

ly in a freezer until they could be later analyzed for corticosterone levels.

We determined plasma corticosterone levels using Enzyme Immunoassay (EIA) kits (Cat No. 901-097, Assay Designs). For our EIAs, we used the optimization protocol suggested by Wada et al (2007) with slight modifications to determine the plasma dilution and amount of steroid displacement buffer (SDB) that worked best for Prothonotary Warbler plasma corticosterone. We found that a plasma dilution of 1:50 with 1% SDB (per raw plasma volume) was needed to optimize our assays. To determine corticosterone levels in each individual sample, 4  $\mu$ L 1:100 dilution of SDB buffer were added to 4  $\mu$ l plasma. After 5 min, 192  $\mu$ l assay buffer were added to each sample, then samples were vortexed and added to individual wells in the assay plate (100  $\mu$ l/well) with each sample run in duplicate. The standard curve was also measured in duplicate, with eight standards ranging from 40,000 to 6.4 pg/ml (100  $\mu$ l/well). A separate, external standard of 200 pg/ml was run in duplicate on every plate. We then followed the assay procedure as outlined by the kit's directions. After adding stop solution, the plates were immediately read on a Molecular Devices FilterMax F3 multi-mode microplate reader at 405 nm. The microplate reader was optimized prior to reading each plate. Samples were completely randomized within and across four plates, with a roughly equal representation of males and females across both sites on each plate.

## RESULTS

### Male Behavioral Observations

In 2010, we observed 41 males during 55 different observation bouts for a total of 3:43:50 hrs of observation. Twenty-three observations were conducted during the pre-incubation stage; 15 observations were conducted during incubation; 17 observations were conducted during the nestling stage. Observation bouts ranged from 00:30 to 11:53 min; the mean observation time per male per nesting stage was 04:09 min. Males were highly stereotyped in the type of prey attack maneuvers that they used while foraging. We observed 203 attempts at attacking prey; the majority of attempts were made using gleans (75.4%) and probes (17.2%)(Fig. 1). Sally hovers and sally strikes combined (7.4%) were rarely observed; therefore, we combined all attack maneuvers into one "prey attack rate" category for analysis. Hopping and walking rates were highly positively correlated with each other and were combined into a "movement rate" category. Preening was rarely observed and was dropped from further analysis.

Male song rates did not vary in relation to neighbor density or across stages of the nesting cycle (Table 2). Movement rates appeared somewhat responsive to conspecific density; we observed a significant neighbor density x stage interaction on male movement rates (Fig. 2). Males moved more during the nestling stage compared to the pre-incubation and incubation stages when they had more neighbors. Neither flight rates nor prey attack

rates changed in response to neighbor density, however (Table 2).

### Warbler Reproductive Output

We did not find strong evidence for neighbor density effects on clutch size, hatching success, or fledging success (Table 3). Hatching success did vary slightly in relation to a neighbor density x year interaction (Table 3; Fig. 3). In 2008 and 2009, hatching success was lower at low neighbor densities and increased slightly with neighbor density. This pattern switched in 2010 and 2011 when hatching success was higher at low neighbor densities. Not surprisingly, hatching success was significantly affected by Brown-headed Cowbird parasitism status (Table 3). Nests that received a cowbird egg had lower hatching success ( $EQ \ O(x, \tau) = 85.0\%$ ,  $SE = 0.03$ ) compared to non-parasitized nests ( $EQ \ O(x, \tau) = 93.0\%$ ,  $SE = 0.01$ ). Neighbor density had no effect on the likelihood of a female attempting a second brood (Table 3). The likelihood of attempting a second brood was significantly affected by ordinal date, however, and decreased as the breeding season progressed. For females that successfully raised a first brood and attempted a second brood on our plots, neighbor density had no effect on the total number of young that they produced each year (Table 3; Fig. 4). Production of young decreased with ordinal date (Table 3) but, on average, females that at least attempted a second brood produced 5 warbler fledglings per year ( $SE = 0.19$ ).

### Adult Baseline Stress Hormones

We successfully bled 72 adult warblers across all four breeding seasons. Additional birds were bled, but their samples had to be eliminated from analysis because we either did not collect enough blood from them or we did not collect it in under 3 mins. The 3 min time limit was very important. Upon capture and handling, the acute stress response kicks in and increased corticosterone levels can be detected in songbird blood samples after 3 mins (Wingfield et al. 1982). In order to measure baseline levels, we needed to finish bleeding birds within 3 mins of them hitting the mist-net.

For our warblers, the mean baseline corticosterone levels was 12.84

**Table 4.** Results of generalized linear mixed models comparing baseline plasma corticosterone concentrations in adult Prothonotary Warblers in southern Illinois, 2008-2011.

	<i>F</i>	<i>P</i>	<i>df</i>
Neighbor density	0.38	0.54	1, 60
Year	1.68	0.18	3, 60
Sex	0.52	0.47	1, 60
Brown-headed Cowbirds <sup>a</sup>	1.55	0.22	1, 60
Age	4.61	0.04	1, 60
Neighbor density x year	1.03	0.37	3, 60

<sup>a</sup>As part of our larger study, 50% of parasitized nests in each of our subplots in 2011 had one cowbird egg left in it so that warbler adults had to raise a cowbird nestling. Although those results are not highlighted in this paper, the variable was included in this analysis and is presented here.