J. Field Ornithol., 69(4):595–602

EFFECTS OF BLOOD SAMPLING IN GREAT TITS

THOMAS LUBJUHN
Institut für Angewandte Zoologie
Rheinische Friedrich-Wilhelms-Universität
An der Immenburg 1
D-53121 Bonn, Germany

JÖRG BRÜN
Institut für Angewandte Zoologie
Rheinische Friedrich-Wilhelms-Universität
An der Immenburg 1
D-53121 Bonn, Germany

WOLFGANG WINKEL
Aussenstation Braunschweig des Instituts für Vogelforschung
"Vogelwarte Helgoland"
Bauernstr. 14
D-38162 Cremlingen, Germany

STEFANIE MUTH
Abteilung Allgemeine Zoologie
FB 9 Biologie
Universität GH Essen
D-45117 Essen, Germany

Abstract.—Because of legal requirements in different countries as well as moral and ethical considerations, the effects of behavioral studies on animal welfare should be quantified. In this study, the effects of blood sampling on juvenile and adult Great Tits (*Poecile major*) were examined. Direct injuries were looked for as a possible short-term effect. We also quantified survival until fledging of nestlings that were bled versus those that were not bled as a possible medium-term effect, and survival until the next breeding season as well as the reproductive success in the year following the treatment were investigated as possible long-term effects. We found no effects of blood sampling in each of these instances.

EFECTO DE TOMAR MUESTRAS DE SANGRE EN *POECILE MAJOR*

Sinopsis.—En estudios de conducta, la salud de los animales debe ser tomada en consideración. Debido a los diferentes requerimientos de ley en diferentes países y consideraciones éticas y morales, se deben cuantificar los efectos de los estudios de conducta en la salud de los animales. En este trabajo, se examina el efecto de tomar muestras de sangre en juveniles y adultos de *Poecile major.* A corto alcance se estudio el efecto de heridas directas a las aves. A mediano alcance, se cuantificó la supervivencia hasta el periodo de volantón. Esto se hizo en pichones a los cuales se les había sacado sangre en comparación con un grupo que no fue sangrado. Como efecto a largo alcance, se estudió la supervivencia, hasta la próxima temporada de cría, y el éxito reproductivo de los adultos que fueron sangrados. No se encontró ningún efecto detrimental al sacarle sangre a los diferentes grupos experimentales.

Although there is considerable agreement in the scientific community that animal welfare should be considered whenever possible (e.g., Dawkins and Gosling 1992), specific impacts of behavioral studies are rarely quantified. If one is able and willing to do such investigations, one has to distinguish between different kinds of effects. First, there may be direct, short-term effects of a treatment (e.g., stress, pain, injuries, or even death). Secondly, there may be influences in the medium-term. For example, in birds parents may care less for their young and thus the nestlings may have a lower chance to fledge or survive. Finally, long-term effects, such as reduced survival to the next breeding season, may occur.

Studies involving blood sampling as a treatment have become more important since the development of techniques like isozyme analyses (e.g., Evarts and Williams 1987, Brown and Bomberger Brown 1988, Sherman and Morton 1988, Gowaty and Bridges 1991) and DNA fingerprinting (e.g., Burke and Bruford 1987, Wetton et al. 1987, Birkhead et al. 1990, Westneat 1990, Lifjeld et al. 1991). Many of these studies were done in birds, but few investigations have measured at least some of the possible effects of blood sampling (e.g., Raveling 1970, Bigler et al. 1977, Colwell et al. 1988, Lanctot 1994). Because our work on Great Tit behavior also involves blood sampling for the purpose of parentage analyses via multilocus fingerprinting (e.g., Lubjuhn et al. 1993a,b; 1994), we examined whether this treatment has negative effects on the birds.

METHODS

Study species.—Great Tits (*Poecile major*) are small passerine birds that are socially monogamous and territorial cavity nesters (Perrins 1979). The sexes are easily distinguished by the size of the black breast stripe (larger in males). During the breeding season females can also be identified by their incubation patch. Like many other bird species, Great Tits show biparental care (both sexes guard their nest from potential predators and feed the nestlings and fledglings).

Study area and bird-banding activities.—The study was carried out during the breeding seasons in 1993 and 1994 in a mixed forest near Bahrdorf (Lower Saxony, Germany [52.20 N, 11.01 E]; for further details see Berndt and Winkel 1967, Curio et al. 1984). This area belongs to the research station "Aussenstation Braunschweig des Instituts für Vogelforschung Vogelwarte Helgoland" where all young of cavity-nesting bird species that settle in any of the 690 nestboxes were banded routinely with unique numbered aluminum bands of the "Vogelwarte Helgoland." Females, which were caught while they were incubating eggs or brooding young, were also banded routinely. Male Great Tits, however, were only banded in context with blood sampling (see below) because they were not involved in the incubation or brooding.

Capture of birds and blood sampling.—Adult birds were captured using nestbox traps when the young were at least 10-d old. Approximately 50 μl of blood were taken from the ulnar vein (*vena ulnaris*) of the adults and the young by puncturing the vein with a small needle (0.4 × 20 mm) and removing the extravasating drop of blood with a microcapillary tube. If bleeding continued after sampling we applied "Clauden™" wadding that efficiently accelerates coagulation. All birds that did not already wear an aluminum band were banded.

Treatments.—In 1993 we sampled blood from 36 complete Great Tit families containing 268 nestlings. Additionally, blood samples were taken from six families where either only the female (n = 4) or the male (n = 2) was caught. These broods contained 39 nestlings. Lastly, in some broods we took blood only from a single parent (females: n = 1; males: n = 12). Thus 41 females, 50 males, and 307 nestlings were bled. This group will be refered to as "bled."

Because of the bird-banding activities in the study area (see above) there were also broods without blood sampling where either only the young were banded (n = 39 broods containing 225 nestlings) or the female and the young were banded (n = 14 broods containing 87 nestlings). In the 12 instances where we took blood only from the males (see above), we also banded the young in 11 cases (n = 74) and the females in 10 cases. Additionally, in two broods only the females were banded. Thus 26 females and 386 nestlings were banded but not bled. This group will be refered to as "unbled."

The sample of birds that were bled was taken randomly from the whole population. Effects from factors other than blood sampling such as time of breeding season or territory quality were therefore unlikely to covary systematically with bleeding status.

Investigation of possible effects of blood sampling.—Possible negative effects of blood sampling on the birds investigated could be attributed to one of the following groups:

(1) Short-term effects: Such effects were considered to result directly from the blood-sampling procedure and to occur during or shortly after blood sampling. For example, birds may die because of blood loss and/or stress or may retain visible injuries.

(2) Medium-term effects: Blood sampling may also generate negative effects within a few days after blood sampling. For example, as a consequence of the stress or injury associated with the blood-sampling procedure, parents may care insufficiently for their young. Additionally, undetectable injuries to the nestlings and/or adults may lead to a delayed death.

(3) Long-term effects: Blood sampling may also harm the birds in the long-term by generating negative effects on their physical condition. In 1994 we sampled blood from 88.8% of all Great Tit families in the study area. Therefore we were able to compare data on recapture rates and dispersal (measured as distance between the nestboxes the birds occupied in 1993 and 1994 using standardized maps) that can be used to evalute such effects. In addition, we compared the reproductive success in the year following the treatment for females that were bled versus those that were not.

RESULTS

Short-term effects.—Short-term effects of blood sampling such as serious injuries or death did not occur in 1993 or 1994. Sometimes small hematomas remained under the skin, but this type of injury did not seem to harm the birds detectably because adults could always fly away after

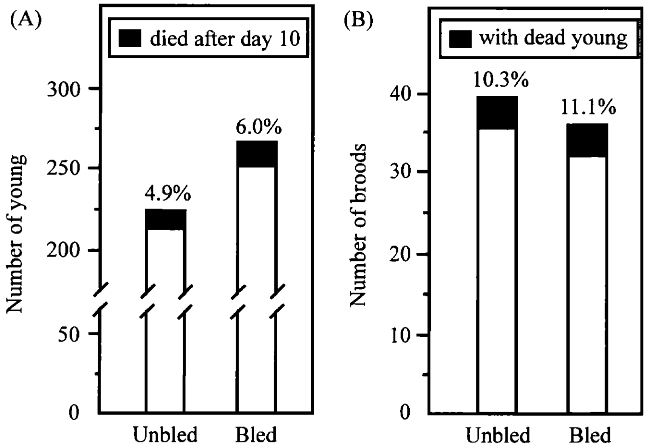

FIGURE 1. Proportional number of young that died after day 10 of hatching (A) and proportional number of broods that contained at least one nestling that died after day 10 of hatching (B) with respect to the different treatments in 1993. No significant differences were found between broods where solely the young were banded ("unbled") and broods where both adults and all young were banded and bled ("bled").

treatment without any visible handicap, and in the young these hematomas disappeared within a few days.

Middle-term effects.—To investigate possible medium-term effects, we compared the number of young that died after day 10 of hatching in broods where the young and both parents were bled versus those in broods where only the young were banded. These two samples were chosen because they exhibited the greatest difference in treatment, and possible negative effects should be most obvious. In the unbled group, 11 of 225 nestlings died after day 10 of hatching, while in the bled group 16 of 268 nestlings died after day 10 (Fig. 1A). This difference was statistically not significant ($\chi^2 = 0.11$, df = 1, $P > 0.7$). Our samples were sufficiently large to detect an approximate doubling of mortality due to bleeding.

One may argue that the nestlings within a given brood are dependent samples in relation to the effects of possibly reduced parental care. However, no differences were found when comparing both groups with respect to the number of broods in which one or more young died (Fig. 1B; unbled: 4 of 39 broods, bled: 4 of 36 broods; Fisher's Exact Test, $P = 1.0$). Here, our samples were sufficiently large to detect an increase in mortality due to bleeding, if the percentage of broods with one or more dead young would have increased by factor 3.

Long-term effects.—The survival of nestlings and adults was investigated as possible long-term effects. First, we compared the recapture rates in 1994 with respect to the different treatments in 1993. No significant differences were observed between young that were only banded and those who were additionally bled (Fig. 2; unbled: 5 of 386 recaptured, bled: 10

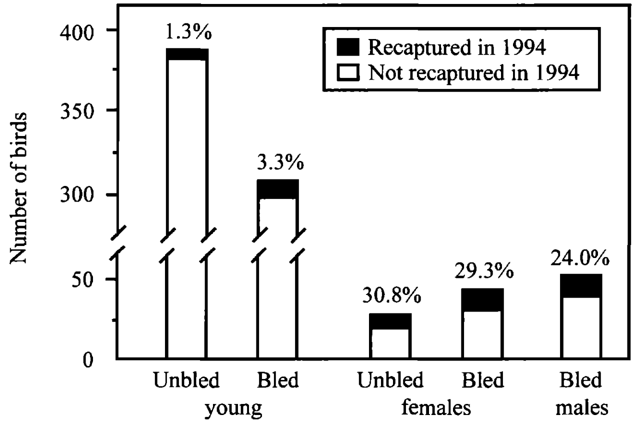

FIGURE 2. Recapture rates in different groups of birds that were either only banded ("unbled") or banded and bled ("bled"). No significant effects of blood sampling were found. Number over the bars represent percentage of recaptures.

of 307 recaptured; $\chi^2 = 2.25$, df = 1, $P > 0.1$). This also holds true for females (Fig. 2; unbled: 8 of 26 recaptured, bled: 12 of 41 recaptured; $\chi^2 < 0.01$, df = 1, $P > 0.9$). Because no males were only banded, we compared the recapture rates of bled males with both bled and unbled females, assuming no sex-specific differences. No significant effects were found (Fig. 2; bled males: 12 of 50 recaptured; $\chi^2 \leq 0.13$, df = 1 and $P > 0.7$ in both comparisons). With the exception of the recapture rates of young, our samples were sufficiently large to detect a reduction in the recapture rates for bled birds of 80% relative to the control group. For the young such a value can not be calculated because even if no bled nestling had been recaptured, this would not have resulted in a statistically significant difference. However, because the recapture rates for bled young are more than twice as high as for unbled nestlings, it seems unlikely that negative effects of blood sampling would be found on the basis of greater samples.

Recapture rates may not be a good estimate of survival of birds because the individuals of one group may disperse farther than those of another (Högstedt 1981). Therefore, we also compared the dispersal of the different groups. No significant effects of treatment were observed in young or females (Fig. 3; unbled vs. bled young, *t*-test: $t = -0.43$, df = 13, $P = 0.68$; unbled vs. bled females, *t*-test: $t = -1.08$, df = 18, $P = 0.30$). Also no significant differences were found comparing bled males with either unbled or bled females (Fig. 3; *t*-tests: each $P > 0.15$).

Another possible long-term effect is reduced reproductive success in the year following the treatment. However, in 1994 neither clutch size nor the number of young at day 10 after hatching differed between females that were bled and those that were unbled in 1993 (clutch size in 1994 [mean ± SE]; females bled in 1993: 9.6 ± 1.5 [n = 12], females unbled

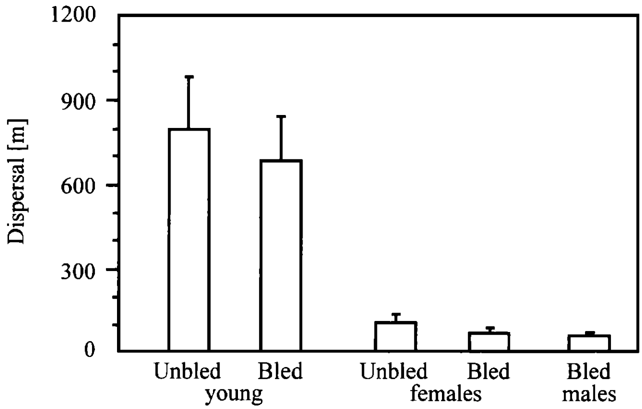

FIGURE 3. Mean (+SE) dispersal in different groups of birds that were either only banded ("unbled") or banded and bled ("bled"). No significant effects of blood sampling were found. Dispersal was measured as distance between the nestboxes the birds occupied in 1993 and 1994.

in 1993: 10.0 ± 1.1 [n = 8]; t-test: $t = 0.68$, df = 18, $P = 0.51$; number of young at day 10 [mean ± SE]; females bled in 1993: 8.2 ± 2.7 [n = 12], females unbled in 1993: 7.1 ± 2.4 [n = 8]; t-test: $t = -0.88$, df = 18, $P = 0.39$).

DISCUSSION

Though not designed to test the effects of bleeding, our data on Great Tits indicate that blood sampling exerts no detectable negative effects on the birds. Similar, but not as comprehensive, investigations have shown no noticeable effects of blood sampling on other bird species (Canada Geese, *Branta canadensis* [Raveling 1970], Mourning Doves, *Zenaida macroura* [Bigler et al. 1977], and Buff-breasted Sandpipers, *Tryngites subruficollis* [Lanctot 1994]). However, Colwell et al. (1988) reported that up to 3% of Red-necked Phalaropes (*Phalaropus lobatus*) died during blood sampling or suffered debilitating injuries. Thus, each species needs to be investigated before negative effects of blood sampling could be excluded. Effects attributable to individual investigators also have to be considered. Some fieldworkers may bleed almost every species without problems, whereas others may struggle with easily handled species.

The effects of blood sampling may also vary with the amount of blood taken. In our study the volume of blood samples was 50 µl. If the total blood volume contributes approximately 8% to the body mass of an individual Great Tit (Sturkie 1965:110–111), we removed approximately 3–4% of the total blood volume of the adults and 4–5% of that of the nestlings. Increasing the volume of blood samples—as may be necessary for methods other than DNA fingerprinting—may create effects not predictable from our investigations.

It should also be noted that our statements on short-term effects are restricted to direct injuries and death; other effects are possible. For example, mass of young within a few days after blood sampling was not measured. It is possible, even likely, that parents stop feeding the nestlings for a more or less short period after blood sampling. This may result in a mass loss of nestlings. However, survival until fledging, recapture rates, and dispersal ranged similarly as described by Winkel (1981). This shows that at least in the medium and long-term, nestlings are not affected by blood sampling. Even if there was a minimal mass loss, it appears to be compensated by fledging. Otherwise one would expect a decreased survival of those nestlings (e.g., Tinbergen 1987, Tinbergen and Boerlijst 1990).

Finally, none of the effects we have examined allow us to evaluate pain or stress of the birds during or after blood sampling, because indicators of stress (e.g., corticosterone, heart rates) or pain were not measured. Future researchers should also consider these variables while judging if the aims (and/or achievable results) of their study justify the method(s) used.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (Lu 572/1-1 and Cu 4/34-1). We thank K. P. Sauer, H. Burda, and J. T. Epplen for critical comments on the manuscript, the Vogelwarte Helgoland for making available the unique numbered aluminum bands, H.-H. Berndt, D. Berndt, and H.-J. Schultz for their help during the regular controls of the nestboxes and A. Schlüter for his permanent support during our field work. We also wish to thank C. Ray Chandler for improving our English.

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Received 14 Jul. 1997; accepted 25 Nov. 1997.