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Does use of doubly labeled water in metabolic studies alter activity levels of Common Poorwills?—The doubly labeled water (DLW) technique is the major method currently used to estimate field metabolic rates (FMR) in free-ranging animals (Lifson and McClintock 1966, Speakman and Racey 1988a, Nagy 1980). The procedure involves injecting small amounts of radioactive or stable isotopes (^3H or ^2H and ^{18}O). After the isotopes have equilibrated with body water, a blood sample is taken. The animal is then released, and a second blood sample is taken one to several days later. Measurements of isotopic loss then provide a means of estimating CO_2 production, from which FMR can be calculated. Validation studies suggest that DLW provides an estimate of FMR accurate to 5–10% in vertebrates (Nagy 1980, 1989; Nagy and Costa 1980; Williams and Nagy 1984a, b; Goldstein and Nagy 1985; Speakman and Racey 1988b; Nagy et al. 1990; Gabrielsen et al. 1991). To our knowledge, the only rigorous assessment of the effect of the DLW protocol (e.g., injection, holding in captivity during equilibration, and blood sampling) on behavior, and hence energy expenditure, has been done under laboratory conditions (Speakman et al. 1991). These authors call for an assessment of the protocol on free-ranging animals.

We made an independent set of measurements to determine if the injection, equilibration period, and blood sampling associated with the DLW technique alters foraging activity of free-ranging Common Poorwills (*Phalaenoptilus nuttallii*). We used telemetry and chose to measure feeding activity as an indicator of altered behavior, since this is probably the most energetically costly behavior undertaken by these birds and because it is easily assessed using telemetry. Poorwills are nocturnal insectivorous birds which have the ability of enter torpor (Brigham 1992), making them interesting subjects for energetic studies. However, since entry into torpor would confound estimates of activity, we collected data only for incubating and brooding birds who do not usually enter torpor (Kissner and Brigham, in press).

We collected these data in June–August 1991 and May–June 1992 in the Okanagan valley near Oliver, British Columbia (49°18′N, 119°31′W) and July–August 1992 in the Cypress Hills, 60 km south of Maple Creek, SK (49°34′N, 109°53′W). We measured the activity of 11 different adult birds (9 males and 2 females, mean mass 49 g) using a Merlin 24 telemetry receiver (Custom Electronics, Urbana, IL) and 5-element Yagi antennae. Radio transmitters (model PD-2T, Holohil Systems Ltd., Woodlawn, Ontario) were affixed in a backpack with an elastic harness (Brigham 1992). The transmitter package weighed 2.4 g, which represents <5% of the bird's mass. Birds carrying transmitters acquired mates, nested normally, and appeared to forage normally by sallying from the ground. The behavior of each bird was classified as active or stationary every 5 min for 2 h after foraging began (approximately sunset) and for 2 h before foraging ended (approximately sunrise). During each 5-min interval, we monitored 20 pulses and assumed that any change in signal direction or strength reflected movement. Direct observations of birds feeding during twilight confirmed that the "movements" inferred by telemetry were actually foraging sallies (Brigham and Barclay 1992).

Telemetric measurements of poorwill activity were made on the two nights prior to the DLW protocol being undertaken and on the two nights following for six different birds on one occasion each. We divided the night into three time periods with different solar and lunar influence on light levels and poorwill activity (Brigham and Barclay 1992). We defined dusk as the period of nautical twilight after the sun sets until it is 12° (generally about 1 h; Anawalt and Boksenberg 1987) below the western horizon; dawn is defined as the period of nautical twilight before sunrise beginning when the sun is 12° below the eastern horizon (approximately 1 h before sunrise); and "true night" is the period between dawn and dusk

when the sun is >12° below either horizon. Thus, on any given night, we assessed poorwill activity for all of the dusk and dawn periods and approximately 2 h of the true night period.

The DLW protocol or sham treatments (see below) were always applied between 06:00 and 10:00 h CST after night 2. Birds were flushed into a mist net and then injected with 150–170 ml of $^3H_2O^{18}$ intra-peritoneally using a 10-mm needle. The bird was weighed and then held in a cloth bag for one hour to allow the isotopes to equilibrate with body fluids. Microcapillary tubes were used to collect a blood sample (50–150 ml) after brachial vein puncture. In almost every instance, a small hematoma formed after the bleeding stopped. Two sham experiments were also conducted. First, three different birds were flushed off a nest on the ground three times in 30 min but not captured or injected. This procedure was the same used when we were attempting to capture birds for injection or blood sampling. The second sham experiment involved two different birds who were caught, weighed, held for one hour and had a blood sample taken, but no injection was administered. The sham experiments were conducted as a control for the effects of handling stress and the injection itself on behavior. For both sham experiments, foraging activity was monitored in precisely the same way as for the experimental birds.

For each of the four nights that activity was monitored, we generated activity scores (percentage of measurements that were classified as moves) and assigned them to one of the three time categories (dusk, dawn, or true night). Scores were arcsin transformed before analysis. Although we found significant heterogeneity of variance using Bartlett's test (Zar 1984), we used a parametric two-tailed ANOVA to minimize the chance of a type II error (accepting the null hypothesis when it is actually false).

Of the eight birds subjected to the full DLW protocol (capture, injection, equilibration, and blood sample), two left the study area shortly after release. There was nothing clearly different in our application of, or the birds' direct response to, the protocol for these two individuals. For the six birds that remained, we found no significant difference in foraging activity before and after the protocol for any of the three time periods (dusk: $F = 2.00$, df $= 23$, $P > 0.10$; night: $F = 1.00$, df $= 23$, $P > 0.40$; and dawn: $F = 0.43$, df $= 23$, $P > 0.70$; Fig. 1). Likewise, for the five birds involved in sham experiments, there were no significant differences in activity scores (dusk: $F = 0.93$, df $= 19$, $P > 0.30$; night: $F = 0.37$, df $= 19$, $P > 0.50$; and dawn: $F = 1.16$, df $= 11$, $P > 0.20$). When the data for the six experimental and five sham birds were pooled, there were no differences in activity scores for the three time periods (dusk: $F = 1.08$, df $= 43$, $P > 0.30$; night: $F = 0.98$, df $= 43$, $P > 0.40$; and dawn: $F = 0.38$, df $= 33$, $P > 0.60$).

One of the two individuals that left the study area after the DLW protocol abandoned its nest but returned to the study area subsequent to the monitoring period. The second bird, which was not nesting at the time, also left the study area during our two-day monitoring period. The mate of this bird, caught and treated on the same day, remained in the same area for the rest of the summer. On at least six other occasions during 1991, tagged poorwills left the study area for short periods (1–3 days). These departures were not associated directly with disturbance due to capture attempts.

For the DLW technique to be a valid means of measuring FMR, it is essential that the procedure have minimal effects on the activity patterns of the study animal. If activity changes significantly as a result of the DLW protocol, an accurate measurement of energy expenditure may not reflect the actual amount of energy expended by an animal that is behaving normally. Our results support the conclusions of two other studies which report negligible effects of the DLW protocol, at least for the birds that remained in the study area. Bryant and Westerterp (1983) found that the energy expenditure by a single injected House Martin (*Delichon urbica*) differed from that of a control bird by only 2%. In a larger study, Speakman et al. (1991) found no discernable effect on the behavior of laboratory

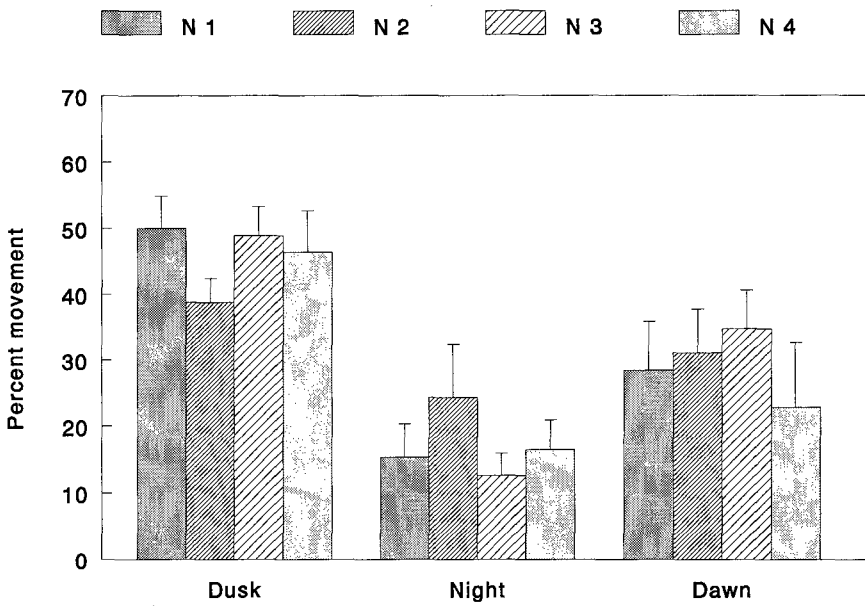

FIG. 1. Histogram (mean ± SE) of untransformed activity scores for dusk, night and dawn for six birds treated with the DLW protocol. Nights 1 (N1) and 2 (N2) occurred before the protocol was applied, nights 3 (N3) and 4 (N4) after the protocol was applied.

white mice (*Mus musculus*), although they conceded that the effect could have been masked by the stable laboratory conditions and the fact that they used domesticated animals. Our work extends that of Speakman et al. (1991) by suggesting that the DLW technique has a negligible effect on free-ranging poorwills that remain in the study area, although different species may be more or less sensitive to the disturbance caused by handling.

Because two birds left our study area after being injected, it is important for the DLW protocol to monitor the free-ranging organisms under study to confirm that "normal" behavior is not dramatically altered. Our manipulations appear to have had an all-or-none effect in that birds either behaved normally or left the study area. It was not uncommon for non-experimental birds to leave the study area for brief periods. These brief departures could not be ascribed to a clear cause and thus may represent a normal behavior (e.g., foraging, mate acquisition). Obviously, more studies to assess the impact of the DLW protocol on other free-ranging animals are required to establish the generality of our results.

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