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From the Field

Effects of Measuring Nestling Condition on Nest Success in the Dickcissel (*Spiza americana*)

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ABSTRACT Handling songbird nestlings is often necessary to investigate a variety of basic and applied research questions, but this may also introduce observer effects that could compromise indices of reproductive success. To test for potential observer effects while measuring nestling condition in a study population of dickcissels (*Spiza americana*), we handled nestlings from 18 of 30 nests in Prairie, Mississippi, USA. We used generalized linear mixed models to compare nest success rate with nests that were monitored but where nestlings were not handled. Confidence intervals for nest success rates overlapped broadly for nests with handled (0.84, 95% CI = 0.57–0.95) and unhandled nestlings (0.77, 95% CI = 0.44–0.93), and a likelihood ratio test indicated the effect of treatment did not approximate the data better than a null model. We also did not find evidence of adults rejecting or abandoning handled and banded nestlings. This suggests that it is unlikely we incurred negative impacts on nest success when measuring nestling condition in our study. © 2014 The Wildlife Society.

KEY WORDS disturbance, investigator activities, observer effects, passerines, predation.

A variety of basic bird biology questions often require researchers to handle nestlings, including (but not limited to) energetics (e.g., Sullivan and Weathers 1992), immunology (e.g., Saino et al. 1997), ecotoxicology (e.g., Brasso and Cristol 2008), and paternity and social mating systems (e.g., Perlut et al. 2008). Handling and banding nestlings may also be useful for applied questions, such as investigating fledgling habitat use (e.g., Jones and Bock 2005), or quantifying nestling condition as an index of habitat quality (Johnson 2007) because condition may vary with food availability (Naef-Daenzer and Keller 1999, Brickle et al. 2000, Chalfoun and Martin 2007). However, handling nestlings may also bias nesting outcome through observer effects (Götmark 1992), which may compromise other indices typically collected in the field, such as estimates of daily nest survival rates (Rotella et al. 2000).

The act of measuring and banding chicks typically involves visiting the nest and temporarily removing, handling, and replacing nestlings. Potential negative observer effects on nesting success include increased predation rates when observers offer visual cues to predators (Westmoreland and Best 1985, Gutzwiller et al. 2002) or leave trails and scents (Whelan et al. 1994). The presence of observers near the nest site may also affect behavior of adults, for example reducing food provisioning rates for nestlings (McDonald et al. 2007, Mitchell et al. 2012). Conversely, observers may inadvertently solicit distress calls from attentive adults (Götmark 1992), and increased adult activity could increase nest predation rates (Martin et al. 2000). Among certain species, observer actions may cause nestlings to leave the nest prematurely (force-fledge, Pietz et al. 2012, but see Streby et al. 2013), adults to abandon their nest (Pietz and Granfors 2000, Renfrew and Ribic 2003), or adults to react negatively toward nestlings with bands (Berger 1953, Blackbill 1954, Calvo and Furness 1992) or throat ligatures (Little et al. 2009, Robinson et al. 2010).

Despite these potential sources of negative observer effects, reviews and meta-analyses generally report a neutral or positive effect from research activities, particularly for passerines (Götmark 1992, Richardson et al. 2009, Ibáñez-Álamo et al. 2012). However, these reviews did not include studies that handled or marked nestlings. Because recommendations are available for minimizing observer effects (Martin and Geupel 1993, Winter et al. 2003, Fair et al. 2010), researchers often assume (implicitly or otherwise) that their actions have minimal influence on their study organisms. Still, effects of handling and banding nestlings on nest fate are relatively understudied, so it is unclear whether researchers meet their assumptions of minimal observer effects.

We hypothesized that handling and banding nestlings introduces observer effects, either through predation or abandonment of nestlings by adults, and we predicted this

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would reduce nest success relative to nests with nestlings that were monitored but not handled and banded. We tested this hypothesis while measuring nestling condition in a study population of dickcissels (*Spiza americana*) breeding in Mississippi, USA. This species is a neotropical migrant that constructs open cup nests in forbs, shrubs, and bunchgrasses and lays an average clutch of 4 eggs (range = 3–6; Temple 2002). The dickcissel is an ideal organism for investigating observer effects in grasslands because it is a commonly studied grassland obligate species and telemetry studies documented a positive relationship between nestling mass and fledgling survival (Suedkamp Wells et al. 2007, Conover 2009, but see Berkeley et al. 2007), but effects of handling nestlings on nest success have yet to be formally tested for this species. In addition, evaluating nest success for nests with handled nestlings will permit researchers to consider our methods and results when designing and conducting future studies.

STUDY AREA

We conducted our study at Mississippi State University's Prairie Research Unit in Monroe County, Mississippi. The Research Unit is situated in the Black Belt of Mississippi and Alabama (USA), which historically was the largest of the blackland prairies although much of its tallgrass prairie was lost to cultivation (Peacock and Schauwecker 2003). We searched for nests in 12 pastures (6.7–11.7 ha), including a non-native grass mix of bermudagrass (*Cynodon dactylon*) and tall fescue (*Schedonorus arundinaceus*), monocultures of Indian grass (*Sorghastrum nutans*), and a native grass mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and Indian grass. Three pastures of native grass mixes remained ungrazed during the study period; the rest were grazed from May through mid-September with autumn-born steer calves stocked at one calf/0.4 ha. We lack data on specific dickcissel nest predators at our study sites, though a previous study from Mississippi in similar habitat reported nest predation primarily from imported fire ants (*Solenopsis* spp.) and snakes, and one case of mammalian predation from a nine-banded armadillo (*Dasypus novemcinctus;* Conover et al. 2011*b*). This nest predator community is consistent with birds nesting in the southern United States (Thompson and Ribic 2012).

METHODS

Every 2 weeks from 16 May to 14 July 2011, teams of 3 observers systematically dragged a 30-m rope weighted at 2-m intervals with noise-makers (i.e., bottles with several rocks), which induced incubating females to flush. We marked grass 5 m north of nests with spray paint (Winter et al. 2003) and recorded the location with a global positioning system. We also candled eggs to estimate age and hatch date (Lokemoen and Koford 1996), or we used reference photographs to age nestlings.

We returned to nests every 2–4 days to check nest status throughout the nesting period. We considered nests successful if at least one nestling fledged, and other signs of fledging were evident, such as feces and feather scales in and around the nest cup, fledglings detected within the vicinity of the nest, or adults nearby scolding the observer and/or carrying food items (Grant et al. 2005). We considered a nest failed if we found it damaged or displaced or if nestlings were absent before their potential fledging date, typically nestling age Day 8–10 for dickcissels (Temple 2002). Initially these indicators were not reliable when visiting nestlings near fledging age every 2–4 days because 2 nests were found empty after their earliest potential fledge date yet typical indices of failure or fledging were not apparent. We therefore increased visits to every 1–2 days when nearing the anticipated fledge date. Observers minimized disturbance to nests during monitoring by avoiding trampling vegetation surrounding nests, keeping visits during nest checks brief ($\bar{x}$ visit time = 0.3 min, max. = 5 min), and recording information away from the nest site (Winter et al. 2003). Observers also wore rubber boots when vegetation was wet from rain or dew to minimize leaving human scent (Churchwell et al. 2008).

Counting hatch date as age Day 1, we handled nestlings at age Day 5–6 to avoid force-fledging (Berkeley et al. 2007) or causing nestlings to leave the nest prematurely. Because of the sequential, random, and often uneven entry of nests into our sample, we systematically selected nestlings from every other nest in each pasture to be handled for measuring nestling condition (hereafter, treatment) or not handled (control). Nests that failed before they reached handling age were not considered in this selection.

Arriving at nest sites between 0600 hours and 0930 hours Central Standard Time, we collected nestlings in a cloth bag and moved them approximately 20 m from the nest to minimize observer presence at the nest site. We left an orange surveyor flag at the nest site to relocate nests quickly when we returned nestlings. We fitted each nestling with an aluminum U.S. Geological Survey band, weighed nestlings with a spring scale, and measured right tarsus length with digital calipers. We then returned nestlings to their nest, sometimes by briefly covering nestlings with one hand over the nest to allow them to settle before retreating from the nest. Mean time between first arriving at the nest site and replacing all nestlings in the nest was 12.3 minutes (range = 5–19 min). The Mississippi State University Institutional Animal Care and Use Committee approved our methods (permit no. 11-020). Control nests were visited as part of regular nest checks, and therefore our study measures the cumulative effect of temporarily removing, handling, and banding nestling dickcissels.

We used generalized linear mixed models with a logit link (logistic regression) to compare the proportion of successful nests between treatment and control nest groups. We used this approach rather than modeling daily nest survival (e.g., with logistic exposure; Shaffer 2004) because 1) we assigned treatment or control only to nests with nestlings surviving to age Day 5, and thus restricted analysis to a small proportion of all nests monitored; and 2) handling occurred within 2–5 days of their potential fledge date so estimates of daily survival rate would be severely constrained compared with estimates from a 24-day nesting cycle. Logistic regression is also preferable for analyzing nest fate data because of its greater flexibility and power compared with other tests such as chi-square (Lewis 2004). We fit generalized linear mixed models with the package *lme4* (Bates et al. 2013) in Program R (R Development Core Team 2013). We specified pasture type (non-native grass mix, Indian grass, grazed or non-grazed native grass mix) as a random effect to account for the potential non-independence of nests among pasture types and because we were not interested in directly estimating effect of cover type for this analysis. We back-transformed parameter estimates to compute proportion of nest success between treatment and control nests and we simulated their 95% confidence intervals (CI) using the R package *arm* to account for uncertainty from random effects (Gelman et al. 2013). We also used a likelihood ratio test to assess whether the covariate of handling improved model fit compared with the null (i.e., intercept-only) model.

Aspects of our study protocol, aside from handling and banding nestlings, could create additional disturbance and confound testing of observer effects if applied unevenly between our treatment and control nest groups. For example, we did not perform egg candling when nests were found during the lay or nestling stage because we could use other indices to age nests. In addition, nests first found during the lay stage were under observation for longer than nests found with nestlings, whereas nests first found at the nestling stage may have been visited with an overall higher frequency because we increased visits to determine nest fate. Similarly, nest site characteristics could influence nest survival rates (Martin et al. 2000), which may potentially confound testing of observer effects in our study. For dickcissels, these include nest height (Conover et al. 2011*b*), grass coverage, and vegetation density (Winter 1999, Conover et al. 2011*a*). To assess nests for these potential confounding variables, we measured nest-site vegetation after nesting attempts were completed, including nest height measured from the ground to the base of each cup, grass coverage averaged from five 0.25-m^2 frames (Daubenmire 1959) positioned around each nest (Winter 1999), and visual obstruction measured with a Robel pole (Robel et al. 1970) at nest sites. We tested whether the proportion of candled nests differed between treatment and control nest groups using logistic regression and estimated 95% CIs. We used Welch's *t*-test for unequal variances (Zar 2010) to compare nest height, grass coverage, visual obstruction reading, days under observation, and visit frequency (days:visit ratio) between treatment and control nests, and we considered statistical significance at $\alpha = 0.10$.

RESULTS

We monitored 85 dickcissel nests, of which 61 (71.8%) failed. Vertebrate predation appeared to be the most common source of failure (73.8%, $n = 45$), followed by fire ants (9.8%, $n = 6$), abandonment (8.2%, $n = 5$), unknown (4.9%, $n = 3$), and cattle trampling (3.3%, $n = 2$). Nestlings from 33 nests (38.8%) reached handling age, and of these we selected 19 nests (57.6%) for treatment. However, nest fate was uncertain for one treatment nest and 2 control nests, so we excluded these from our comparison. This resulted in a sample of 18 treatment nests and 12 control nests. Although we attempted to handle nestlings at age Day 5 or 6, nestlings from one nest were handled on age Day 7. When this nest was checked 2 days later, we found evidence of recent fledging, including female calling, feather scales in the nest cup, and feces on the upper leaves, so we did not infer force-fledging. One nest in the treatment group was parasitized by brown-headed cowbirds (*Molothrus ater*) before its discovery, but we inferred that both host and cowbird nestlings fledged. The parasitization rate for the greater study population was 5.9% ($n = 5$), and no nests in the control group were parasitized by cowbirds.

The act of removing, measuring, banding, and replacing nestlings increased mean nest visit time from 0.7 min/visit (max. = 7 min) in our control group to 2.6 min/visit (max. = 19 min) for treatment nests. Proportion of nests fledging at least one offspring did not differ statistically between treatment (0.84, 95% CI = 0.57–0.95) and control nests (0.77, 95% CI = 0.44–0.93). Confidence intervals overlapped broadly, and our sample size likely did not offer the statistical power to detect slight differences between the 2 groups. Likelihood ratio test indicated that including effects of handling in our model did not approximate the data better than the null ($\chi^2 = 0.24$, df = 1, $P = 0.63$). At nest sites, mean nest height, visual obstruction reading, and grass coverage did not differ significantly between the 2 nest groups (Table 1). Nests in our sample also did not differ statistically by days under observation or visit frequency, and confidence intervals of proportion of nests with egg candling

Table 1. Mean, standard error, and test statistics for variables that could potentially confound comparisons of observer effects between nests with handled (treatment, $n = 18$) and unhandled (control, $n = 12$) dickcissel nestlings at the Mississippi State University Prairie Research Unit in Prairie, Mississippi, USA, 2011.

	Nest group						
	Treatment		**Control**				
Nest variable	$\bar{x}$	**SE**	$\bar{x}$	**SE**	t^a	**df**	***P*-value**
Visual obstruction reading (cm)	32.5	4.6	32.7	4.5	−0.04	27.13	0.97
Grass coverage (%)	59.1	4.4	64.4	5.6	−0.75	23.03	0.46
Nest ht (cm)	11.8	2.6	15.9	3.0	−1.02	24.88	0.32
Days under observation	10.8	1.1	13.7	1.3	−1.66	24.23	0.11
Visit frequency (days:visit ratio)	2.4	0.1	2.7	0.1	−1.57	27.97	0.13

[a] Test statistic for Welch's *t*-test.

overlapped between treatment (0.67, 95% CI = 0.44–0.85) and control (0.83, 95% CI = 0.57–0.97). Of nests in the treatment group that did not fail or fledge prior to the first visit after handling ($n = 13$), 12 nests (92.3%) had the same number of nestlings during the first visit after they were handled. During the handling visit for one nest, we observed an adult eject a severely emaciated dickcissel nestling (that we did not band) before we returned the other nestlings to the nest.

DISCUSSION

We found no evidence of negative impacts on nest success when observers measured nestling condition among dickcissels in our study. We also did not observe instances of abandonment or rejection by adults after nestlings were temporarily removed from the nest and banded. Measures of nest vegetation and additional potential observer effects that could influence nest fate were largely similar between the 2 nest groups, so we do not believe this confounded our analysis. The ability to handle and band nestlings without compromising estimates of nest success could encourage the pursuit of a variety of ecological study questions *in situ*. However, we purposely used procedures that are purported to minimize disturbance to the nest (Winter et al. 2003), and our results do not preclude the potential of disturbance for other species or banding procedures because observer effects may be species- and study-specific (Götmark 1992, Weidinger 2008). Our study is important because it is, to our knowledge, the first to assess effects of banding and handling grassland bird nestlings and provides a protocol for future studies. It is also among a growing number of studies reporting negligible observer effects on nesting outcome (e.g., Weidinger 2008, Jacobson et al. 2011, Ibáñez-Álamo et al. 2012).

Predation is reported to be the primary cause of nest failure for grassland birds (Pietz and Granfors 2000), and effects of investigator activities are studied mainly in the context of predation (Götmark 1992, Weidinger 2008, Ibáñez-Álamo et al. 2012). Nest predation rates are reportedly higher for grass- and shrubland birds than for species in other cover types (Martin 1993), and multiple predator species are implicated (Renfrew and Ribic 2003, Thompson and Burhans 2003). Predator diversity and predator specificity for different attributes of nest-site vegetation make it difficult to anticipate causes of nest failure (Dion et al. 2000) and, consequently, to mitigate observer effects. Olfactory cues left by observers may reveal nest locations to mammalian predators (Whelan et al. 1994), though the importance of scent trails is questioned (Götmark 1992, Skagen et al. 1999). Mammals might also be less important nest predators than snakes for grass- and shrubland birds in the southern United States (Thompson and Ribic 2012), especially for dickcissels (Zimmerman 1984, Klug et al. 2010). Snakes may be attracted to nest sites by activity of adults and nestlings (Stake et al. 2005), and although the handling of nestlings agitated adults during our study, we strived to minimize the duration of our banding visits and did not infer predation of nests from snakes after banding (i.e., nest found empty and intact prior to the earliest potential fledge date; Thompson and Burhans 2003). Finally, the overall nest predation rate observed in our study (73.8%, or 83.6% when including fire ants) was at the middle- to lower end of the range reported in other dickcissel studies (range = 76–94% [Churchwell et al. 2008; Conover et al. 2011*a*, *b*]), and this may have reduced the effects of observers on nest predation relative to other studies.

Rejection or abandonment of nestlings by adults after observer actions is also a concern. Of the handled nestlings that had not fledged or been depredated, only one nest had one fewer nestling on the visit after treatment, though it is unclear whether observer actions caused or precipitated ejection of the severely emaciated (and unbanded) nestling. Although rejection of banded nestlings is documented in several studies (Calvo and Furness 1992), this behavior may vary among species and was not reported in recent grassland bird studies when nestlings were banded or radiomarked (Berkeley et al. 2007, Suedkamp Wells et al. 2007, Perlut et al. 2008, Conover 2009). In addition, adult harassment and ejection of nestlings with ligatures may be related to nest sanitation behaviors (Robinson et al. 2010). When we replaced banded nestlings in the nest cup, their bands were typically concealed underneath them, and this may avoid eliciting a nest sanitation response by adults, if such a response exists for dickcissel nestlings with leg bands.

Our results suggest that handling and banding dickcissel nestlings in our study did not reduce nest success rates compared with control nestlings. Nevertheless, our study is limited because of its sample size and lack of temporal or spatial replication for a commonly studied species with a relatively wide distribution in the southern United States (Temple 2002). Our results also do not rule out the possibility of cumulative effects from repeated visits, which may be necessary to measure nestling growth. We still recommend that researchers take precautions to minimize disturbance at and around the nest site and consider how the local predator community may respond to observer actions. We also encourage researchers to test their assumptions of neutral observer effects when handling nestlings in other systems, so we may garner a more general understanding of this concept.

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