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BLACK FLY SURVEY OF A WHOOPING CRANE REINTRODUCTION AREA IN EASTERN WISCONSIN

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***Abstract*:** Nest desertion due to harassment by black flies (*Simulium annulus* and *S. johannseni*) during incubation has been a major factor inhibiting success of the reintroduced Eastern Migratory Population of whooping cranes (*Grus americana*). To avoid this problem, which was prevalent in the core reintroduction area in central Wisconsin, the Whooping Crane Eastern Partnership changed the primary reintroduction area to eastern Wisconsin in 2011. However, a 2010 assessment of black flies in that area had concentrated predominantly on the southern portion of the new area. In 2017-2018, we collected the first samples of black flies in Green Lake County, including a new primary reintroduction site on White River Marsh (WRM), by sweep-netting over taxidermic crane mounts on artificial nests. In 2017, peak mean numbers of *S. johannseni* per sample at WRM and Grand River Marsh were 3,077 (maximum 6,838) and 891specimens, respectively. Numbers of black flies of this magnitude (and lower) collected during sampling by the same technique have been associated with nest desertion at Necedah National Wildlife Refuge in the core area. Numbers of *S. johannseni* were much lower in Green Lake County in 2018, and *S. annulus* was not abundant in either year. In contrast, an additional survey of black flies at WRM in 2021 recorded numbers of *S. annulus* potentially large enough to affect whooping crane nesting. Multi-year studies of black flies at WRM and other new reintroduction sites, coordinated with monitoring of whooping crane nesting, are needed to ascertain the impact of black flies and implement plans to promote success of this whooping crane population.

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Key words: black flies, Eastern Migratory Population, eastern Wisconsin, *Grus americana*, nest desertion, reintroduction, *Simulium annulus*, *Simulium johannseni*, White River Marsh, whooping crane.

Reintroduction of a migratory population of whooping cranes (*Grus americana*) in Wisconsin began in 2001. Central Wisconsin, especially Necedah National Wildlife Refuge (NWR), Juneau County, was the original reintroduction site. Reproduction began in 2005 and during subsequent years largely failed because of nest desertions attributable to harassment of incubating cranes by black flies (primarily *Simulium annulus*), which breed in enormous numbers near Necedah NWR (Urbanek et al. 2010, King and Adler 2012, Urbanek et al 2018*a*, Adler et al. 2019). Coincident with whooping crane nesting, synchronous mass emergence of *S. annulus* from flowing water occurs in early spring (late March-early May); the exact date is dependent on degree-days (Urbanek 2010). Black flies then disperse up to 10 km from their natal sites in search of blood meals (Bennett and Fallis 1971).

Based in part on a black fly sampling survey as well as on the presumption of fewer problem black flies elsewhere, the primary reintroduction area was shifted to eastern Wisconsin in 2011 (Van Schmidt et al. 2014; Fig. 1). Black flies had been sampled as adults and larvae at various locations within this reintroduction area in 2010 (Fig. 1; Adler 2010, Adler et al. 2019). One of 2 primary whooping crane release areas was the 12,950-ha Horicon Marsh, consisting of Horicon National Wildlife Refuge NWR and Horicon Marsh State Wildlife Area (SWA) (Dodge and Fond du Lac Counties), which together comprise the largest cattail marsh in North America. Originally, this unique marsh was dominated by common broad-leaved cattail (*Typha latifolia*), but since the mid-1900s invasion by narrow-leaved cattail (*T. angustifolia*) has resulted in dense monotypic stands of these species and their hybrid (*T.* x *glauca*) (Beule 1979). Horicon Marsh is surrounded by intensive agriculture and a higher human population than areas farther north and west in Wisconsin. Resulting effects have probably made streams in the Horicon area largely unsuitable for larvae of *S. annulus* and *S. johannseni* (Adler 2010).

The other primary whooping crane release site within the new reintroduction area was White River Marsh (WRM) (Green Lake County), but that area was not sampled in the 2010 survey. In addition, other than specimens collected at a failed whooping crane nest in 2014, no sampling of black flies had occurred in the new reintroduction area prior to the current study

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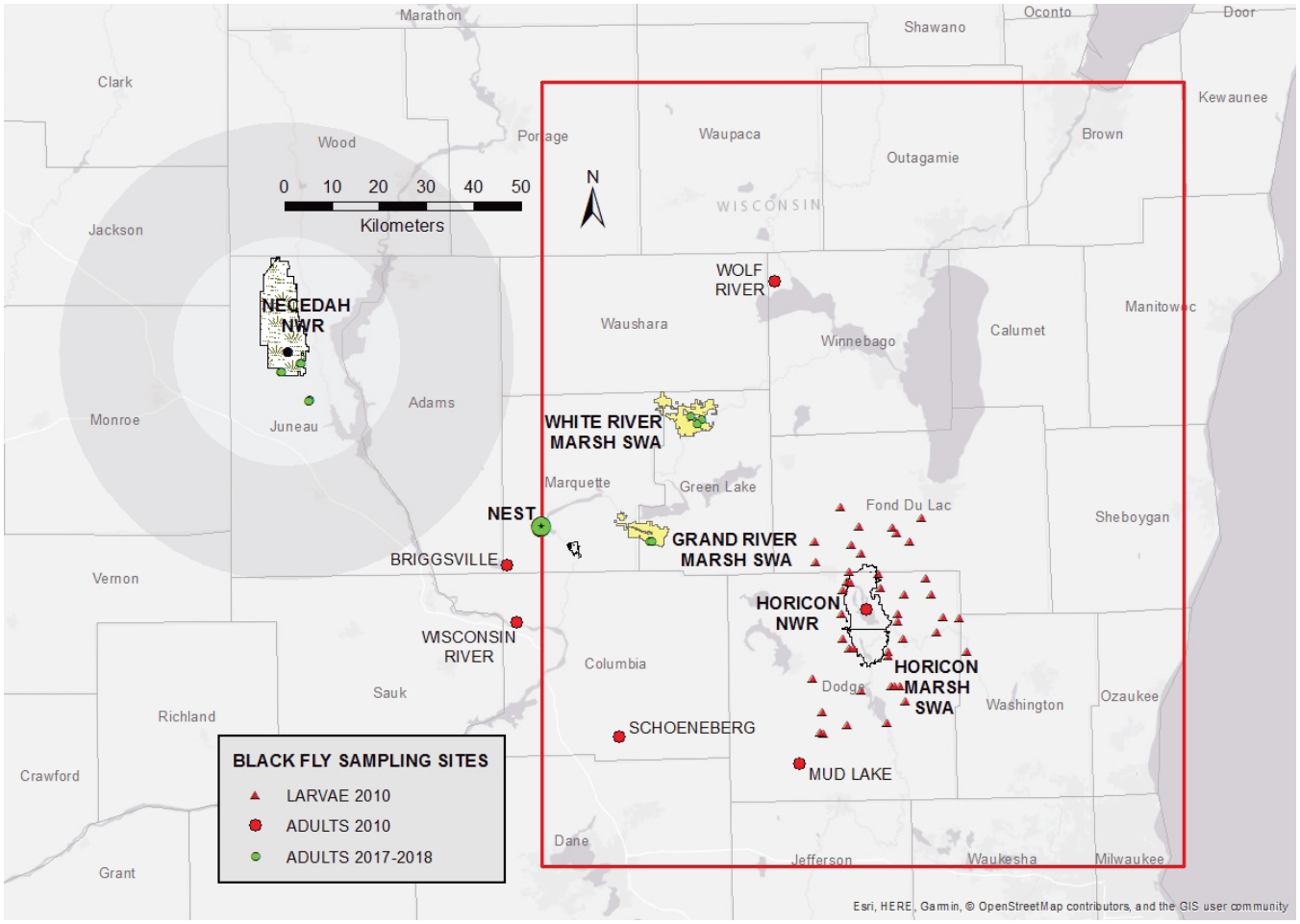

Figure 1. Black fly sampling sites in the eastern reintroduction area of Wisconsin. The boundary (red rectangle) is approximated from Van Schmidt (2014). Locations of sampling sites in 2010 are from Adler (2010) and Adler et al. (2019). Sites sampled for adults in 2010 are approximate. Location of sites in Green Lake County in 2017-2018 (this study) are shown at larger scale in Figure 2. Also shown are black fly sampling sites where comparative data were collected concurrently outside the boundary but <6 km from Necedah National Wildlife Refuge (black dot is centroid of whooping crane rearing locations) in the central Wisconsin core reintroduction area (within outermost concentric circle) (Cannon 1999, Urbanek et al. 2018*b*). Additional sites sampled since 2009 in other studies that included the core area are not shown on this map. Black flies were also collected from the failed whooping crane nest in Marquette County on the western boundary of the rectangle in 2014.

in Green Lake County. This paper reports on the first survey (2017-2018) of the target black fly species (*S. annulus* and *S. johannseni*) on White River and Grand River Marshes in Green Lake County, centrally located within the western portion of this eastern reintroduction area.

STUDY AREA

We sampled black flies at 5 sites in Green Lake County, Wisconsin (Fig. 2). Three sites were on the 4,856-ha WRM SWA, and 2 sites were on the 2,833-ha Grand River Marsh (GRM) SWA. Sites on WRM were 0.3-1.3 km from the White River, which flows through the SWA (Fig. 3). Dominant vegetation in the marsh varied by site and included tussock sedge (*Carex stricta*), common burreed (*Sparganium eurycarpum*), cattails (primarily narrow-leaved cattail), beaked sedge (*C. utriculata*), bluejoint grass (*Calamagrostis canadensis*), meadow willow (*Salix petiolaris*), and invasive reed canary grass (*Phalaris arundinacea*). One whooping crane pair nested in WRM in 2017 and 2018. Two sites sampled on GRM were each composed of monotypic stands of reed canary grass and were not in close proximity to rivers as were the sites at WRM. One whooping crane pair nested on GRM in 2018 but in the

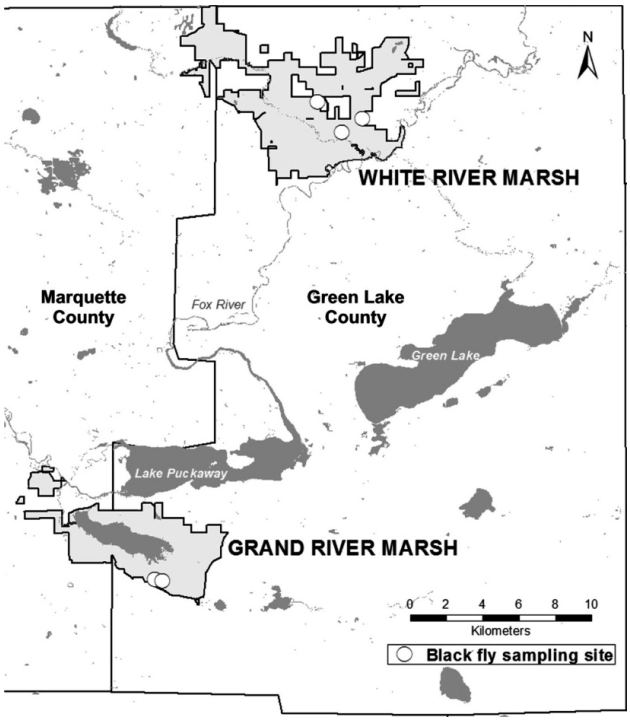

Figure 2. Black fly sampling sites on White River Marsh and Grand River Marsh State Wildlife Areas, Green Lake County, Wisconsin, 2017-2018.

western portion (Marquette County) 8.0 km from the area sampled for black flies. Results from concurrent sampling of black flies at 3 locations near Necedah NWR (Fig. 1) are provided for comparison.

METHODS

An incidental collection from a wet portion of a farm field adjacent to the northeastern boundary of WRM on 24 April 2017 produced 6 black flies (*S. johannseni*) and prompted further investigation into the abundance of black flies in this whooping crane reintroduction area. A limited sampling plan was devised to complement concurrent sampling ongoing near Necedah NWR. Three collection sites on WRM and 2 sites on GRM were selected (Fig. 2) based on location in open marsh (no woody overstory) containing potential sandhill or whooping crane nesting habitat and on accessibility (entry permission, near drivable road, and efficient walk-in time). Sites were 0.44 km from each other at GRM and even farther apart at WRM; therefore, number of black flies collected at each site was not affected by simultaneous sampling at other sites within the same area. Sites were sampled according to the standard protocol (below) on 4, 11, and 26 May and 14 July in 2017. Sampling in May provided numbers of the ornithophilic species emerging in early spring (*S. annulus* and *S. johannseni*) while the July date provided some information on the late spring and summer-emerging *S. meridionale*. We expanded the sampling program in 2018 to 13 sampling dates, 20 April-3 August, to cover the seasonal period of adult black fly activity relevant to breeding whooping cranes.

We used a rapid assessment sampling technique that consisted of placing a taxidermic mount of a lesser sandhill crane (*Grus canadensis canadensis*) on an artificial nest (pile of raked marsh vegetation). After a 1-hour waiting period, flies that appeared on and over the mount during a 5-minute period were collected with a 64 x 80-cm oval-rim sweep net (Urbanek et al. 2018*a*). At least 3 back-and-forth individual sweeps of the net were typically made over the mount during each minute of the 5-minute period. More individual sweeps were made as needed to remove additional black flies as they appeared. A single 5-minute period of sampling was conducted per site per sampling date. Specimens were transferred into 70% ethanol and later identified to species according to the dichotomous key of Adler et al. (2004). All collected

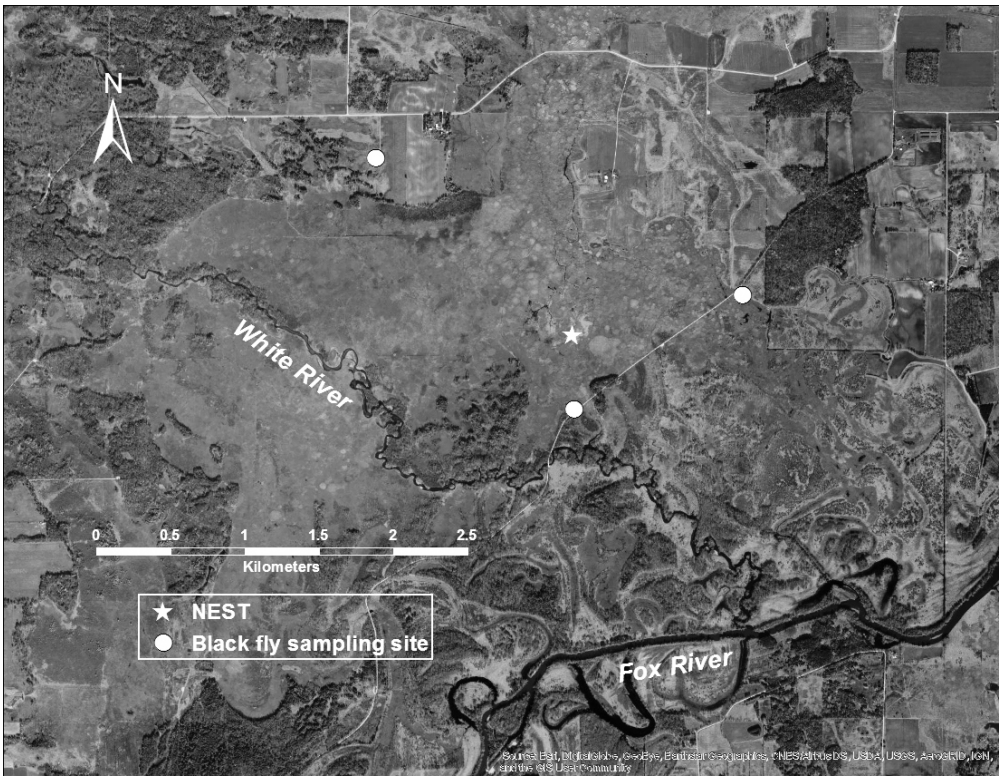

Figure 3. Black fly sampling sites and whooping crane nest location on White River Marsh State Wildlife Area, Green Lake County, Wisconsin, 2017-2018.

individuals were identified to species level except in samples containing >1,000 specimens from Green Lake County. In the latter a subsample of 200 specimens was randomly extracted from a 150-mm-diameter petri dish over a grid. Individuals in the subsample were identified, and species composition was then extrapolated to the entire sample. Except for 2 males among the 1,867 identified specimens of *S. annulus and S. johannseni* from Green Lake County, all specimens of these 2 species referred to below were females, including all 7,383 specimens of these 2 species collected in Juneau County.

RESULTS

Both SWAs demonstrated similar abundance patterns with large numbers of *S. johannseni* collected on 4 May (Fig. S1, available online in Supplemental Material). The greatest number of black flies occurring in a single sample was 6,838 (a subsample of 200 were all identified as *S. johannseni*) and occurred at WRM. Another site on WRM and 1 site on GRM produced 2,338 and 1,425 black flies (>99.7% *S. johannseni*), respectively, per 5-minute sweep period on the same date. Other than *S. johannseni*, only small numbers of *S. annulus* and other black fly species were collected in Green Lake County in 2017. Of the total black flies collected at WRM in 2017, 99.4% were *S. johannseni*, 0.4% were *S. annulus*, and 0.2% were other species. Similarly, at GRM, 98.7% were *S. johannseni*, 0.3% were *S. annulus*, and 1.0% were other species.

Although sampling in 2018 had been expanded to 13 sampling dates from 20 April to 3 August, resulting black fly numbers in that year were much lower than in 2017. A small peak (31-36 flies/sample) of *S. johannseni* occurred 14-20 May 2018 in Green Lake County (Fig. 4). Other than that, only small numbers of other species were collected, and the only other peak was 111 flies/sample on 5 July 2018 on GRM. All of those latter specimens were *S. meridionale*.

Numbers of *S. annulus* were much greater near Necedah NWR than in the areas sampled in Green Lake County during the same periods (Fig. 5). *S. johannseni* predominated in Green Lake County, but differences in

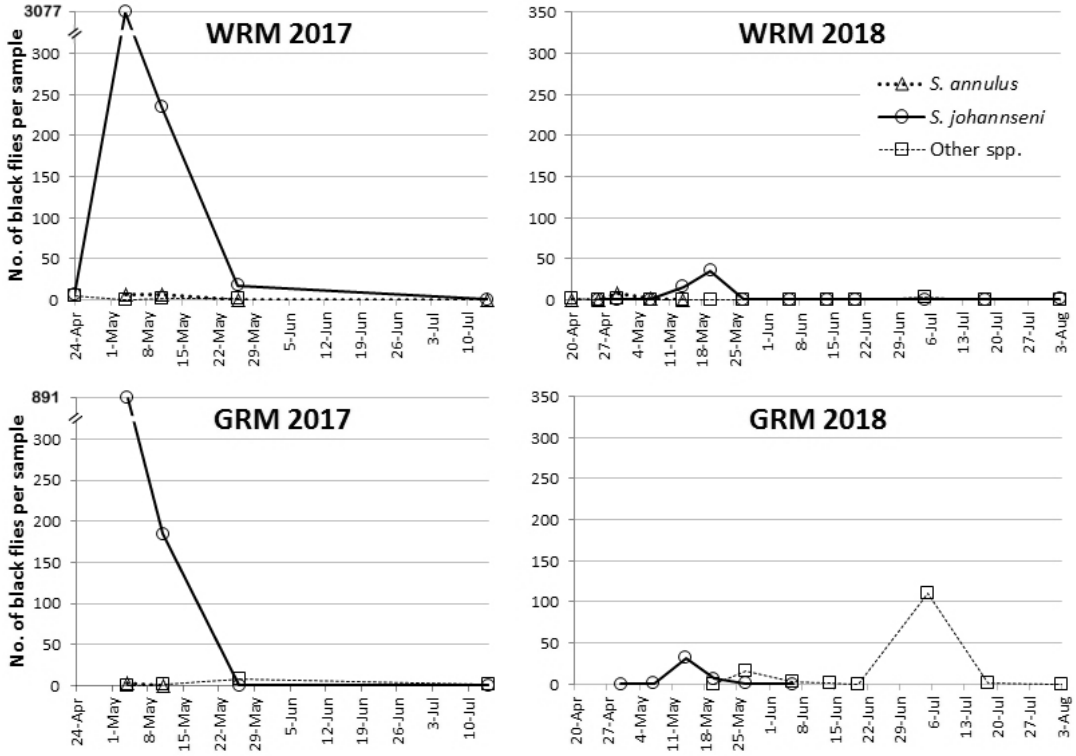

Figure 4. Mean number of black flies collected per 5-minute sweep period in Green Lake County, Wisconsin, 2017-2018. WRM = White River Marsh (*n* = 3 sites per sampling date). GRM = Grand River Marsh (*n* = 2 sites per sampling date). Note that upper horizontal tier in 2017 graphs is not to scale.

numbers of that species were more variable.

DISCUSSION

The 6,838 black flies collected in 1 sample on WRM on 4 May 2017 far exceeded the number ever collected in any other sample by the sweep-over-taxidermic-mount technique, including during 7 years of sampling on or near Necedah NWR in Juneau County (Urbanek et al. 2018*a*; R. Urbanek, unpublished data). Large numbers in a single sample from another site on WRM and 1 site on GRM on the same date were similar to the record and atypically high numbers ever collected per sample by this technique at Necedah NWR (2,899 and 1,244 specimens on 24 April 2020; identification pending but expected to be mainly *S. annulus*). The specimens collected in these samples from Green Lake County were nearly all *S. johannseni*. The numbers of specimens collected by this sweep technique are method-specific and not directly comparable to much greater numbers collected by CO_2 trapping used in other studies (e.g., Adler 2010, Barzen et al. 2018, Adler et al. 2019)

A small peak of *S. meridionale* occurred on GRM in July 2018. This smaller ornithophilic species of black fly also occurs commonly in Juneau County and appears mainly after the nesting season. No evidence linking this species to additional negative effects on reproduction of whooping cranes has yet been found.

Both *S. annulus* and *S. johannseni* have been implicated in whooping crane nest desertions on Necedah NWR (Urbanek et al. 2018a), but *S. annulus*, which emerges earlier, has caused most of the impact, and nearly all in most years (Barzen et al. 2018). In 2014, a peak of nest desertions occurred on Necedah NWR amidst large numbers of *S. johannseni*. This pattern followed an extremely cold winter and late spring, and relatively few *S. annulus* were found on the landscape (Urbanek et al. 2018*a*; R. Urbanek, unpublished data). In typical years when *S. annulus* is abundant, 2 factors should be considered in interpretation of results: 1) *S. annulus* emerges first and causes mass nest desertion, so most nests have already been deserted when the majority of *S. johannseni* emerges, and 2) the initial black fly attack each spring appears to be much more damaging to

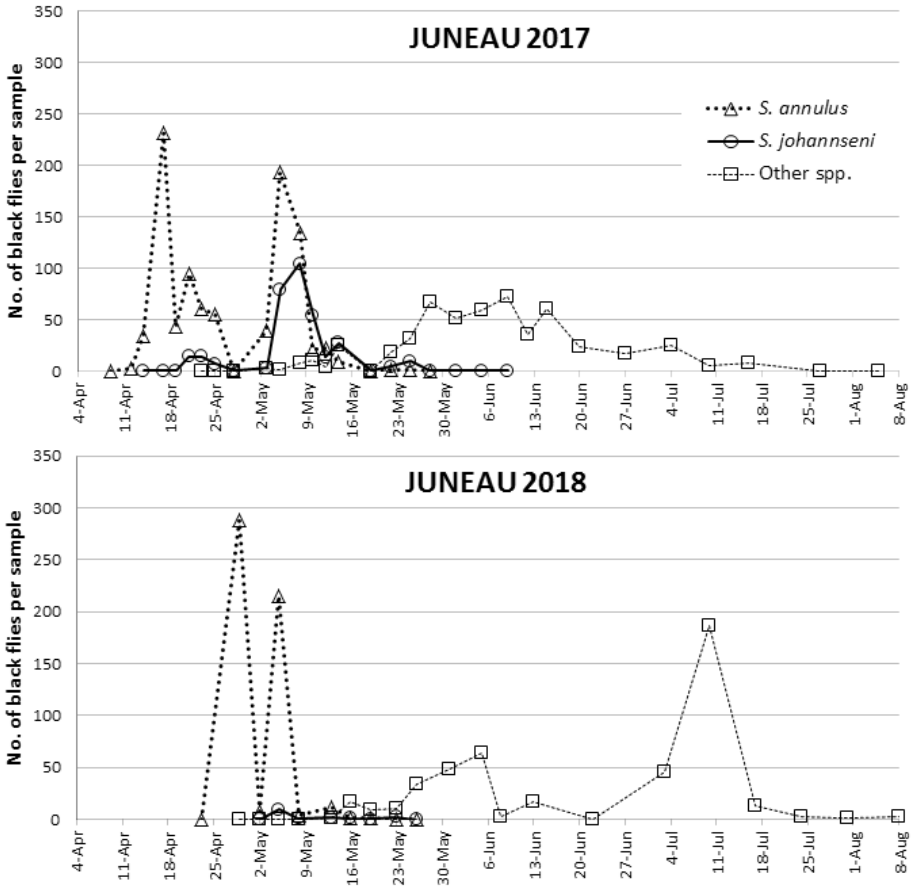

Figure 5. Mean number of black flies collected per 5-minute sweep period outside the boundary but <6 km from Necedah National Wildlife Refuge (Fig. 1), Juneau County, Wisconsin, 2017-2018 (*n* = 4 sites [2 in each of 2 different areas] per sampling date).

whooping crane nesting than do larger numbers of black flies that may occur later, implying increased tolerance develops later in the nesting season. Therefore, in most years the potential negative effect of *S. johannseni* on whooping crane nesting is not realized.

Both *S. annulus* and *S. johannseni* were collected in Green Lake County during sampling in 2017 and 2018, but *S. johannseni* was by far the predominant **s**pecies at both WRM and GRM in each year. However, formal sampling began late (May) in 2017, and if a peak of *S. annulu*s had occurred, it might have been missed. *Simulium johannseni* occurred in huge numbers in 2017 but in much smaller numbers in 2018. *Simulium annulus* was not abundant in either year, and *S. johannseni* numbers were much reduced in 2018 in comparison to 2017.

Our preliminary study indicated that *S. johannseni* could occur in numbers large enough to result in nest failures (e.g., as compared to effects of *S. johannseni* on Necedah NWR in 2014; Urbanek et al. 2018*a*), whereas *S. annulus* probably occurred in numbers too low to affect nesting success. The effect could differ greatly in different years. The whooping crane nest at WRM in 2017 proceeded almost full-term in spite of the huge black fly numbers, and it failed on 8 May during the peak emergence of *S. johannseni* (Fig. 4). However, the proximal cause of failure, captured on video as well as observed firsthand by an observer (B. Pennypacker, Operation Migration, personal communication), was predation of the eggs by a coyote (*Canis latrans*) immediately after the incubating whooping crane left the nest to assist its mate in territorial defense against a newly arrived intruding whooping crane. The video clearly showed that black flies had previously been harassing the cranes during incubation, but they endured the irritation and continued to incubate.

On 10 May 2014, dead specimens of *S. johannseni* were found entrapped in the contents of egg remains in

a nest near Endeavor Marsh (Marquette County). That nest was on the boundary of the eastern rectangle (Fig. 3) and 16.1 km west of the nest location of the same male on western GRM in 2018. At Necedah NWR, black flies entrapped in egg remains were a consistent indicator of nest desertion (Urbanek et al. 2010, 2018*a*).

Numbers of *S. johannseni* at WRM during 2017 were much larger than typical numbers of *S. annulus* collected by the sweep-over-taxidermic-crane-mount technique on or near Necedah NWR (Urbanek et al. 2018*a*; R. Urbanek, unpublished data; Fig. 5), where much smaller sample numbers had resulted in nest desertions. Larger numbers at WRM might have been related to closer proximity to probable larval habitat, i.e., the adjacent and imbedded White and Fox Rivers (Fig. 3), than at Necedah NWR, where the Yellow River (major source of *S. annulus*) is farther (4-12 km) from nests (Urbanek 2010) or to the much smaller expanse of wetland in the adjacent landscape at WRM and possible resulting concentration of emerging flies.

At Necedah NWR black fly flight and attack were weather dependent, being greatest on warm, sunny days; lower on cold, windy, or overcast days (especially between periods of rain); and insignificant during rain, winds > 40 km/h, and temperatures <13° C (Urbanek et al. 2010; R. Urbanek, personal observations). Even during periods of peak black fly emergence, intermittent days of inclement weather could greatly reduce numbers available to harass cranes. In the present study and the long-term study at Necedah (Urbanek et al. 2018*a*), sampling was intentionally avoided on days of inclement weather, but some collections were made on marginal days to adhere to the sampling schedule in addition to the preferential sampling that occurred on good weather days. This procedure introduced irregularities in general numerical peaks and additional variability into the data (e.g., Fig. 5, Necedah NWR). In addition, the number of sample dates in Green Lake County was small in 2017, and variability (not addressed here) among the few sites sampled was high. However, despite these factors, the patterns were clear and consistent for each year.

MANAGEMENT IMPLICATIONS

Because whooping crane nest desertion due to black flies has been a major problem in the Eastern Migratory Population reintroduction near Necedah NWR, survey of black flies in potential new reintroduction areas should be included in planning. This has been done in the past for many areas, but others such as Green Lake County, reported here, still require study to increase probability of a successful reintroduction.

The nest desertion problem has 4 possible solutions (Barzen et al. 2018, Adler et al. 2019): 1) control black flies with the biological control agent *Bacillus thuringiensis israelensis* (*Bti*) (Golden 2010); 2) remove first clutches to encourage or "force" renesting by cranes after peak black fly emergence has declined; 3) move reintroduction to areas with fewer problem black flies; or 4) do nothing, i.e, anticipate that cranes may eventually nest successfully in spite of harassment by black flies. Each of these alternatives has specific advantages and disadvantages. In some cases, older whooping crane pairs have been able to tolerate the black fly attack and continue incubating (R. Urbanek, unpublished data); however, this response has been observed in just a few pairs of cranes per year and incurs an unknown toll on their health. Neither is there any known biological mechanism or a means to induce whooping cranes in a small reintroduced population to delay onset of nesting to avoid black fly activity that will not occur until later, a hypothesis that was also tested and rejected by Barzen et al. (2018). Options 1-3 each have potential to significantly reduce the problem, but each also has political, logistical, and biological drawbacks. In addition, solution of the black fly problem does not address other problems such as high chick mortality, not related to black flies, on Necedah NWR. Some preliminary identification and quantification of causative factors of chick mortality have been attempted and some preliminary solutions proposed (Urbanek 2015, McLean 2019), but further work has been too limited to resolve this problem. High chick mortality could likely be a problem at any site in Wisconsin. Unlike at Necedah NWR, water control and other habitat management options that may be needed for successful establishment of the population may be beyond the logistical capability of other areas. New sites with little or no previous study may also conceal additional limitations to successful reintroduction that will not become evident until after the practical lifetime of the reintroduction (limited by number of birds, funding, political support, and solution of existing problems) has been expended.

From the above alternatives, the Whooping Crane Eastern Partnership chose to proceed with forced renesting for the existing core population on Necedah NWR. In addition, beginning in 2011, the Partnership moved the primary reintroduction area to eastern

Wisconsin after habitat assessment (Van Schmidt 2014) and preliminary sampling for black flies (Adler 2010). The latter 20,218-km^2 area needs black fly sampling at additional sites and of longer duration. The limited sampling in Green Lake County in 2017-2018 confirmed abundance of 1 species of black fly (*S. johannseni*) implicated in whooping crane nest failure. However, limited sampling during only 2 years was not adequate to assess the potential threat. If the status of black fly species that could cause nest desertion continues to be a major criterion for release of whooping cranes in the eastern rectangle or elsewhere, then additional multi-year studies at potential release sites, including WRM, will be needed during the nesting season to determine black fly abundance and distribution. These studies must be coordinated with monitoring of whooping crane nesting to assess the impact of black flies and to develop and implement plans to manage and promote success of the whooping crane Eastern Migratory Population.

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SUPPLEMENTAL MATERIAL

Figure S1. Black flies (predominantly *Simulium johannseni*) swarming a taxidermic sandhill crane mount on an artificial nest at White River Marsh, Wisconsin, 4 May 2017. Video by Richard P. Urbanek, Whooping Crane Technical Assistance Group. <https://www.nacwg.org/workshop15_urbanek_and_adler_S1.mp4>

ADDENDUM

In 2021 additional sampling was conducted on White River Marsh (17 Apr–13 Jul) and near Necedah NWR (6 Apr–13 Jul). At the same 3 sites sampled earlier on White River Marsh, large enough numbers of *Simulium annulus* to affect nest success (based on earlier work at Necedah) were discovered, although not nearly as large as numbers of *S. johannseni* in Green Lake County in 2017 or of *S. annulus* in the Necedah area in most years (Fig. 6). Sampling began relatively late at White River Marsh in 2021, so peak numbers could have occurred earlier. The variability in the 3 years of data (2017, 2018, 2021) collected at White River Marsh indicates that more study is needed before the long-term abundance and potential effect of black flies on whooping crane nesting in the eastern reintroduction area can be fully characterized.

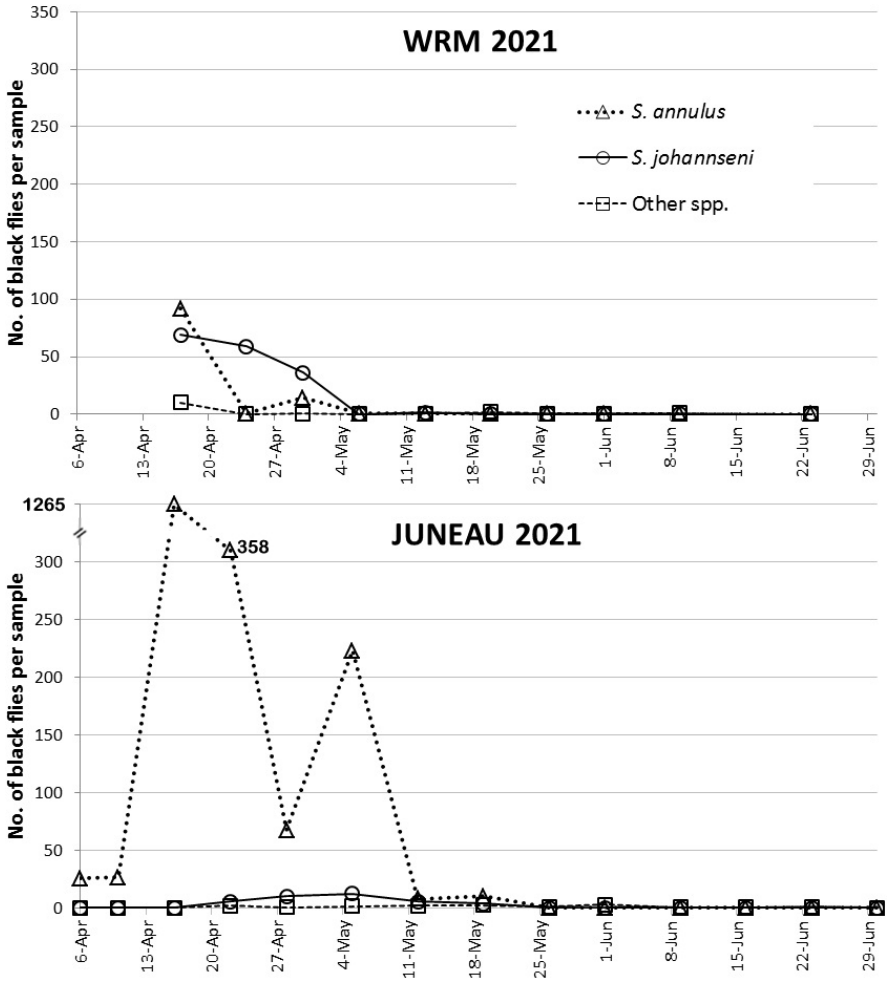

Figure 6. Mean number of black flies collected per 5-minute sweep period on White River Marsh, Green Lake County (*n* = 3 sites per sampling date), and within 300 m of the boundary of Necedah National Wildlife Refuge, Juneau County (*n* = 2 sites per sampling date), Wisconsin, 2021. Note that upper horizontal tier in bottom graph is not to scale.