

12-2012

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Recommended Citation

Weinandt, M. L., Meyer, M. Strand, M., and Lindsay, A.R. (2012). Cues used by the black fly, *Simulium annulus*, for attraction to the common loon (*Gavia immer*). *Journal of Vector Ecology*, 37(2), 359-364.

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Cues used by the black fly, *Simulium annulus*, for attraction to the common loon (*Gavia immer*)

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Received 11 March 2012; Accepted 1 June 2012

ABSTRACT: The parasitic relationship between a black fly, *Simulium annulus*, and the common loon (*Gavia immer*) has been considered one of the most exclusive relationships between any host species and a black fly species. To test the host specificity of this blood-feeding insect, we made a series of bird decoy presentations to black flies on loon-inhabited lakes in northern Wisconsin, U.S.A. To examine the importance of chemical and visual cues for black fly detection of and attraction to hosts, we made decoy presentations with and without chemical cues. Flies attracted to the decoys were collected, identified to species, and quantified. Results showed that *S. annulus* had a strong preference for common loon visual and chemical cues, although visual cues from Canada geese (*Branta canadensis*) and mallards (*Anas platyrhynchos*) did attract some flies in significantly smaller numbers. *Journal of Vector Ecology* 37 (2): 359-364. 2012.

Keyword Index: Black fly, conservation, common loon, decoy experiments, host specificity, nest parasites, chemical cues.

INTRODUCTION

Empirical data (Hudson 1998) and population models (Anderson and May 1978) indicate that parasites can radically affect host population dynamics, yet the dynamics of the interactions between loons and their parasites remain largely understudied. Storer (2002) reviewed descriptions of metazoan loon parasites and noted a lack of attention paid to black flies (Diptera: Simuliidae) and their influence on loon reproduction and ecology. Incubating loons are particularly vulnerable to black fly attacks, which when severe can lead to nest abandonment (McIntyre and Barr 1997). Black flies serve as vectors for a number of avian disease organisms and can transmit pathogenic protists, filarial nematodes, arboviruses, and possibly bacteria (Adler et al. 2004). Weinandt³ found *Leucocytozoon* and *Plasmodium* in the blood of common loons from northern Wisconsin. *Leucocytozoon* protists are vectored by black flies, suggesting that *S. annulus* effects on loon health and fitness may include the transmission of blood-borne pathogens.

One black fly species, *Simulium annulus* (Lundström) (junior synonym *S. euryadminiculum* Davies), has been noted for its high host preference for common loons (Adler et al. 2004). The purported preference of *Simulium annulus* to the common loon is unusual since most black fly species are generalists that feed on several host species of similar sizes within particular habitats (Adler et al. 2004). It is also unusual that only one of the over 250 Nearctic black fly species has been reported to feed on common loons. The first report of the specificity of *S. annulus* to the common loon (Lowther

and Wood 1964) indicated that *S. annulus* were attracted to chemicals specific to loons. Fallis and Smith (1964) reported similar importance of chemical cues, but they also collected nearly equal numbers of *S. annulus* from common loon and American Black Duck (*Anas rubripes*) carcasses. Location of hosts by most black fly species involves a series of steps that include habitat features, host size and shape, odor, and temperature (Adler et al. 2004). Although the specificity of *S. annulus* to the common loon has been repeatedly asserted, *S. annulus* females have been collected from penned moose (*Alces alces*), ruffed grouse (*Bonasa umbellus*), and domestic fowl (Adler et al. 2004). More recent evidence indicates *S. annulus* feeds on cranes (Malmqvist et al. 2004, Urbanek et al. 2010).

It seems evident that black flies negatively affect loons directly through feeding and indirectly through disease transmission, thus the details of this host-parasite relationship should prove useful in loon conservation efforts. In this study, we tested hypotheses of host preference and black fly specificity and performed a series of field experiments where several types of bird decoys were presented on lakeshores of common loon breeding habitats. Our study design evaluated the specificity of *S. annulus* to common loons and delineated the cues used by the black flies to target their hosts.

MATERIALS AND METHODS

This study was conducted in northeastern Wisconsin, U.S.A., in Vilas, Oneida, and Forest Counties, an area dominated by northern hardwood and coniferous forests and marked by a high density of glacial kettle lakes used by common loons for breeding territories. This region is populated by an estimated 1,200 loons during the breeding season, 800 of which were individually color-banded in 1992-

³Weinandt, M.L. 2006. Conservation implications of common loon (*Gavia immer*) parasites: Black flies, haematozoans, and the role of mercury. Thesis. Northern Michigan University.

2005 (Fevold et al. 2003). The 26 lakes used in this study were oligotrophic to mesotrophic, ranged from 0.057 to 1.465 km² in size, and ranged in maximum depths from 2.7 to 13.7 m.

General decoy presentation and black fly collection

To simulate different nesting species that vary in visual and chemical cues, three types of bird decoys were presented on lakeshores. The decoys were used to attract and collect black flies. Black flies were collected with 40 mm x 65 mm glueboards ("Monitor Glueboard", Professional Pest Control, Columbus, GA) affixed to the tops of decoy heads ("head") and on the dorsal surfaces immediately anterior to the tails of the decoys ("back"). Each decoy was attached to the lid of a cream-colored plastic tub and encased within the overturned tub when not being used for an experimental presentation. Each decoy was exposed on lakeshore sites for 10 min and then covered again by the tub. All decoys for each set of tests were presented at each lake in a randomized order. After each presentation, glueboards were removed and placed in containers of 95% ethanol, euthanizing all attached flies. Fly collections from presentations of decoys are reported as numbers of flies (and percent of total flies) for each presentation type and the mean (\pm SE) number of flies per presentation replicate.

The host specificity of loon-associated black flies was first tested by presenting four types of decoys (of three species) on lakes with a history of common loon nesting. The three species of decoys were: common loon, Canada goose (*Branta canadensis*), and hen mallard (*Anas platyrhynchos*) (Figure 1). The Canada goose and mallard decoys (hereafter, "goose" and "duck" decoys, respectively) were chosen as potential alternative host species since they are common waterfowl that breed in this region. The decoys used for these species were a Greenhead Gear Life-Size Series™ active style Canada goose decoy and a Greenhead Gear hen mallard decoy (Greenhead Gear, Memphis, TN). Common loon decoys (hereafter, "loon" decoys) were a modified version of custom-made loon decoys

crafted for a previous study (Kenow et al. 2003).

To test the importance of chemical cues, we compared abundances of black flies captured on unmanipulated decoys to abundances captured on decoys fitted with wings from a deceased loon recovered from the area (Figure 1). The loon wings used for this presentation were amputated from a deceased adult loon carcass recovered on Manson Lake in Oneida County, WI in May, 2005, and then subsequently frozen and stored. Before the decoy presentations were initiated, muscle was removed from the wings to reduce the possible attraction of black flies to decaying tissue and wings were stored on ice between field presentations. This group of four decoy types was presented on ten lakes from 07 May 2005 to 09 May 2005. Comparisons of results from this set of presentations, involving the four decoy types (loon with no wings, loon with wings, goose and duck each without wings), will be referred to as the "one-wing" presentations.

Mallard and Canada goose wings were obtained from the Wisconsin Department of Natural Resources Wildlife Health Lab, Madison, WI. These wings were placed on two additional decoys (of their respective species), creating a six-decoy presentation scheme – two decoys of each of the three species (loon, goose, and duck) where one decoy was presented with wings, the other without wings. Wings were prepared and presented on their respective decoys in a manner similar to the loon decoy. All six decoys were presented on 22 lakes that were visited during the period of 27 May 2005 through 02 June 2005. Comparisons of results from this set of presentations, involving the six decoy types (loon, goose and duck with and without wings), will be referred to as the "three-wing" presentations.

Presentations on four additional lakes visited on 03 June and 04 June 2005 were augmented with a "wing-swap" set of decoys. The "wing-swap" decoys were a loon decoy with Canada goose wings applied to its dorsal surface and a goose decoy with common loon wings applied to its dorsal surface.

Data collection

Total numbers of black flies on each piece of glueboard (from the head and back for each decoy presentation) were quantified in the lab using a dissecting microscope, an external light, and the following general procedure. First, all flies on each piece of glueboard were scanned for gross differences from one another and any non-simuliids were removed (e.g., arachnids, hymenopterans, coleopterans). Next, from the three-wing and wing-swap collections, five simuliids were selected at random from each piece of glueboard and then identified to species using character descriptions (largely genitalia morphology) provided by Adler et al. (2004). For species identification, the abdominal sections of flies were removed from the carcasses, soaked in a 10% KOH solution, and cleared with 95% ethanol. Voucher specimens were stored in glycerine and deposited in the Northern Michigan University Insect Collection. The remaining flies from the three-wing and wing-swap glueboards, and all flies from the one-wing glueboards were visually compared to the subset that had been keyed to species without abdominal excision, soaking, and clearing.

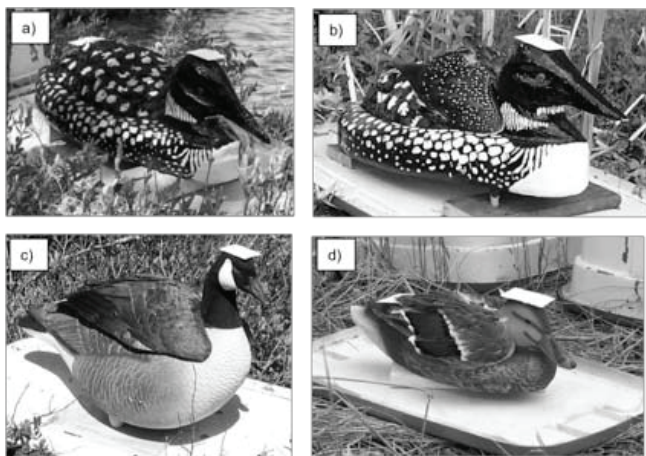


Figure 1. Photographs of decoys used for black fly collection; a) loon decoy without wings, b) loon decoy with wings, c) goose decoy with wings, and d) duck decoy with wings. Glueboard locations can be seen on the decoys' heads and backs.

Environmental conditions (e.g., air temperature, wind speed and direction, barometric pressure, wave action, cloud cover, precipitation) were recorded at each lake during the decoy presentations. The presence or absence of living (non-decoy) loons, geese, and ducks was noted at each lake during decoy presentations, as well as a general assessment of black fly abundance. Geospatial coordinates were taken at each decoy presentation site and a general description of vegetation and substrate was recorded.

Stepwise multiple linear regression analyses were used to determine which factors had the greatest effect on the number of black flies collected. The following factors were used as independent variables and were added into models in a stepwise manner when the probability of F was less than 0.05 and removed when more than 0.10: time of day, temperature, wind speed, barometric pressure, wave action, cloud cover, precipitation, the number of adult loons present, the number of loon chicks present, the presence or absence of geese, the presence or absence of ducks, the vegetation type at the site of presentation (i.e., sphagnum bog, cattails, leatherleaf), the nesting status of the loon pair at the time of presentation (i.e., current nest or no nest), the order of decoy presentation, and the decoy type (species of decoy with or without wings). Two separate MLR analyses (for the one-wing and the three-wing presentations) were run using SPSS ver. 13.0 (2004).

To determine if black fly numbers varied among decoy types, we performed Kruskal-Wallis analyses (the data did not conform to the assumptions of parametric analyses) that compared black fly abundances between presentation types for each set of tests (one-wing and three-wing). For each presentation type, differences were compared among fly numbers collected 1) on the heads, 2) the backs, and 3) for the whole decoy (head and back combined). Post-hoc tests between pairs of treatments were performed using Mann-Whitney U-tests, with alpha values Bonferroni-corrected depending on the number of tests performed.

RESULTS

During the one-wing presentations (four decoy types each at ten different lakes), a total of 3,467 black flies were collected (Figure 2a). The majority of black flies (80.2%) were collected from heads of the loon decoys with wings ($= 277.9 \pm 39.98$). Backs of the loon decoys with wings attracted a further 16.8% of the total flies ($= 58.3 \pm 19.36$), and 0.66% ($= 2.3 \pm 1.98$) were collected from heads of the loon decoys without wings. Black flies were less numerous on the goose and duck decoys, yet decoy heads remained more attractive than decoy backs. Goose heads attracted 2.2% of black flies ($= 7.5 \pm 5.17$), while only one black fly was collected on a goose back. Duck heads attracted only five black flies ($= 0.5 \pm 0.40$), and only one black fly (0.03%) was captured on a duck back.

During the three-wing presentations (six decoy types at 22 lakes), 552 black flies were collected on the glueboards (Figure 2b). Again, loon decoys with wings attracted the greatest number black flies (94.7%), with the most collected on heads ($= 23.8 \pm 10.69$) and a smaller number collected on backs ($= 0.86 \pm 0.72$). Goose decoys with and without wings

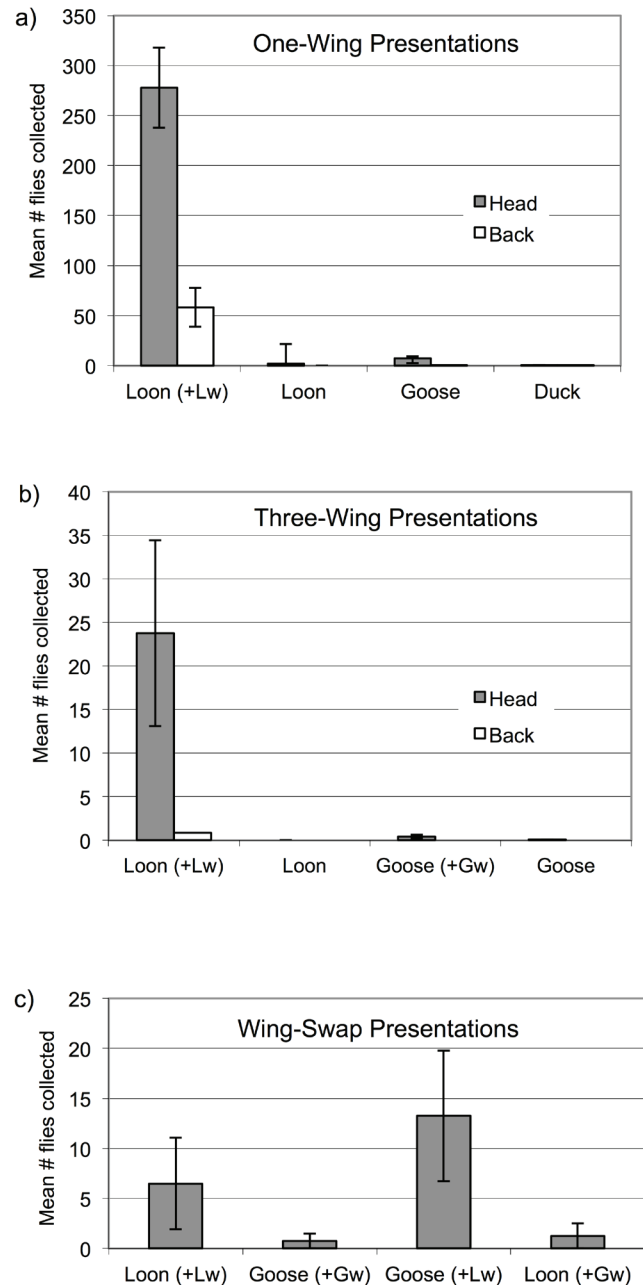


Figure 2. Mean abundances \pm SE of black flies for various presentations (x-axis) of decoy/wing combinations at different lakes. The “+Lw” and “+Gw” categories were decoys presented with amputated loon and goose wings, respectively. In all cases, the mean number of black flies gathered from glueboards on the head are presented with shaded bars and from the back with open bars. Specific results are shown from a) the “one-wing” (four decoy-type) presentations, each at ten lakes, b) the “three-wing” six decoy-type presentations, each at 22 lakes - neither of the duck presentations (with or without wings) collected any flies so data are not shown, and c) results from the wing-swap decoy presentations at four lakes.

were the only other decoy types to attract black flies during this presentation period. Nine black flies ($= 0.41 \pm 0.20$) were captured on heads of the goose with wings decoys, and one black fly was collected from the head of a goose decoy without wings.

Black flies were also captured on the four lakes where the “wing-swap” decoy presentations were made (Figure 2c). No black flies were collected from the backs of any of the decoy types. Heads of the goose decoys with loon wings attracted the majority of the captured flies (53 of 87, 60.9%), while heads of the loon decoys with loon wings attracted 26 flies (29.9%). Three flies were captured on heads of goose decoys with goose wings (3.4%) and five black flies (5.7%) were captured on heads of loon decoys with goose wings.

All examined black flies ($N = 125$) from the three-wing and wing-swap presentation periods were identified as *Simulium annulus*. No other species of black fly was found on any glueboard.

Results from multiple linear regression analyses showed that decoy presentation type (species type with or without wings) was the only significant factor that influenced the number of black flies captured on glueboards (Table 1). Inclusion of environmental variables did not improve the model that best explained variation in the number of black flies collected on the different decoy types.

During the one-wing presentations, significant

differences were found among the number of black flies collected from the heads of the four decoy types ($\chi^2 = 26.104$, $df = 3$, $P < 0.001$). The number of black flies captured on the backs of the decoys and the total number of black flies captured on the decoys also differed significantly ($\alpha = 0.0083$) among decoy types ($\chi^2 = 28.092$, $df = 3$, $P < 0.001$ and $\chi^2 = 26.061$, $df = 3$, $P < 0.001$, respectively). Post-hoc tests on the total numbers of black flies showed the number of black flies captured on the loon-with-wings decoy was greater than the total numbers of black flies captured on the other three decoy types (Table 2). Black flies were equally attracted to the loon decoy without wings and the goose and mallard decoys (Table 2).

Comparisons from the three wing (six decoy-type) presentations also showed the numbers of black flies collected on the heads of the six different decoy types were significantly different among decoy types ($\chi^2 = 52.389$, $df = 5$, $P < 0.001$), as were the number of black flies collected on the backs among decoy types ($\chi^2 = 20.465$, $df = 5$, $P < 0.001$). The total numbers (heads and backs combined) of black flies collected among the six decoy types were also significantly different among the six decoy types ($\chi^2 = 52.389$, $df = 5$, $P < 0.001$; $\alpha = 0.0033$ for 15 comparisons). The post-hoc tests showed that the total number of black flies collected on the loon decoy with wings was significantly greater than the number of flies collected on each of the other decoy types. Black flies did not show a preference among the remaining decoy types (Table 3).

Data from the wing-swap presentation indicate that the loon wings were associated with increased attraction of black flies (Figure 2c), although a small sample size precludes an evaluation of statistical significance.

Table 1. Results of multiple linear regression analyses for the two sets of experimental conditions: one-wing and three-wing presentations.

	EXPERIMENTS	
	One-wing	Three-wing
F	21.226	9.621
df	63	327
p-value	< 0.001	0.002
R ²	0.255	0.029
Regression coefficient: Decoy type	-13.255	-0.633
Constant	122.391	6.313

DISCUSSION

This study demonstrates the importance of visual, and more clearly, chemical cues for host location by black flies and provides evidence that *Simulium annulus* is strongly attracted to loon-specific chemical stimuli and, to a lesser-degree, visual stimuli. In particular, our data demonstrate that *S. annulus* is attracted both to tall, dark objects (common cues for black flies) and those that emit chemical signals associated with loon plumage.

S. annulus was the only black fly species collected and identified in these experiments, and although some individual flies were captured on decoys with other stimuli (e.g., goose and duck decoys, goose wings), the vast majority of black flies

Table 2. Post-hoc comparisons of the total number of black flies captured during the one-wing presentations. Decoys comparisons were replicated by presentations at ten different lakes. Asterisks indicate significance with Bonferroni-corrected $\alpha = 0.0083$.

Decoy Comparison	Mann-Whitney U	P-value
Loon with wing vs Loon	0	< 0.001*
Loon with wing vs Goose	1.0	< 0.001*
Loon with wing vs Mallard	0	< 0.001*
Loon vs Goose	38.0	0.306
Loon vs Mallard	44.5	0.585
Goose vs Mallard	32.0	0.110

Table 3. Post-hoc comparisons of the total number of black flies captured during the three-wing presentations. Decoys comparisons were replicated by presentations at 22 different lakes. Asterisks indicate significance with Bonferroni-corrected $\alpha = 0.0033$.

Decoy Comparison	Mann-Whitney <i>U</i>	<i>P</i> -value
Loon w/wing vs Loon	99.0	< 0.001*
Loon w/wing vs Goose w/wing	126.5	0.002*
Loon w/wing vs Goose	104.0	< 0.001*
Loon w/wing vs Mallard w/wing	99.0	< 0.001*
Loon w/wing vs Mallard	99.0	< 0.001*
Loon vs Goose w/wing	187.0	0.019
Loon vs Goose	231.0	0.317
Loon vs Mallard w/wing	242.0	1.000
Loon vs Mallard	242.0	1.000
Goose w/wing vs Goose	197.0	0.076
Goose w/wing vs Mallard w/wing	187.0	0.019
Goose w/wing vs Mallard	187.0	0.019
Goose vs Mallard w/wing	231.0	0.317
Goose vs Mallard	231.0	0.317
Mallard w/wing vs Mallard	242.0	1.00

were attracted to the decoy or the wings that presented loon-specific cues. Our data corroborate past reports that suggest *S. annulus* is the only black fly that regularly feeds on common loons (Adler et al. 2004). Urbanek et al. (2010) reported that *S. annulus* is also an important pest of whooping cranes, which indicates that the relationship between loons and *S. annulus* is not exclusive, but is nonetheless highly restricted relative to other black fly species. Our data also indicate that chemical cues play a significant role in the attraction of *S. annulus* to common loons. During the one-wing and three-wing presentations, more black flies were captured on the loon decoy with wings than on all other decoy types combined, demonstrating the importance of chemical cues (Figures 2a,b). During all three types of presentations (one-wing, three-wing, and wing-swap), all decoys fitted with common loon wings captured greater numbers of flies than the other decoy types.

Fallis and Smith (1964) pinpointed the tail and uropygial gland as the main source of black fly attraction to common loons. However, they also recognized the importance of visual and tactile cues since the flies swarmed close to, but did not alight on, a paper soaked in uropygial gland extract but at close distances were more inclined to land on raised objects than on flat surfaces and were more apt to crawl among the soft head and neck feathers of a loon while only staying in brief contact with the more rigid feathers of the back (Fallis and Smith 1964). The results presented here support this observation, since the vast majority of black flies were captured on the heads of the loon decoys. The numbers of black flies collected from the backs of loon decoys indicate that region was less attractive than decoy heads. Results from the “wing-swap” presentations support the inference that proximate visual targeting occurs after initial chemical

attraction to a prey individual (Gibson and Torr 1999), since goose decoys with loon wings captured more flies than the heads of the loon decoys with loon wings.

During this study, more black flies were captured during the one-wing presentation period than during the three-wing and wing-swap presentations. This discrepancy is perhaps best explained by the emergence time and lifespan of black flies rather than by the change in decoy presentations. Adult black fly emergence generally occurs in late spring and early summer (Adler et al. 2004) and black fly numbers were declining by the time the wings from the other two species were obtained and used for the three-wing presentations. This time delay likely decreased the chance of capturing many blood-thirsty females during the three-wing and wing-swap presentations.

Our data indicate that *S. annulus* are attracted to objects with visual cues that grossly resemble those presented by incubating common loons (e.g., a dark, erect bird head), and that the attraction is greatly improved when a loon-specific chemical cue is simultaneously presented. The reports of *S. annulus* attraction to common cranes (*Grus grus*) in Europe (Malmqvist et al. 2004, Hellgren et al. 2008) and to whooping cranes (Urbanek et al. 2010) in Wisconsin, indicate this species of black fly is not entirely loon-specific, although the importance of chemical cues is amplified by the noted attraction of *S. annulus* to the eggs of whooping cranes (Urbanek et al. 2010), which obviously present no visual cues similar to the head or neck of a waterbird. Future studies might compare the response of *S. annulus* to crane and loon chemical and visual cues.

The direct effects of *S. annulus* on common loon reproductive success should be quantified to better understand the population level effects of this host-parasite

relationship. As vectors of *Leucocytozoon*, black flies also have the potential to transmit host-specific blood parasites to loons. Many species of *Leucocytozoon* are also host-specific, affecting only closely-related hosts within an avian family (Fallis and Smith 1964). The high level of specificity of *S. annulus* to the common loon is unusual (Adler et al. 2004), and the potential effects of this relationship on loon reproduction are of special interest since the probability of transmission of *Leucocytozoon* potentially increases as a result of the specific nature of the relationship between vector and host.

Acknowledgments

We applied the FLAE approach for the sequence of authors (Tscharntke et al. 2007). The Wisconsin DNR and USGS (Kevin Kenow) provided field support for this project and numerous landowners allowed access to private lands. Financial support for this research was provided by the Sigurd T. Olson Loon Research Award, the Wisconsin Society of Ornithology, and Northern Michigan University.

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