*	*P*
Adult	Not recaptured	101	73.3	25.4	0.04	0.96 NS
	Marked	18	71.6	21.1		
	Biopsied	32	72.5	26.9		
First-year	Marked	35	51.7	27.1	3.41	0.07 NS
	Biopsied	19	65.7	25.6		

[1] For the returning adults that were not recaptured during the current season, we used the number of days the male was present after 21 May, the median date of capture of males that were captured by 1 July.

marked than if they were both marked and biopsied ($\chi^2 = 4.14$, 1 df, $P < 0.05$). The effect of biopsy was apparently associated with the nesting status of a first-year male at the time of biopsy.

To test whether the tendency of first-year males to desert after biopsy might be related to a lower androgen level before they had bred than the levels in nesting birds, we compared two structures that may be androgen-sensitive, the cloacal protuberance and the bill color. The cloacal protuberance of male buntings increases in length from 3 or 4 mm early in the breeding season to 6 to 8 mm in breeding individuals and is a sperm maturation and storage organ. The bill of newly returned first-year males is yellowish at the base but turns gray during the breeding season. The size of the cloacal protuberance (<6 mm; ≥6 mm) was associated with bill color (yellowish, not yellowish) among first-year male buntings captured from 1978 through 1982 ($n = 174$, $\chi^2 = 16.5$, 1 df, $P < 0.01$). However, the proportion of first-year males observed on the study area at least eight days after capture was not significantly associated with either cloacal protuberance size ($n = 96$, $\chi^2 = 1.04$, 1 df, $P > 0.1$) or bill color ($n = 97$, $\chi^2 = 3.74$, 1 df) in 1983 and 1984, though it was nearly so in the latter case ($P = 0.053$). Nor was it significantly different in birds with both a large cloacal protuberance and a non-yellow bill color than in birds without both of these characteristics of breeding males ($\chi^2 = 1.81$, 1 df, $P > 0.05$). Although both the cloacal protuberance and the bill color tend to indicate a lower sexual activity in birds that did not remain after capture, additional factors are apparently involved in the disappearance of nonbreeding males after biopsy.

The effect of capture and biopsy on the total number of days that the resident males were active on the study area was compared with an analysis of variance (ANOVA), including only those males that were first sighted or captured before 1 July and were seen on the study area for at least seven days. The results showed no effect of capture or biopsy on adults or on first-year males (Table 3). Biopsied first-year males tended to remain longer than the nonbiopsied first-year males, but the difference was not significant. The ANOVA was repeated for only those birds that were known to nest, and for only those birds that were captured before they had nested. In none of the tests did the biopsied birds have a significantly shorter season.

MALE MATING AND BREEDING SUCCESS

Males captured at least eight days before the first egg was laid in their first nest of the season were selected for comparison of the proportion of birds that nested. This time interval was selected because buntings usually initiate copulation and the females begin to build a nest at least eight days before the first egg is laid. Most males that remained were successful in attracting a female (Table 4). The proportion that mated was not different in uncaptured control, marked, and biopsied adults ($\chi^2 = 0.51$, 2 df, $P > 0.05$) or in captured and biopsied first-year males ($\chi^2 = 2.35$, 1 df, $P > 0.05$).

The number of males that initiated a new nest after capture was compared in birds captured before 1 July and seen at least seven days after release. The proportion that nested after capture was no lower in biopsied birds than in birds that were only marked and released (Table 5; adults that did not nest were too few for comparison; first-year males, $\chi^2 = 3.46$, 1 df, $P > 0.05$).

TABLE 4. Effect of capture and biopsy on the number of male Indigo Buntings that acquired a mate after capture.[a]

Age	Group	No. of males that acquired a female: Yes	No
Adult	Not recaptured	73	1
	Marked	12	0
	Biopsied	25	0
First-year	Marked	14	6
	Biopsied	12	1

[a] Sample includes those males that remained on the study area for at least seven days after capture or initial observation (Group 1) and captured at least eight days before the first nest of the season (if any nest). Birds of uncertain mating history are excluded.

TABLE 5. Effect of biopsy on the number of male Indigo Buntings that nested after capture.[a]

Age	Group	No. of birds that had a subsequent nest: Yes	No
Adult	Marked	16	0
	Biopsied	27	2
First-year	Marked	21	9
	Biopsied	15	1

[a] Sample includes only those males that were caught before 1 July and remained on the study area for at least seven days after capture, and whose nesting histories were determined.

The effect of biopsy on the success of a male's current nest was tested in males captured when their nest had eggs or nestlings no older than four days. The sample is small (total 38 nests) and the adults and first-year males were combined for comparison (Table 6; $\chi^2 = 1.47$, 1 df, $P > 0.05$, ns). The comparison is complicated by the biopsy of the females and the nestling brood in most cases. However, male biopsy had no evident detrimental effect on the likelihood that the current nest would fledge.

The effect of biopsy on breeding success was also tested by comparing the proportion of males that had fledglings either from their current nest or from a later nest (including only those males caught before 1 July and sighted at least seven days after capture; Table 7). In neither adults or first-year males did biopsy have a detrimental effect on breeding success (adults, $\chi^2 = 1.21$, 1 df, $P > 0.05$; first-year males, $\chi^2 = 2.26$, 1 df, $P > 0.05$).

MALE SURVIVAL

The effects of capture and biopsy on males observed in a later year are summarized in Table 8, which indicates whether or not the marked birds seen on the study area in 1983 or 1984 were resighted in the following year. The proportion that returned to the study area was independent of capture and of biopsy in adults ($\chi^2 = 3.42$, 2 df, $P > 0.05$, ns) and in first-year males ($\chi^2 = 1.22$, 1 df, $P > 0.05$, ns). Ten nonrecaptured adults that did not return in the next year were found dead on the road; they are included in the totals. One was 1.0 km from the study area and 2.3 km from its breeding site of the previous year. A few other birds that were not seen again may have returned outside the study area; fewer than 10% of the adult males seen in a year had not been banded in an earlier year, so had dispersed across the margin of the study area. Three males that did not remain for at least seven days after capture in the previous year were seen again the next year; an adult and one first-year male had been biopsied and the other first-year male had not. Survival as indicated by return in the next year was not affected by capture and marking or by biopsy.

TABLE 6. Effect of biopsy on the fledging success of males that were captured during an active nest (laying, incubation, or nestlings no older than four days).

Group	Nest success: did one or more young buntings fledge? Yes	No
Captured, not biopsied	3	9
Captured and biopsied	12	14

TABLE 7. Effect of biopsy on breeding success: number of males that had fledglings after capture, either from a current nest or from a later nest.[a]

Age	Group	Breeding success: did one or more young buntings fledge? Yes	No
Adult	Marked	9	7
	Biopsied	21	8
First-year	Marked	10	20
	Biopsied	9	7

[a] Sample includes only those males that were caught before 1 July and remained on the study area for at least seven days after capture. "Current nest" is the nest that was active at the time of capture; not all males had a current nest when captured.

EFFECT OF BIOPSY ON FEMALES

We compared the effect of capture and biopsy on the survival and breeding success of females. Most females survived biopsy and remained with their nest. Two females apparently deserted nests with nestlings after they were captured and biopsied. One nested again later in the same territory; the other deserted a nestling Brown-headed Cowbird (*Molothrus ater*) and was not seen again. Another female deserted a nest with three eggs after she was captured but not biopsied. Female survival was determined from the number that either fledged their brood or were resighted after the nestlings were taken by a predator (Table 9). Survival was independent of capture and biopsy ($\chi^2 = 0.33$, 2 df, $P > 0.05$).

The tendency of females to initiate another nest in the same season after they were captured at an earlier nest was not related to

TABLE 8. Effects of capture and biopsy on the number of male Indigo Buntings that returned to the study area in a later year.

Age	Group	No. of birds observed in a later year: Yes	No
Adult	Not recaptured	55	54
	Marked	13	16
	Biopsied	36	21
First-year	Marked	22	33
	Biopsied	22	21

TABLE 9. Number of females that were seen again on the study area within the season after capture and biopsy.[a]

Group	Observed after release	
	Yes	No
Not recaptured	32	5
Marked	43	3
Biopsied	62	9

[a] Numbers of marked and biopsied females indicate cases where female was seen at least seven days after capture or whose brood fledged after her capture (no broods were reared by males alone). For females that were not recaptured, the numbers indicate females observed at a nest with nestlings of four to seven days of age at any time during the season.

whether they were biopsied at the earlier nest (Table 10). Females were no less likely to nest again following biopsy than following capture without biopsy ($\chi^2 = 0.89$, 1 df, $P > 0.05$).

The proportion of females that returned in a later year was compared among the three experimental groups (Table 11). A few females were sighted one year, not sighted the next, then sighted again in a later year; we suspect they nested off the study area in the middle year. The field observations were intensive locally, and females switch territories from year to year more often than do males (Payne 1983b). Neither biopsy nor capture appeared to affect the probability that a female would return from year to year ($\chi^2 = 1.08$, 2 df, $P > 0.05$, ns).

EFFECT OF BIOPSY ON NESTLINGS

A total of 261 nestlings were biopsied in 1983 and 1984. There was no sign that biopsy affected the growth, development, or activity of the nestlings. The only instance of biopsied young that were found dead in the nest was associated with female desertion. Most nestlings survived both in biopsied broods and in broods that were not biopsied but were banded (Table 12). No difference in nest success was noted in nests where both the female and the young were biopsied and in nests where the female and young were only banded. The overall survival of the biopsied nestlings was the same as the overall survival of the marked nestlings (77% of marked broods, 80% of biopsied broods, $\chi^2 = 0.175$, 1 df, $P > 0.05$, ns).

TABLE 10. Number of females that nested again on the study area after capture and biopsy.[a]

Group	Nested after capture and release	
	Yes	No
Not recaptured	8	1
Marked	7	6
Biopsied	10	4

[a] Samples of marked females and biopsied females include only those birds that were caught at a nest before 1 July. For females that were marked in an earlier year and not recaptured during the current year, the sample includes all birds that were observed at a nest with nestlings at least four days of age by 1 July. This selection of cases includes all females with a nest likely to have fledged by 8 July, and who had time to nest successfully again during the same breeding season.

TABLE 11. Effects of capture and biopsy on the number of female Indigo Buntings that returned to the study area in a later year.

Group	No. of birds observed in a later year	
	Yes	No
Not recaptured	28	37
Marked	24	24
Biopsied	30	44

EFFECT OF FEMALE CAPTURE ON SURVIVAL OF NESTLINGS

In addition to the cases listed in Table 12, we had a few cases where a female was captured at a nest when the nestlings were too young to be biopsied and the nestlings were never marked or biopsied because they disappeared from the nest before they had time to fledge. In five additional cases the female was captured, marked, and biopsied. Comparing the sums from Table 12 in Rows 1 and 2 with the sums of Rows 3 and 4 (including the nine cases where the female was captured but the young were not marked), we found that nests where the female was captured were significantly less likely to survive to fledging than were nests where the female was not captured but had been marked at an earlier nest or in an earlier year ($\chi^2 = 5.14$, 1 df, $P < 0.05$). In all cases the nests were unsuccessful because the young were taken by a predator, not because they were deserted (no young were found dead; the emptied nests usually were tipped or the lining pulled up where a nestling apparently gripped while being removed by a predator). Some predators may have used the disturbance of vegetation to locate the nest; we avoided netting or visiting a nest if we saw Blue Jays (*Cyanocitta cristata*) nearby. Although our netting may have increased the risk of predation of the nestlings, most nests with young did in fact fledge (Table 12).

SURVIVAL OF YOUNG TO THE NEXT BREEDING SEASON

Although most buntings banded as nestlings were not seen in a following year and probably most survivors returned to their breeding range

TABLE 12. Number of broods that fledged: comparison of biopsy and nonbiopsy treatments.

Female treatment	Nestling treatment	No. of broods	
		Fledged (%)	Not fledged (%)
Not recaptured	Marked	45 (78)	13 (22)
Not recaptured[1]	Biopsied	34 (89)	4 (11)
Marked	Marked	16 (76)	5 (29)
Biopsied	Biopsied	45 (74)	16 (26)

[1] These females were biopsied at an earlier nest.

to breed away from their natal site, several did return to their natal area. The proportion of buntings that returned to their natal area at Niles was compared for birds biopsied as nestlings and for birds that were only banded as nestlings. Only those birds that were recaptured are included. Of the 228 biopsied nestlings that fledged, 13 were captured in the following year. Of the 195 control nestlings that fledged, 26 were captured in the following year. The return rate was 5.7% for biopsied young and 13.3% for nonbiopsied young. The proportion of returning birds was significantly lower for the biopsied young ($\chi^2 = 7.31$, 1 df, $P < 0.01$). We conclude that there was a negative effect of biopsy on nestling survival from fledging to return to the natal area in a following year.

DISCUSSION

Our results indicate that biopsy of the pectoral muscle has little effect on survival and breeding success of adult Indigo Buntings. The main effect of biopsy appears to be a tendency for first-year males to desert the study area early in the season if they have not yet mated and initiated a nest. Males and females that were captured after they had begun a nest were likely to continue the current nest and to nest again later in the season if the initial capture was earlier than late June. The increase in handling time and the possibility of injury in stretching the wings may present a greater risk to the bird than the incision and biopsy procedure itself. The risk in biopsy appears to be no greater than in field laparotomy to measure gonad development; and as in laparotomies (Miller and Miller 1968), the birds sang and resumed their nesting activities shortly after release. Neither capture and marking nor biopsy had any apparent effect on the number of days that a male remained on the study area after capture, or on his chances of nesting again. Survival of the adults from year to year as determined by the proportion that were sighted again on the study area also was independent of capture and biopsy in the preceding year.

The tendency of first-year males to desert their territories when biopsied early in the breeding season probably indicates their assessment of the area as risky and to be avoided. The extra handling and trauma involved in biopsy beyond that involved in being captured and color-banded may have behavioral effects on unmated males. We suspect that most of these males moved to a new territory outside the study area. The disappearance of some unmated first-year male buntings is similar to that observed in the Pied Kingfisher (*Ceryle rudis*) where unmated male auxiliaries that were not closely related to the breeding pair were more likely to desert after being captured and sampled for metabolic data than were primary helpers or the breeding birds (Reyer 1984).

Biopsy also had a negative effect on the survival of young buntings in the months after fledging. The slightly lower proportion of young buntings that returned in the following year from biopsied broods than from broods that were only banded dampens our enthusiasm for a general use of biopsy in population studies. No decreased survival of biopsied nestlings was apparent. Many fledglings were sighted for weeks after biopsy as nestlings. Our observations of biopsied broods were more intensive so we have no reliable data to compare survival during the weeks after fledging in the biopsied and control broods. Several surviving yearlings were found to return at distances of 1 km or greater from their natal territory and we usually were unable to locate them if they dispersed at greater distances.

Muscle biopsy generally seems to have little adverse effect on small songbirds. Dark-eyed Juncos (*Junco hyemalis*), White-crowned Sparrows (*Zonotrichia leucophrys*), and White-throated Sparrows (*Z. albicollis*) have all been biopsied in field conditions with no obvious detrimental result (Baker 1981; Baker and Fox 1978; Westneat, 1986). In the present study on a breeding population of Indigo Buntings, the procedure had little adverse effect and that appeared to be mainly behavioral, though we did not trace all cases of disappearing birds to dispersal into another area. The potential usefulness of the technique in genetic analysis leads us to encourage a selective and judicious use in other populations.

In addition to testing the effects of biopsy, the comparison of the seasonal activity, breeding success, and survival across years of the older buntings that were captured and banded with birds of the same age that were identified by observation in the field from their color bands on an earlier year indicates that there is no significant effect on the birds of capture and marking. The captured and the nonrecaptured birds of both sexes had similar success and similar survival. Capture and color-marking of small songbirds did not greatly affect their lives, though it allowed us to identify individual birds and to follow their breeding success and return from year to year.

We offer a few recommendations that may improve results with biopsy as a field technique. We routinely sutured the incision to minimize the area of exposed muscle tissue, though suturing added 3 to 5 min to handling time. Second, subjects should be caught when the weather is not too hot. Females should be

caught when their young are partly grown and after the brood patch has regressed, to avoid desertion of the nest and to facilitate the surgical procedure of biopsy by avoiding the cutting of vascular or edematous area. Capture of female buntings for banding often causes desertion shortly after laying, but desertion is rare after the first week of incubation. Finally we suggest continued monitoring of the technique by comparing the behavior, breeding success, and survival of a control sample in any population study.

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LITERATURE CITED

BAKER, M. C. 1981. A muscle biopsy procedure for use in electrophoretic studies of birds. Auk 98:392–393.

BAKER, M. C., AND S. F. FOX. 1978. Dominance, survival, and enzyme polymorphism in Dark-eyed Juncos *Junco hyemalis*. Evolution 32:697–711.

BARROWCLOUGH, G. F. 1983. Biochemical studies of microevolutionary processes, p. 223–261. *In* A. H. Brush and G. A. Clark [eds.], Perspectives in ornithology. Cambridge University Press, New York.

FLEISCHER, R. C. 1983. A comparison of theoretical and electrophoretic assessments of genetic structure in populations of the House Sparrow (*Passer domesticus*). Evolution 37:1001–1009.

FLEISCHER, R. C. 1985. A new technique to identify and assess the dispersion of eggs of individual brood parasites. Behav. Ecol. Sociobiol. 17:91–99.

GOWATY, P. A., AND A. A. KARLIN. 1984. Multiple maternity and paternity in single broods of apparently monogamous Eastern Bluebirds (*Sialia sialis*). Behav. Ecol. Sociobiol. 15:91–95.

JOHNSON, M. S., AND J. L. BROWN. 1980. Genetic variation among trait groups and apparent absence of close inbreeding in Grey-crowned Babblers. Behav. Ecol. Sociobiol. 7:93–98.

MARSDEN, J. E., AND B. MAY. 1984. Feather pulp: a nondestructive sampling technique for electrophoretic studies of birds. Auk 101:173–175.

MILLER, A. H., AND V. D. MILLER. 1968. The behavioral ecology and breeding biology of the Andean Sparrow, *Zonotrichia capensis*. Caldasia (Ecologica) 10:83–154.

MOCK, D. W. 1983. On the study of avian mating systems, p. 55–84. *In* A. H. Brush and G. A. Clark [eds.], Perspectives in ornithology. Cambridge University Press, Cambridge, England.

MUMME, R. L., W. D. KOENIG, R. M. ZINK, AND J. A. MARTEN. 1985. Genetic variation and parentage in a California population of Acorn Woodpeckers. Auk 102:305–312.

PAYNE, R. B. 1982. Ecological consequences of song matching: breeding success and intraspecific song mimicry in Indigo Buntings. Ecology 62:401–411.

PAYNE, R. B. 1983a. Bird songs, sexual selection, and female mating strategies, p. 55–90. *In* S. K. Wasser [ed.], Social behavior of female vertebrates. Academic Press, New York.

PAYNE, R. B. 1983b. The social context of song mimicry: song-matching dialects in Indigo Buntings (*Passerina cyanea*). Anim. Behav. 31:788–805.

REYER, H.-U. 1984. Investment and relatedness: a cost/benefit analysis of breeding and helping in the Pied Kingfisher (*Ceryle rudis*). Anim. Behav. 32:1163–1178.

SHERMAN, P. W. 1981. Electrophoresis and avian genealogical analyses. Auk 98:419–422.

WESTNEAT, D. F. 1986. The effects of muscle biopsy on survival and condition in White-throated Sparrows. Wilson Bull. 98:280–285.