PROPOSED EXPERIMENTAL SUPPRESSION OF BLACK FLIES IN SURROUNDING AREAS OF THE INTRODUCED EASTERN MIGRATORY POPULATION OF WHOOPING CRANES: EFFECTS TO NONTARGET SPECIES OF CONCERN

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Summary

Nest abandonment as a result of black fly outbreaks has been hypothesized as an explanation for the continued reproductive failure in the captive-bred whooping crane (*Grus americana)* population introduced to Necedah National Wildlife Refuge in Wisconsin. A two-year experimental suppression of two black fly species associated with nesting cranes, *Simulium annulus* and *Simulium johannseni*, has been proposed as a means to determine the validity of this hypothesis. Surveys of black flies in waters outside refuge property revealed five waterways within the 10 km dispersal distance of target species that contain breeding populations. Due to its high efficacy and specificity to black flies, application of *Bacillus thuringiensis israelensis* (*Bti*) can provide almost complete suppression of target species while resulting in minimal impacts to nontarget organisms in the ecosystem. As such, we propose a 2011 application of *Bti* at breeding sites of *S. annulus* and *S. johannseni* within dispersal distance of crane nests. As studies of field use of *Bti* for black fly suppression consistently result in findings of no adverse effects to nontarget organisms, we anticipate no adverse effects to birds, mammals, reptiles, fish, mussels, and invertebrate species exposed to *Bti*, including those species of special concern. Minor effects to susceptible nontarget dipterans, such as certain chironomid midges, and predatory insects that use these species as a significant food source cannot be ruled out. However, effects would only be anticipated to nontarget insects if targeted black flies make up a significant and necessary portion of their diet. We deem these food chain effects unlikely due to the brief emergence by adults of both black fly species during early spring, alternative sources of insect food available from untreated streams and other wetland types, and availability of black fly larvae and resulting adults that emerge later in the season. In the event that any adverse effects were to occur, they would be expected to be of short-term duration due to the two-year limitation of the study.

Introduction

The whooping crane (*Grus americana*) is the rarest of 15 species of cranes worldwide and is a critically endangered species, with only one naturally occurring population in North America. The whooping crane recovery plan (Canadian Wildlife Service and U.S. Fish and Wildlife Service 2007) calls for establishment of two self-sustaining wild populations in addition to the natural wild population before the species can be downlisted to threatened status. The reintroduction of an eastern migratory population of whooping cranes began in 2001 by the Whooping Crane Eastern Partnership, a consortium of governmental agencies and private organizations. As of 29 October 2010, there were 107 whooping cranes in the wild in eastern North America. Resulting survival, migration, and human avoidance behavior of reintroduced birds have been adequate to make successful establishment of a population possible. However, adequate reproduction has not been achieved. Nesting first occurred in 2005, and through 2009 there had been 41 nesting attempts by 16 different pairs. All 34 first nesting attempts during these 5 years failed. Three of 7 renesting attempts resulted in hatching of 4 chicks, of which 1 fledged. In 2010, 12 pairs nested and incubated eggs. All early nests (initiated in the first week of April) failed, but 3 late nests and 3 renests were incubated full term, resulting in 2 fledged chicks by summer's end.

During 2005-09, nests were monitored by videography, radiotracking of attending adults, visual observation, aerial observations, radio dataloggers, and examination after failure. Nest failures were evenly distributed during the period of incubation, varying from 1 to 29 days. First nest failures were synchronous, occurring within the same few calendar days in some years but were spread over sixteen days in 2009. Renest failures have occurred over 28 days.

Several hypothetical causes of nest failure were not supported by evidence or could not wholly account for widespread nest abandonment:

- Monitoring of nests during 2005-09 confirmed that neither predation nor water level fluctuations were the primary cause of the nest failures.

- Poor body condition of returning birds due to inadequate food resources on the wintering grounds does not explain the consistent widespread nest failure among all pairs because most pairs winter at different, often widely separate, locations.

- Human disturbance is unlikely to have had widespread affects on nesting whooping cranes because: 1) Access all of Necedah National Wildlife Refuge (NWR), including whooping nest areas, is regulated and 2) whooping cranes appear to be tolerant of disturbance around their nests based upon the lack of nest failures following numerous nest visitations at Wood Buffalo National Park and limited visitation to nests in Florida and Wisconsin.

- Low nutrient levels in aquatic habitats on Necedah NWR may result in limited availability of food for whooping cranes after incubation has started, but does not explain the widespread desertions occurring over multiple years. This assumption is based on the fact that whooping cranes eat a large variety of food items including acorns, fish, eggs, and ducklings, all of which are available during nesting. Additionally, supplemental food in the form of kernel corn was provided to half of the nesting whooping crane pairs on Necedah NWR in 2009 and had no affect on nest success or failure.

- Though costume-imprinting is known to affect whooping crane behavior (Kreger et al. 2004, 2005), cranes have exhibited normal formation of breeding pairs and nesting behaviors early in incubation appear to be normal. Additionally, most nests of costume-imprinted non-migratory whooping cranes introduced in Florida were incubated full-term, and no differences were noted in reproduction between costume and parent-imprinted cranes.

- Inexperience by the cranes nesting at Necedah NWR is a potential explanation for low reproduction. Whooping cranes are slow to reproduce, reaching reproductive age at three years. However, this can be delayed to as great as ten years of age as evidenced with non-migratory whooping cranes from Florida. Analysis of nest data indicates that incubation length is increasing as the age of the nesting pair increases, and the minimum age of the pair is valuable for predicting daily survival of whooping crane nests. However, inexperience alone does not account for the widespread nest abandonment as pairs with individuals who are eight years old have abandoned nests.

- Captive exposure effects have been shown to affect a wide range of reintroduced taxa (Darwin 1868, Price 1984, Lickliter and Ness 1990, Carlstead 1996, and Price 1998), and offer an explanation for the slightly higher nest success rates reported for the Florida, non-migratory population. Although birds used in both the Wisconsin and Florida experiments came from the same captive-breeding facilities, the Florida experiment began approximately a decade earlier. As captive exposure effects are proportional to the number of generations a population is removed from the wild, we would predict less affect on the Florida cranes. Nest success rates in both the Florida and Wisconsin populations are well below that reported for the wild, Wood Buffalo/Aransas population. The effects of captive breeding remains untested but alone cannot explain the widespread nest desertion.

Black fly outbreaks offer another explanation of widespread and simultaneous desertion of multiple nests. Although nest desertion due to black fly harassment of incubating birds of other species has been documented (Smith et al. 1998, Bukacinski and Bukacinska 2000), sandhill cranes (*Grus canadensis*) in the Great Lakes region coexist with black flies and routinely nest successfully (Urbanek and Bookhout 1992). Therefore, this factor had not been initially predicted as a cause of nest desertion by whooping cranes. The black fly *Simulium annulus* – the same species identified here as swarming around whooping cranes and their nests –has also been implicated in nest abandonment by common loons (*Gavia immer*) (McIntyre 1988). Field observations in 2008 and 2009 indicate that some nesting cranes were attacked by black flies and that they reacted by flicking their bill, preening, and rubbing their head against their body or vegetation.

Whooping crane decoys deployed at abandoned nests in 2009 were swarmed by three black fly species specific to birds including *Simulium annulus, S. johannseni, and S. merdionale*.

In 2009, the abandonment of 8 whooping crane nests (incubated 3-20 days) coincided with the emergence of adult *S. annulus*. An additional nest located off of Necedah NWR (Wood County) also failed at this time. Black flies were photographed on deserted eggs in Wood County and on Necedah NWR. One damaged egg collected on April 24, contained more than 700 drowned black flies, all *S. annulus*. Four nest attempts failed when both *S. annulus* and *S. johannseni* were on the landscape, with one damaged egg containing 2,272 black flies (462 *S. annulus*, 1,810 *S. johannseni*). Eggs from two successful nests hatched when neither *S. annulus* or *S. johannseni* were on the landscape. However, both species were on the landscape during the beginning of the 30 day incubation period for both successful nests. Three nests failed when no black flies were present on the landscape.

Surveys of adult and larval black flies were performed in 2009 and 2010 to determine species identity, distribution, and abundance in the whooping crane reintroduction area. All major ditches on Necedah NWR were sub-sampled at 56 locations for early season black fly larvae and pupae (Adler 2009). During larval/pupae surveys conducted between April 1 and 10, 2009, early season black fly were collect from the following species: *Simulium vitattum, Stegopterna mutate, Cnephia dacotensis, Simulium tribulatum, Simulium venustum, Prosimulium fuscum, Stegopterna mutate, S. annulus, Prosimulium arvum, S. johannseni,* and *Greniera denaria*. Only *S. annulus* and *S. johannseni* are attracted to whooping crane nests and their larvae were found on only four stretches of ditch totaling 3.2 miles. These sections of ditch were an average of 2.2 miles from whooping crane nests used in 2009. Three sites sampled off refuge (2 in Yellow River to east, 1 in Beaver Creek to west) all contained *S. annulus*. In 2010, larval surveys within a 10-km zone surrounding crane nests found the Yellow River to be a primary breeding site for both *S. annulus* and *S. johannseni* (P. Adler, personal communication). Smaller populations of both species were found residing in the South Branch of the Yellow River, Lemonweir River, Cranberry Creek and Beaver Creek. Populations of both species in the Spencer-Robinson Ditch on Necedah NWR were smaller in 2010 than in 2009 and attributed to year-to-year variability of stream conditions. Analysis of stream variables at the larval sites indicated that *S. annulus* is most likely to breed in larger, more basic, forested streams with sandy streambeds and remote from impoundments.

Pilot study: 2010 macroinvertebrate sampling

In 2010, a pilot study of *Bti* efficacy and effects to nontarget macroinvertebrates was conducted in conjunction with larval surveys of the Yellow River by Peter Adler of Clemson University and Elmer Gray of the University of Georgia. Macroinvertebrate samples were collected in April of 2010 from a *Bti* treatment site in the Yellow River (downstream of the 4th Street; 44 degrees 12.816'N, 90 degrees 07.162'W) before and after application. Nontarget samples were taken 100 meter above and below the treatment site. Pre-application samples were collected on April 5th and post-application samples on April 6th.

Samples were sorted and analyzed for a variety of community indicators by the University of Wisconsin – Stevens Point (Attachment B). Specimens were sampled using a randomized subsampling procedure to ensure that each was representative of the whole. All samples were dominated by Simuliidae, which accounted for 81% - 87% of individuals counted at each site (Tables 1, 2). The percent Simuliidae sorted from the pre- and post-treatment sites downstream of the application was similar, likely due to the tendency of black fly larvae to remain attached to their substrates (e.g., leaves) after death and thus remain available for collection. Investigators report that all Simuliidae larvae were upon collection in the post-application downstream sample. No differences were observed following *Bti* application at either site for nontarget taxa, including chironomids, and specifically, individuals within the genus Rheotanytarsus, for which sensitivity to *Bti* has been observed in prior studies. No individuals within the order Odonata were observed in any of the four samples. Indices of diversity, richness, and biotic integrity were similar among samples (Table 3).

Table 1. Number of individuals and percent of total sample for macroinvertebrate genera collected upstream of a *Bti* application in the Yellow River.

UPSTREAM OF BTI APPLCATION				
	pre-application		**post-application**	
GENUS	# INDIV	%	# INDIV	%
Simulium	137	84.0%	347	87.6%
Rheotanytarsus	11	6.7%	13	3.3%
Tanytarsus	3	1.8%	11	2.8%
Rheocricotopus	3	1.8%	3	0.8%
Microtendipes	2	1.2%	5	1.3%
Shipsa	1	0.6%	3	0.8%
Pteronarcys	1	0.6%	1	0.3%
Orthocladius	1	0.6%	2	0.5%
Macronychus	1	0.6%	0	0.0%
Isoperla	1	0.6%	0	0.0%
Cricotopus	1	0.6%	2	0.5%
Conchapelopia	1	0.6%	0	0.0%
Thienemannimyia "complex"	0	0.0%	2	0.5%
Thienemanniella	0	0.0%	1	0.3%
Stylodrilus	0	0.0%	1	0.3%
Stempellinella	0	0.0%	1	0.3%
Prostoia	0	0.0%	1	0.3%
Physa	0	0.0%	1	0.3%
Parakiefferiella	0	0.0%	1	0.3%
Atherix	0	0.0%	1	0.3%
TOTALS	**163**		**396**	

Table 2. Number of individuals and percent of total sample for macroinvertebrate genera collected downstream of *Bti* application in the Yellow River.

DOWNSTREAM OF BTI APPLCATION				
	pre-application		**post-application**	
GENUS	# INDIV	%	# INDIV	%
Simulium	134	85.9%	131	81.4%
Rheotanytarsus	10	6.4%	10	6.2%
Shipsa	3	1.9%	3	1.9%
Tanytarsus	3	1.9%	5	3.1%
Conchapelopia	2	1.3%	1	0.6%
Stempellinella	2	1.3%	0	0.0%
Parametriocnemus	1	0.6%	0	0.0%
Microtendipes	1	0.6%	1	0.6%
Pycnopsyche	0	0.0%	1	0.6%
Ancyronyx	0	0.0%	1	0.6%
Parakiefferiella	0	0.0%	1	0.6%
Rheocricotopus	0	0.0%	4	2.5%
Rheosmittia	0	0.0%	2	1.2%
Cricotopus/Orthocladius	0	0.0%	1	0.6%
TOTALS	**156**		**161**	

Despite the identification by University of Wisconsin taxonomists of 4 species of black flies in these samples (*Simulium emarginatum*, *Simulium anatinum*, *Simulium longistylatum*, and *Simulium aestivum)*, Dr. Adler believes that these are largely misidentifications of *S. annulus* and *S. johannseni* based on his two seasons of sampling in the Yellow River and expertise in black fly taxonomy. Dr. Adler is the primary author of *The Black Flies (Simuliidae) of North America* (Adler et al. 2004), which contains the latest and most complete keys for identification of this taxon. Neither *S. anatinum* or *S. aestivum* is likely to be found in the Yellow River; *S. anatinum* breeds in small streams that run through swamps and bogs, and *S. aestivum* has never been identified in the Yellow River in Dr. Adler's surveys. *S. emarginatum* is morphologically very similar to both *S. annulus* and *S. johannseni*, but has been distinguished by both morphological and genetic techniques by Dr. Adler in his surveys. *Simulium longistylatum* is believed to be a misidentification of *Prosimulium magnum*, a very small population of which occurs in the Yellow River. Dr. Adler also believes that the unidentified Simuliidae larvae, which accounted for the greatest numbers of insects sorted, are likely *S. annulus* and *S. johannseni*, which made up the overwhelming majority of flies that he surveyed and identified on the Yellow River.

Table 3. Indices of diversity, richness, and biotic integrity in macroinvertebrate samples collected upstream and downstream of *Bti* application in the Yellow River.

UPSTREAM OF BTI APPLCATION								
	COUNT	IBI	HBI	FBI	SR	GR	COUNT_EPT	GR_EPT
pre-application	163	3.16778	5.520	5.884	14	12	2	25
post-application	397	3.89886	5.391	5.928	21	17	1	17
	CHI_PC_CNT	DIV	SCR	FIL	SHR	GAT	HBI10	
pre-application	0.14286	1.079	0	94	2	3	5.500	
post-application	0.10859	0.949	0	95	2	3	5.333	
DOWNSTREAM OF BTI APPLCATION								
	COUNT	IBI	HBI	FBI	SR	GR	COUNT_EPT	GR_EPT
pre-application	157	0.25740	5.227	5.912	10	8	2	13
post-application	162	1.90422	5.462	5.886	14	12	3	17
	CHI_PC_CNT	DIV	SCR	FIL	SHR	GAT	HBI10	
pre-application	0.12500	0.916	0	96	2	1	5.227	
post-application	0.16667	1.238	0	91	3	6	5.462	

COUNT	Count of individuals in subsample
IBI	Index of Biotic Integrity (Weigel 2003)
HBI	HBI (Hilsenhoff 1977, 1982, 1987)
FBI	FBI (Hilsenhoff 1988)
SR	Species richness of subsample
GR	Generic richness of subsample
COUNT_EPT	# EPT individuals in subsample
GR_EPT	% of genera in subsample that are EPT taxa
CHI_PC_CNT	% of individuals in subsample that are Chironomidae
DIV	Shannon's diversity index (base 2)
SCR	% of individuals in subsample that are scrapers
FIL	% of individuals in subsample that are filterers
SHR	% of individuals in subsample that are shredders
GAT	% of individuals in subsample that are gatherers
HBI10	HBI 10 Max Modification (Hilsenhoff 1998)

Proposed Experiment

In order to determine the contribution of black fly harassment to whooping crane nest desertion, a two-year experimental suppression of larval *S. annulus* and *S. johannseni* within a 10-km radius of nests on Necedah NWR is proposed. The 10-km radius selected for surveying breeding habitats of black flies reflects known dispersal distance of adult *S. annulus* (Bennett and Fallis 1971).

Suppression of *S. annulus* and *S. johannseni* will be achieved by application of *Bacillus thuringiensis* var *israelensis* (*Bti*) as Vectobac® 12AS in larval breeding sites as determined by surveys in 2009 and 2010, and prior to application in 2011. *Bti* is economical and environmentally safe, and can be applied with ease by pouring it into target areas from hand-held containers (Molloy 1982). Downstream carry of *Bti* can be achieved for up to 9 km from the point of introduction, with mortality rates of larval black flies routinely exceeding 90% (Abbott Laboratories 1999). All applications of *Bti* will include pre and post-treatment black fly larval surveys. Data from these surveys will be used to measure the effectiveness of *Bti* applications as it relates to larval black fly mortality.

Based on *S. annulus* and *S. johannseni* larval surveys, the breeding sites, and potential areas of treatment, for both species include the following locations (Attachment A):

Primary breeding site

- Yellow River, essentially from Babcock (and above) to slightly below the dam in Necedah

Secondary breeding sites (smaller populations)

- South Branch of the Yellow River from south of 22nd Street to about Hwy 80
- Lemonweir River from 25th Street to 14th Street
- Cranberry Creek from about 5th Street to the confluence with the Yellow River
- Beaver Creek from about 9th Street to 15th Street
- Spencer-Robinson Ditch, Necedah NWR

Final treatment locations within these sites will be determined via larval surveillance in 2011. Only areas found to contain significant populations of the pest species will be treated.

Potential effects of *Bti* to off-refuge resources of concern

<u>*Bti* mode of action</u>

The biological control agent *Bti* is a variant of the common soil bacterium *Bacillus thuringiensis.* To assess the potential toxicity of *Bti* to both target and nontarget organisms, it is helpful to understand its mode of action. *Bti* is a spore-forming bacterium that induces toxicity in insects via activation of a crystalline endotoxin contained within the bacterium. Activation of this toxin can only take place under specific conditions inside the gut of appropriate insect larva. Until the time of ingestion and transport to the gut, Bt is dormant in the environment and its toxin exists as an inactivated protoxin.

Bti has been shown to have activity against only again black flies, mosquitoes and certain species of midge. This specificity is believed to be due, in part, to the unusually alkaline gut of black flies and mosquitoes, with typical pH's ranging from 10-12. A wide body of literature reveals no direct effects to other invertebrate or vertebrate species.

Different strains of Bt are specific to different insects, and can only be activated following their ingestion. The specificity of the various strains is related to the biological properties of the insect that interact with the toxin, including the pH in the insect's gut and species-specific membrane receptors within the gut. In order to be activated, the crystal containing the toxin must dissolve within the gut of the insect. This activation can only occur within a very narrow range of pH, which varies by Bt strain, to conform to the physiological properties of the target insect. If a *Bti* crystal is ingested to a gut environment with the wrong pH, it will have no adverse effects upon the organism. Once the toxin is activated, it must bind to receptors on the gut membrane to result in adverse effects. These receptors are believed to be species specific, so that even an activated toxin may pass through an organism that does not possess the proper receptors.

Regulation of toxic impurities

Previously, a number of fermentation-based products tested at high doses were found to exhibit intrinsic toxicity to nontarget organisms including *Daphnia magna*, honeybees, insects, rainbow trout, bluegill, mice, and rats. The most sensitive species to these toxic effects was *Daphnia magna.* Investigations conducted to determine the cause of this toxicity found that these effects were not the result of the Bt endotoxin, but heat-labile soluble exotoxins contaminating the technical material. The Environmental Protection Agency's (EPA) 1998 Reregistration Eligibility Decision addressed this issue by mandating standardization of the manufacturing process for all Bt production and introducing new quality control measures, including toxicity testing on mice and *Daphnia magna* prior to sale. In order to meet these new guidelines, production batches must contain negligible quantities of toxic impurities to be eligible for sale. As a result of these changes instituted by EPA, adverse effects due to exotoxins in Bt products are not anticipated to result from use of *Bti* to treat black flies.

Environmental Effects: General

Virtually every field evaluation of effects to nontarget organisms has demonstrated a lack of adverse environmental effects from *Bti* use for black fly control. These tests have been performed throughout much of North America, and often span multiple years. Below is an examination of the literature for specific taxa of concern in potential treatment sites near Necedah, Wisconsin. (Status of each species refers to State of Wisconsin, except where otherwise specified.) It should be noted that a number of studies examine the effects of *Bti* following its use for mosquito control. While these studies can provide insight into possible risk to nontarget organisms, special consideration should be used in translating the results of these studies to black fly control. Unlike black flies, which breed only in flowing water, mosquitoes breed only in standing water. A reduction or absence of flow can reduce the dissipation of the pesticide and may increase its concentration and persistence in the area of application. In addition, the consideration of indirect effects must take into account the role of each element of the food web and whether loss of mosquitoes from the food web would be comparable to a loss of black flies as a prey item.

<u>Effects to Invertebrates</u>

Species of concern that may be present in and around treatment areas:

- Frosted Elfin (*Callophrys irus*) – Threatened, Beaver Creek
- Karner Blue Butterfly (*Lycaeides melissa samuelis*) – Special Concern, Federally Endangered, Beaver Creek
- Persius Dusky Wing (*Erynnis persius*) – Special Concern, Beaver Creek
- Sand Snaketail Dragonfly (*Ophiogompus smith*) – Threatened, Lemonweir River
- Spangled Skimmer (*Libellula cyanea*) – Special Concern, Yellow River
- Ringed Boghaunter (*Williamsonia lintneri*) – Special Concern, Beaver Creek
- Tiger Beetle (*Cicindela patruela huberi*) – Special Concern, Beaver Creek

Laboratory studies of direct effects to nontarget invertebrates:

Bti has been shown to have activity against black flies, mosquitoes and certain species of midge. Treatment with *Bti* is likely to have non-target impacts on other black fly species in larval form at the same time as *S. annulus* and *S. johannseni*. However, these two species make up the majority of black flies present in treatment areas, effects to other black fly species are likely to be minimal.

In toxicity studies performed in support of product registration, *Bti* was not toxic or pathogenic to green lace-wing larvae (order Neuroptera), parasitic hymenoptera, predaceous coleopteran, honey bees, grass shrimp or copepods (EPA 1998). Moderate toxicity to daphnia was attributed to factors other than the delta-endotoxin (see discussion above regarding the regulation of impurities) and is not expected with current formulations due to quality control requirements in product manufacture (EPA 1998).

Direct toxicity testing of *Bti* spores at concentrations of 50 to several hundred times the LD50 for *Culex pipiens* detected no effects to individuals within several orders of crustaceans (Amphipoda, Decapoda, Anostraca, Cladocera, Ostracoda, Copepoda, and Isopoda), insects (Ephemeroptera. Odonata, Hemiptera, Coleoptera, and Trichoptera), tubellarian worms, and Gastropod snails (Garcia et al. 1980). Individuals of several species exhibited normal molting and reproduction during the study period, including notonectids, damselflies, dragonflies, ephydrid flies, and a species of Tricoptera, indicating that growth and development continued to occur during ingestion of *Bti*. No effect was found to dipteran brine flies in the family Ephydridae.

Within the order Odonata, intestinal pH levels ranged from 5.5 to 7.2 in dragonflies from the families Aeshnidae and Libellulidae (Saxena 1978, Joose and Verhoef 1987). In Lepidoptera, midgut pH was measured in 60 species from 20 families (Case 1978). The majority (75%) of species had a pH level between 7.0 and 9.0, with only one species reaching a pH of 10.0. Within the family Lycaenidae, pH levels ranged from 8.2 to 8.7. Within the order Coleoptera, digestive tract pH ranged from 5.5 to 9.0 in 4 species and from 7.5 to 10.2 in 2 species (Grayson 1958). Because of the large number of species associated with each of these insect orders, the number of investigations is relatively limited. Results of these studies show a small percentage of species with slight overlap of the typical pH in the gut of a black fly or mosquito, which ranges from 10-12. The gut pH of black flies and mosquitoes is an unusually alkaline environment for the activation of *Bti* that rarely exists within other species. Results for most species investigated in these studies are well below this range and cannot activate the *Bti* toxin.

Effects to chironomids: The only invertebrate taxa other than black flies and mosquitoes to show sensitivity to *Bti* is dipteran midges, and that sensitivity has varied among studies. Midges within the families Dixidae, Ceratopogonidae, and Chironomidae were affected by treatments of *Bti* spores, but at concentrations of 50 to several hundred times the LD50 for *Culex pipiens* (Garcia et al. 1980). Abundance of chironomids exposed to the standard application rate of 9 kg/ha used by the Minneapolis-St. Paul Metropolitan Mosquito Control District for mosquito control did not differ from controls (Liber et al. 1998). *Bti* concentrations required to reduce chironomid abundance in mesocosms by 25, 50 and 75% were 1.5 – 2.0X, 2.1-3.3X and 3.5-11.0X the operational application rate. Charbonneau et al. (1994) detected no reduction in benthic invertebrates, including chironomids, following application of Vectobac® G at up to 5 times the operational application rate for mosquito control. Follow-up studies of chironomid toxicity in the lab found that these species showed lower toxicity under field conditions.

Field studies of effects to aquatic invertebrates following Bti use for black fly control:

Virtually every field evaluation of *Bti* for nontarget organisms in black fly treatments has demonstrated a lack of adverse effects (e.g., Colbo and Undeen 1980, Molloy and Jamnback 1981, Burton 1984, Duckitt 1986, Pistrang and Burger 1984, Gibbs et al. 1986, Merritt et al. 1989, Jackson et al. 1994, Brancato 1996, Jackson et al. 2002). These tests have been performed throughout much of North America. Two studies (Molloy 1982, Merritt et al. 1989) indicated minor impacts to a single genus of chironomid, the filter-feeder Rheotanytarsus, with reductions of 23% and 27%, respectively. A Canadian study (Back et al. 1985) showed that net-winged midges in the genus *Blepharicera* were affected when dosage rates were 3-15 times greater than operational dosages. These net-winged midges, however, occur only in fast-flowing water with beds of large rocks and boulders.

The Merritt et al. (1989) reference is one of the broadest, most comprehensive studies on nontargets and was conducted in nearby Michigan, and revealed no effects to macroinvertebrate diversity or species richness. In Pennsylvania, areas where *Bti* has been used extensively for a quarter of a century (e.g., in more than 30 counties twice per week for more than 4 months each year), no adverse effects have been noted on non-target organisms (Pennsylvania's Black Fly Suppression Program 2008).

Effects on predatory insects: In a study that examined the effects of black fly suppression upon predatory insects, predators continued to consume black fly larvae after killed by *Bti* at recommended application rates for black fly control in artificial and natural streams, and exhibited no adverse effect, even when larvae were treated at levels greatly exceeding the application rate (Wipfli and Merritt 1994). Black fly larvae remained attached to substrate for over two weeks following a *Bti* application. The authors concluded that *Bti* appeared to be harmless to nontarget benthic invertebrates, even at applications rates greatly exceeding those required for black fly control. No effects were detected to survival, emergence, or growth in sixteen taxa of nontarget Ephemeroptera, Plecoptera, Tricoptera, and Diptera.

Field studies of effects to aquatic invertebrates following Bti use for mosquito control:

Two studies detected possible indirect effects to invertebrates resulting from food web disruption following *Bti* use for mosquito control. As stated above, differences between mosquito and black fly breeding habitats and species assemblages must be taken into account when applying the results of these studies to black fly suppression. *Bti* appears to have fewer non-target impacts when used in streams for black fly control than in wetlands when used for mosquito control. In streams the *Bti* moves through as a short-term pulse, is gathered from the water by the filter-feeding black fly larvae, and does not accumulate in the benthos, where most beneficial Diptera such as chironomids occur.

In a review of effects to Odonata exposed to *Bti* for mosquito control, Lacey and Mulla (1990) concluded that *Bti* applied at larvicidal or higher rates had no noticeable adverse effects on odonates either through direct exposure to spores or secondary exposure via consumption of treated mosquito larvae or prey reduction. Their review also assessed effects to Coleoptera and revealed no adverse impact on dytiscid or hydrophilid beetle larvae from lab and field studies.

In a comprehensive 10-year study, 27 wetlands in Wright County, Minnesota were selected in 1988 by the Natural Resources Research Institute (Duluth, MN) to serve as locations to study the long-term effects of mosquito larvicides, including *Bti*. Results of these studies varied in their detection of effects to nontarget invertebrates. Niemi et al. (1999) found that 3 years of *Bti* treatments (applied as Vectobac® G granules) in Minnesota wetlands from 1991-1993 reduced total insect density and richness on treated sites for 2 of the 3 study years. Effects were observed broadly across insect taxa, with Diptera species most strongly affected, especially the Chironomidae (Hershey et al. 1998). The authors (Hershey et al.) suggest that direct effects to dipterans led to indirect effects to other insect groups due to disruption of the invertebrate food web, and cautioned about the use of short-term studies due to a 2 to 3 year lag time to detect these effects. However, no impacts were detected to growth, reproduction, or community composition for zooplantkton despite this reduction (Niemi et al. 1999). In a follow-up study during 1997-1998, no differences were detected in the total mean density of macroinvertebrates (Balcer et al. 1999). The only treatment effects found were a reduction in the density and biomass of chironomids within the subfamily Chironominae alone, which consists mostly on non-predatory species, but no effect of treatment for chironomids as a whole. Differences in the results between these study years and the 1991-1993 study years may have been due to draught conditions in the first study, increasing the susceptibility of organisms (Balcer et al. 1999). In addition, analysis of dosage data for both studies suggests higher than planned doses in 1992 and 1993, when treatment effects were detected, which may have contributed to the difference in results (Read 2002).

Indirect effects were presumed for insect species that prey on Nematocera following mosquito suppression in the Camargue, France (Poulin et al. 2010). Though not measured directly, the number of predatory insects of the orders Odonata and Araneae taken by house martins (*Delichon urbicum*) was significantly reduced at sites were Nematocera were suppressed. Though population level effects of these species were not independently assessed, species within these taxa were presumed to have been reduced based on evaluation of house martin diets and attributed to their high reliance on Nematocera as prey.

Potential effects to invertebrate species of concern:

The Frosted Elfin, Karner Blue Butterfly, and Persius Dusky Wing are members of the order Lepidoptera. Neither direct nor indirect effects have been observed for this order following either laboratory exposure to *Bti* spores or field exposure to *Bti* for black fly control. These species are herbivorous and use wild lupine as a host plant. The use of *Bti* is not anticipated to adversely affect these species in the treatment area.

The Sand Snaketail Dragonfly, Spangled Skimmer, and Ringed Boghaunter are members of the order Odonata. Neither direct nor indirect effects have been observed for this order following either laboratory exposure to *Bti* spores or field exposure to *Bti* for black fly control. One field study concluded that suppression of mosquitoes by *Bti* likely resulted in indirect effects to Odonata due to loss of prey. This contrasts with a black fly suppression study which found no effects to predatory insects. Most dragonflies are generalists in both the larval and adult form, eating whatever suitable prey is abundant. Maturing larvae and females developing eggs eat intensely, and studies have shown food shortages to be a limiting factor in reproduction.

The Tiger Beetle *Cicindela patruela* is a member of the order Coleoptera. They are carnivorous, generally preying upon ants, spiders, flies and other invertebrates, though specific components of the diet are unknown. Neither direct nor indirect effects have been observed for this order following either laboratory exposure to *Bti* or field exposure to *Bti* for black fly control.

The use of *S. annulus* or *S. johannseni* as food sources for the sand snaketail dragonfly, spangled skimmer, ringed boghaunter, and tiger beetle *Cicindela patruela* in the treatment area is unknown. The brief emergence by adults of both species during early spring is an evolutionary strategy that makes buildup of corresponding numbers of predators dependent on them as a potential food source unlikely. There are also alternative sources of insect food available from untreated streams and other wetland types. Additionally, *Bti* use would have no impact on black fly larvae and resulting adults that emerge later in the season. However, depending on local prey availability, it is possible that dipteran flies could make up a significant portion of the diets of predatory insects. If such is the case, individuals may be able to switch prey to other locally abundant invertebrates following black fly suppression. However, if individuals were unable to switch prey, and black flies were a significant source of nutrition, it is possible that indirect effects to predatory insects could occur from black fly control.

<u>Mussels</u>

Species of concern that may be present in and around treatment areas:

- Salamander Mussel (*Simpsonaias ambigua*) – Threatened, Lemonweir River

Potential for direct effects to mussels:

Glochidia: There is no clear *Bti* exposure pathway for glochidia. Glochidia exist in two states: free-living and encysted on the gills of fish. The salamander mussel is the only freshwater mussel known to have a non-fish host, the mudpuppy (*Necturus maculosus*). The free-living glochidial stage occurs after a female mussel releases glochidia either as individuals or as part of a conglutinate, and can last from 1 to 10 days, or until ingestion by a host. During this stage, larvae are non-mobile and either drifting in the water column or are attached to substrate as a part of a lure. Glochidia lack storage tissue and do not feed during this free-living stage (Jansen et al. 2001). Therefore, it is not anticipated that glochidia will be exposed to *Bti* in water or sediments, nor have the ability to activate the toxin by ingestion.

If a glochidium is ingested by a host, it will become encysted in its gills, and will have no exposure to surface water or sediments. Once encysted, glochidia are parasitic to the host, feeding only on host tissues enclosed between the glochidial shells (chiefly epidermis, dermis, and connective tissue) and their own larval adductor muscle (Blystad 1923, Arey 1932, Watters 2005). Exposure to *Bti* is highly unlikely through host tissues, as any *Bti* incidentally ingested by the vertebrate host will be exposed to a much more acidic gut environment than necessary for toxin activation, and will be unlikely to survive this ingestion.

Juvenile and Adult Mussels: No direct effects of *Bti* toxicity have been found for aquatic invertebrates other than black flies, mosquitoes, and midges. For mollusks, no adverse effects were detected in 15 different species including mussels, oysters, and snails exposed to *Bti* (Glare and O'Callaghan 1998). A study of the freshwater Unionid mussel *Obliquaria reflexa* found no mortality or signs of external stress from *Bti* exposure up to 200 times the suggested application rate (Waller 1992). The author reported that these results agree with an earlier study in which no effects were found to the freshwater mussel *Anodonta imbecilis* exposed to twice the rate of *Bti* applied to Tennessee Valley Authority reservoirs. Another study found no effects to mussels (Pelecypoda *sp.*) or freshwater snails (*Physa sp.*) exposed to concentrations of *Bti* over 100 times the lethal dose for mosquitoes (Garcia et al. 1980). In a field study of ponds occurring within a natural stream system, no change was detected in the average number of gastropods between treated and untreated pools (Dickman 2000).

Though stomach and intestinal tract pH are available for only a subset of aquatic species, investigations of several mollusks have found intestinal pH levels to average between 6 and 8 (Yonoe 1925, Barlocher et al. 1989, Areekijseree et al. 2004, Greenfield 2009), well below the range of species known to activate the *Bti* toxin. The typical pH in the gut of a black fly or mosquito ranges from 10-12, an unusually alkaline environment for the activation of *Bti* that is unlikely to exist within other species.

Potential for indirect effects to mussels:

Indirect effects to mussels following *Bti* use for suppression of black flies have not been evaluated in the field.

Potential effects to mussel species of concern:

No direct toxic effects are anticipated to mussels from *Bti* use. Indirect effects may be possible if mussels rely upon *S. annulus* and *S. johannseni* as a significant source of food, or if the suppression of these black flies would reduce other prey species.

The adult salamander mussel is a filter feeder, obtaining nutrition from material suspended in the water column that is pumped in through its siphon. Types of food derived in this manner include algae, bacteria, protozoans, and other organic particles. Glochidial salamander mussels absorb blood and nutrients from the tissue of its host salamander after attachment. As no direct toxic effects have been detected for mussels, and neither adult or glochidia rely upon black fly larvae or adults for food, no indirect effects to the salamander mussel are expected to occur from suppression of black flies with *Bti*.

Fish

Species of concern that may be present in and around treatment areas:

- Pirate Perch (*Aphredoderus sayanus*) – Special Concern, Yellow River
- Weed Shiner (*Notropis texanus*) – Special Concern, Yellow River

Potential for direct effects to fish:

In toxicity studies performed by the registrant, *Bti* was not toxic or pathogenic to either freshwater (trout and bluegills) or estuarine (sheepshead minnow) fish species (EPA 1998). In addition, no effects on behavior and reproduction were observed in resident fish in field studies of *B. thuringiensis* delta-endotoxin contaminated water (EPA 1998). Exposure to *Bti* at 10 times the effective field concentration had no effect on swimming performance of crimson-spotted rainbowfish (*Melanotaenia duboulayi*) (Hurst et al. 2007).

Of teleost fish possessing a true stomach, pH generally ranges from 1-4, and from 6.5 to 9 in the intestine (Kleinow et al. 2008). The typical pH in the gut of a black fly or mosquito ranges from 10-12, an unusually alkaline environment for the activation of *Bti* that is unlikely to exist within fish species.

Potential for indirect effects to fish:

Several studies have evaluated Pennsylvania's long-term use of *Bti* for black fly control and found no effects to fish. Comparison before and after *Bti* treatment of the North Branch of the Susquehanna River found that the fish community was not affected by repeated treatments, despite a high proportion of the diet comprising black flies and midges for darter species in the area (Brancato 1996). Gut analysis found that darters feed on the most abundant and easily accessible aquatic invertebrate in the drift, and likely exhibit switching behavior between prey items as needed. Following application of the Vectobac® 12AS *Bti* formulation for black fly control at 6 locations on the Susquehanna River, fish species composition and abundance did not change, despite the fact that black flies were an important source of food for some of the fish species present (Jackson et al. 2002). Similar results were found in Betsie River, Michigan, where no effects to mortality or weight change of caged rock bass; or fish numbers, species composition, length-weight relationships or rock bass diet were detected following application of *Bti* (Teknar® HP-D) for black fly control (Merritt et al. 1989).

Potential effects to fish species of concern:

Direct effects are not expected from potential *Bti* exposure to any fish species of concern. Indirect effects may still be possible if fish species rely upon *S. annulus* and *S. johannseni* as a significant source of food, or if the suppression of these black flies would reduce other prey species.

<u>Pirate perch</u> are insectivores/carnivores that feed on aquatic insects, small crustacea, and occasional small fish including their own young. The use of *S. annulus* or *S. johannseni* as food sources is unknown. Contents of stomachs from fish collected in Wisconsin included algae, ostracods, Hyallela, ants, and fish scales (Becker 1983). Aquatic Diptera larvae have been reported from specimens captured in other locations. Percentage of diet for each type of prey species varies by location (Hassan-Williams and Bonner 2010), indicating their ability to adapt according to prey availability. The brief emergence by adults of both black fly species during early spring is an evolutionary strategy that makes buildup of corresponding numbers of predators dependent on them as a potential food source unlikely. There are also alternative sources of insect food available from untreated streams and other wetland types. Additionally, *Bti* use would have no impact on black fly larvae and resulting adults that emerge later in the season. For these reasons, indirect effects to pirate perch are not anticipated due to their ability to exploit other available food sources.

Weed shiners are primarily detritivores, exhibiting feeding behavior in which accumulated detritus is scraped from macrophyte leaves. Animal prey, including small invertebrates, has been reported as elements of the diet, and the percent contribution can vary by season (Hassan-Williams and Bonner 2010). Contents of stomachs from fish collected during one study in Wisconsin consisted primarily of plant debris, with animal material present but unidentifiable (Becker 1983). As black fly larvae are not believed to be a major portion of their diet, indirect effects to weed shiners are not anticipated due to their ability to exploit other available food sources. In addition, as emergence of *S. annulus* and *S. johannseni* generally occur before spawning of weed shiners in Wisconsin (late June to July), larvae of this species is not believed to be a food sources during this lifestage.

Birds

Species of concern that may be present in and around treatment areas:

- Cerulean Warbler (*Dendroica cerulea*) – Threatened, Lemonweir River
- Red-shouldered Hawk (*Buteo lineatus*) – Threatened, Lemonweir River
- Yellow-crowned night-heron (*Nyctanassa violecea)* - Threatened
- Trumpeter Swan (*Cygnus buccinators*) – Special Concern
- Bald Eagle (*Haliaeetus leucocephalus*) – Special Concern, Lemonweir River
- Osprey (*Pandion haliaetus*) – Special Concern, Beaver Creek

Potential for direct effects to birds:

Direct effects are not expected from potential *Bti* exposure to birds. *Bti* was not toxic or pathogenic to mallards and bobwhite fed *Bti* at either 3.1 g/kg/day or 5 ml/kg/day for 5 days (EPA 1998).

Based on observations of chickens, pigeons, pheasant, duck, and turkey, pH values in avian digestive tracks ranged from lows of 1.4 to 4.8 in the stomach (proventriculus and gizzard) to highs of 5.6 to 7.2 in the ileum (Denbow 1999). The typical pH in the gut of a black fly or mosquito ranges from 10-12, an unusually alkaline environment for the activation of *Bti* that is unlikely to exist within avian species.

Potential for indirect effects to birds:

While specific studies of indirect effects to birds following black fly control with *Bti* have not been conducted, a limited number of studies have looked at effects following mosquito control. A series of investigations occurred in Minnesota wetlands. Hanowski et al. (1997a) and Niemi et al. (1999) found no evidence that 3 years of *Bti* treatments (applied as Vectobac® G granules) for mosquito control in surrounding wetlands in Minnesota had any negative impacts to reproduction, growth of nestlings, or foraging behavior of adult breeding red-winged blackbirds. This was in spite of reductions in insect density and richness in one of the studies (Niemi et al. 1999). Hanowski et al. (1997b) censused 19 species of birds before, during, and after 3 years of *Bti* treatment for mosquito control in Minnesota wetlands and found no evidence that breading bird communities or individual species were affected by the treatment. In the Camargue, France, reduced breeding success of house martins, (as measured by clutch size and fledgling survival) was correlated with the intake of *Bti*-sensitive Nematocera and their predators at the nest level (Poulin et al. 2010). Nematocera are a significant food source for house martins in this region, accounting for approximately 35% of the diet of birds on control sites.

Potential effects to avian species of concern:

No direct toxic effects are anticipated to any avian species of concern from *Bti* use. Indirect effects may be possible if avian species rely upon *S. annulus* and *S. johannseni* as a significant source of food, or if the suppression of these black flies would reduce other important prey species.

Cerulean warblers eat insects, especially homopterans and larval lepidopterans during breeding season. Food is taken from leaf bases and foliage by gleaning, hover-gleaning, sallying, and other techniques (Hamel 2000). This species has also been reported to eat small amounts of plant mass during migration (Hamel 2000). A study of individual stomach contents indicated large percentages of Hymenoptera (42%), Coleoptera, including weevils (23%), and Lepidoptera (35%) from individuals collected in Alabama (Hamel 2000). A study in West Virginia found Homoptera (52% of total bio-mass of gut contents); Lepidoptera, primarily larvae (37%); Coleoptera (7%); with small amounts of Hymenoptera, Diptera, Hemiptera, Araneae, and other arthropods (Sample et al. 1993). It is possible that cerulean warblers in Wisconsin rivers forage on black flies as a portion of their diet. However, the brief emergence by adults of *S. annulus* or *S. johannseni* during early spring is an evolutionary strategy that makes buildup of corresponding numbers of predators dependent on them as a potential food source unlikely. Additionally, dipterans have never been recorded as a large percentage of their diet, and studies indicate that this species tends to take a variety of insects and would be able to temporarily adapt to other food sources if *S. annulus* and *S. johannseni* were suppressed. Because of their lack of dependence on black flies as a food source, no indirect effects are anticipated for cerulean warblers.

Red-shouldered hawks are predatory species that typically consume small mammals, reptiles, and amphibians. Less often they will take prey opportunistically, such as carrion, grasshoppers during outbreaks, and birds at feeders during winter (Dykstra et al. 2008). Percentage of diet for each type of prey species can vary largely both geographically and temporally (Dykstra et al. 2008). As *Bti* is unlikely to reduce populations of these prey species, adverse effects to red-shouldered hawks are not anticipated to occur.

Osprey are piscivorous, with live fish accounting for 99% of prey taken in almost every published account of food habits (Poole et al. 2002). Some inland populations have been observed to feed on the same species throughout the breeding season, with prey switching recorded according to food availability (Poole et al. 2002). A wide variety of fish can represent the main species taken in local populations. As osprey do not feed on target dipteran species, and fish are not expected to experience declines resulting from black fly suppression, indirect effects to this species are not anticipated to occur.

The yellow-crowned night-heron is a crustacean specialist throughout its range, feeding almost exclusively on crayfish in inland areas. A diversity of other prey constitute minor parts of the diet overall, but may comprise major components in isolated areas or for individuals (Watts 1995). Other prey species may include mussels, frogs, aquatic insects, snails, small snakes, and leeches. As aquatic insects are a minor part of the night-heron's diet, suppression of *S. annulus* and *S. johannseni* is not anticipated to cause indirect effects to this species.

Trumpeter swans are herbivores, taking invertebrates only incidentally. Aquatic insects are a larger part of the diet of cygnets, which utilize this source of protein from ages 2-5 weeks (Mitchell and Eichholz 2010). Types of invertebrates consumed are diverse and include Amphipoda, Arthropoda, Chironomidae, Dytisidae, Ephemoptera, Mollusca, Oligochaeta, and Trichoptera (Mitchell and Eichholz 2010). It is possible that cygnets in Wisconsin rivers forage on black flies as a portion of their diet. However, studies indicate that this species tends to take a variety of insects and would be able to temporarily adapt to other food sources if *S. annulus* and *S. johannseni* were suppressed. In addition, cygnets do not hatch and begin feeding until June, past the typical date for *S. annulus* emergence, making it unlikely that this species is an integral part of their diet. Indirect effects to trumpeter swans are not anticipated.

Bald eagles are opportunistic foragers. Food items vary greatly across range, based on local availability (Buehler 2000). In general, bald eagles have a preference for fish, and often use birds and mammals as carrion. Other species taken include various terrestrial and aquatic vertebrates as well as crustaceans. As bald eagles are generalists and *Bti* is unlikely to reduce populations of prey species, indirect effects to this species are not anticipated to occur.

Mammals

Species of concern that may be present in and around treatment areas:

- Gray Wolf (*Canis lupus*) – Federally Endangered, Lemonweir River

Potential for direct effects to mammals:

To date, no known mammalian health effects have been demonstrated for any strain of *Bacillus thuringiensis*, including *Bti* (EPA 1998).

Potential for indirect effects to mammals:

Indirect effects to mammals following *Bti* use for suppression of black flies have not been evaluated in the field.

Potential effects to mammalian species of concern:

No direct toxic effects are anticipated to any mammalian species of concern from *Bti* use. Indirect effects may be possible if mammals rely upon *S. annulus* and *S. johannseni* as a significant source of food, or if the suppression of these black flies would reduce other prey species.

Gray wolves are terrestrial, so direct exposure to this species or its prey is likely to be minimal. Gray wolves are carnivores. As reported by the Wisconsin Department of Natural Resources on its website, a study in the early 1980's showed that the diet of Wisconsin wolves was comprised of 55% white-tailed deer, 16% beavers, 10% snowshoe hares and 19% mice, squirrels, muskrats and other small mammals. As *Bti* is unlikely to reduce populations of these prey species, indirect effects to gray wolves are not anticipated to occur.

Reptiles

Species of concern that may be present in and around treatment areas:

- Slender Glass Lizard (*Ophisaurus attenuatus*) – Endangered, Lemonweir River
- Eastern Massasauga (*Sistrurus catenatus catenatus*) – Endangered, Yellow River
- Blanding's Turtle (*Emydoidea blandingii*) – Threatened, Lemonweir River
- Wood Turtle (*Glyptemys insculpta*) – Threatened, Beaver Creek

Potential for direct effects to reptiles:

While toxicity studies to reptiles have not been performed, direct effects are not expected from potential exposure to *Bti* as no direct toxic effects have been observed to any other vertebrate taxa (EPA 1998).

Potential for indirect effects to reptiles:

Indirect effects to reptiles following *Bti* use for suppression of black flies have not been evaluated in the field.

Potential effects to reptilian species of concern:

No direct toxic effects are anticipated to any reptilian species of concern from *Bti* use. Indirect effects may be possible if reptiles rely upon *S. annulus* and *S. johannseni* as a significant source of food, or if the suppression of these black flies would reduce other prey species.

The Slender Glass Lizard is terrestrial and exposure to *Bti* for this species or its prey is expected to be minimal. Its main food source is beetles, but crickets, grasshoppers, snails, slugs, spiders, small mice, worms, and eggs of reptiles and ground-nesting birds are alternate food sources. The lizard emerges from hibernation in Wisconsin in May and has been described as a non-selective forager. As slender glass lizards eat a variety of prey and black flies are not believed to be a major portion of their diet, indirect effects are not anticipated for this species due to their ability to exploit other available food sources.

The Eastern Massasauga is a member of the "pit viper" family that preferable preys upon warm-blooded prey like mice and voles, other small rodents and occasionally birds. They will also take cold-blooded prey, such as frogs, lizards and other snakes. As *Bti* is unlikely to reduce populations of these prey species, indirect effects to eastern massasauga are not anticipated to occur.

Blanding's Turtle is an omnivore, eating crayfish, snails, tadpoles, fish, insects, worms, grasses, and berries. In a study of Northern Illinois Blanding's turtles, major components of stomach contents included Mollusca (35%), Decapoda (19.3%), Annelida (12.7%), Insecta (10.3%), Vertebrata (5.9%) and plant material (12.3%) (Rowe 1992). Dipteran species accounted for only 1.7% of stomach contents and occurred in samples 18.2% of the time. As Blanding's turtles are generalist feeders and black flies are not believed to be a major portion of their diet, indirect effects are not anticipated for this species due to their ability to exploit other available food sources.

Wood Turtles are generalists, feeding upon insects, mollusks, carrion, worms, berries, dandelions, grasses, mullen, sorrel, sedges, filamentous algae, and mushrooms and other succulent herbs. Wood turtles spend significant time in forested habitats adjacent to rivers and streams, where they feed on terrestrial plants and invertebrates. As wood turtles eat a variety of prey and black flies are not believed to be a major portion of their diet, indirect effects are not anticipated for this species due to their ability to exploit other available food sources.

Plants

Species of concern that may be present in and around treatment areas:

- Arrowhead Rattlebox (*Crotalaria sagittalis*) – Special Concern, Lemonweir River
- Cross-Leaved Milwort (*Polygala cruciata*) – Special Concern, Beaver Creek
- Eastern Straw Sedge (*Carex straminea*) – Special Concern, Lemonweir River
- Farwell's Milfoil (*Myriophyllum farwellii*) – Special Concern, Beaver Creek
- Roundstem False Foxglove (*Agalinis gattingeri*) – Threatened, Lemonweir River
- Fringed Sagebrush (*Artemisia frigida*) – Special Concern, Lemonweir River
- Maidenhair Spleenwort (*Asplenium trichomanes*) – Special Concern, Lemonweir River
- Virginia Meadow Beauty (*Rhexia virginica*) – Special Concern, Beaver Creek
- Northern Long Sedge (*Carex folliculate*) – Special Concern, Lemonweir River
- Shadowy Goldenrod (*Solidago sciaphila*) – Special Concern, Lemonweir River
- Wooly Milkweed (*Asclepias lanuginosa*) –Threatened, Lemonweir River

Potential for direct effects to plants:

As *Bti* must be ingested and activated to have a toxic effect, there is no clear *Bti* exposure pathway for plants as they have no mechanism for ingestion. In its Reregistration Eligibility Decision for *Bacillus thuringiensis*, EPA was unable to find any reports of adverse effects to plants despite its extensive use on vegetation (EPA 1998). Therefore, it is not anticipated that *Bti* use will result in any adverse effects to plants.

Potential for indirect effects to plants:

Indirect effects may be possible if plants rely upon *S. annulus* or *S. johannseni* as obligate pollinators. Black flies are not known to be obligate pollinators of plants in the treatment area. No indirect effects of *Bti* use are anticipated for these species.

Conclusions

Suppression of black flies via application of *Bti* to waterways surrounding whooping crane nests in Necedah NWR is likely to provide valuable information regarding the role of these pest species in nest desertion and failure. This information may be critical to the recovery of this of this critically endangered species. Studies of field use of *Bti* for black fly suppression consistently result in findings of no adverse effects to nontarget organisms. As such, we anticipate no adverse effects to birds, mammals, reptiles, fish, mussels, and other invertebrate species, including those of special concern. However, minor effects to susceptible nontarget dipterans such as certain chironomid midges and predatory insects that use these species as a significant food source cannot be ruled out. Due to the experimental nature of the study proposed herein, limiting treatment to two years, any effects are expected to be short-term. Data regarding the sensitivity of chironomids to *Bti* is varied, and where reductions are seen, it is at levels lower than target dipterans. Effects to predatory insects are only anticipated if targeted black flies make up a significant and necessary portion of the diet. We deem these food chain effects unlikely due to the brief emergence by adults of both species during early spring, alternative sources of insect food available from untreated streams and other wetland types, and availability of black fly larvae and resulting adults that emerge later in the season.

Literature Cited:

Abbott Laboratories. 1999. Aqueous formulation: VectoBac®. Technical Bulletin. Abbott Laboratories, Agricultural Products, North Chicago, Illinois. 8 pp.

Adler, P.H., D.C. Currie, and D.M. Wood. 2004. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca, NY. xv + 941 pp. + 24 color plates.

Adler, P.H. 2009. Biting Flies of Necedah National Wildlife Refuge, Wisconsin, with Reference to Whooping Cranes. Report to the Whooping Crane Eastern Partnership, September 14, 2009.

Areekijseree M., A. Engkagul, U. Kovitvadhi, A. Thongpan, M. Mingmuang, P. Pakkong, and K. Rungruangsak- Torrissen. 2004. Temperature and pH characteristics of amylase and proteinases of adult freshwater pearl mussel, Hyriopsis (Hyriopsis) bialatus Simpson 1900. Aquaculture 234:575–587

Arey, L.B. 1932. The formation and structure of the glochidial cyst. Biol. Bull. 62:212-221.

Back, C., J. Boisvert, J.O. Lacoursière, and G. Charpentier. 1985. High-dosage treatment of a Quebec stream with *Bacillus thuringiensis* serovar. *israelensis*: efficacy against black fly larvae (Diptera: Simuliidae) and impact on non-target insects. Canadian Entomologist 117:1523-1534.

Balcer, M.D., K.L. Schmude, and J. Snitgen. 1999. Long-term effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macro-invertebrates in wetlands in Wright County, Minnesota (1997-1998) Report submitted to Metropolitan Mosquito Control District February 4, 1999.

Barlocher, F., T.L. Arsuffi, and S.Y. Newell. 1989. Digestive enzymes of the saltmarsh periwinkle *Littorina irrorata* (Mollusca: Gastropoda). Oecologia 80:39-43.

Becker, G.C. 1983. *Fishes of Wisconsin*. Madison, Wisconsin: University of Wisconsin Press, 1052 pp. Accessed online at http://digital.library.wisc.edu/1711.dl/EcoNatRes.FishesWI.

Bennett, G.F., and A.M. Fallis. 1971. Flight range, longevity, and habitat preference of female Simulium euryadminiculum Davies (Diptera: Simuliidae). Canadian J. Zool. 49:1203-1207.

Blystad, C.N. 1923. Significance of larval mantle of freshwater mussels during parasitism, with notes on a new mantle condition exhibited by *Lampsilus luteola*. Bull. U.S. Bur. Fish. 39:203-219.

Brancato, J.C. 1996. Environmental impact of Pennsylvania black fly control programs on fish and aquatic invertebrates. Proceedings of the 83rd Annual Meeting of the New Jersey Mosquito Control Association 83:64-71.

Buehler, D.A. 2000. Bald Eagle (*Haliaeetus leucocephalus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/506

Bukacinski, D., and M. Bukacinska. 2000. The impact of mass outbreaks of black flies (Simuliidae) on the parental behaviour and breeding output of colonial common gulls (*Larus canus*). Annales Zoologici Fennici 37: 43-49.

Burton, D.K. 1984. Impact of *Bacillus thuringiensis* var. *israelensis* in dosages used for black fly (Simuliidae) control, against target and non-target organisms in the Torch River, Saskatchewan. M.S. thesis. University of Manitoba, Winnipeg, Manitoba. 129 pp.

Canadian Wildlife Service and U.S. Fish and Wildlife Service. 2007. International recovery plan for the whooping crane. Ottawa: Recovery of Nationally Endangered Wildlife (RENEW), and U.S. Fish and Wildlife Service, Albuquerque, NM. 162 p.

Carlstead, K. 1996. Effects of captivity on the behavior of wild mammals. In: D.G. Kleiman, M.E. Allen, K.V. Thompson and S. Lumpkin, eds., Wild Mammals in Captivity: Principles and Techniques, University of Chicago Press, Chicago, pp. 317–333.

Case, T.J. 1978. Adaptive significance of midgut pH in larval Lepidoptera. American Naturalist 115:138-146.

Charbonneau, C.S., R.D. Drobney, and C.F. Rabeni. 1994. effects of *Bacillus thuringiensis* var *israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. Env. Toxicol. Chem. 13:267-279.

Colbo, M.H., and A.H. Undeen. 1980. Effect of *Bacillus thuringiensis* var. *israelensis* on non-target insects in stream trials for control of Simuliidae. Mosquito News 40: 368-371.

Darwin, C.R. 1868. The Variation of Animals and Plants under Domestication. Johns Hopkins University Press, Baltimore, MD.

Denbow, D.M. 1999. Gastrointestinal anatomy and physiology. In C. Whittow, ed., Sturkie's Avian Physiology (5th Ed.), Academic Press, New York, pp. 299-326.

Dickman, M. 2000. Impacts of a mosquito selective pesticide, *Bti*, on the macroinvertebrates of a subtropical stream in Hong Kong. Chemosphere 41:209-217.

Duckitt, G.S. 1986. *Bacillus thuringiensis* var. *israelensis*: the bacterium, its use in black fly control and effects on nontarget organisms. Master of Pest Management Professional Paper. Simon Fraser University, Burnaby, British Columbia. 72 pp.

Dykstra, C.R., J.L. Hays, and S.T. Crocoll. 2008. Red-shouldered Hawk (*Buteo lineatus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/107

EPA (Environmental Protection Agency). 1998. Reregistration Eligibility Decision for *Bacillus thuringiensis*. EPA738-R-98-004. Office of Prevention, Pesticides, and Toxic Substances. March 1998.

Garcia, R., B.D. Rochers, W. Tozer, and B. Des Rochers. 1980. Studies on the toxicity of *Bacillus thuringiensis* var. *israelensis* against organisms found in association with mosquito larvae. In C.D. Grant, ed., *Proceedings and papers of the Forty eighth Annual Conference of the California Mosquito and Vector Control Association, Inc.* Anaheim, California. pp. 33-36.

Gibbs, K.E., F.C. Brautigam, C.S. Stubbs, and L.M. Zibilske. 1986. Experimental applications of B.t.i. for larval black fly control: persistence and downstream carry, efficacy, impact on non-target invertebrates and fish feeding. Maine Agricultural Experiment Station Technical Bulletin 123:1-25.

Glare, T.R., and M. O' Callaghan. 1998. Environmental and health impacts of *Bacillus thuringiensis israelensis*. Report to the Ministry of Health, Wellington, NZ.

Grayson, J. 1958. Digestive tract pH of six species of Coleoptera. Annals of the Entomological Society of America 51:403-405.

Greenfield, M.L. 2009. Feeding and gut physiology in *Acanthopleura spinigera* (Mollusca). J. Zool. 166:37-47.

Hamel, P.B. 2000. Cerulean Warbler (*Dendroica cerulea*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/511

Hanowski, J.M., G.J. Niemi, A.R. Lima, and R.R. Regal. 1997b. Response of breeding birds to mosquito control treatments of wetlands. Wetlands 17:485-492.

Hanowski, J.M., G.J Niemi, A.R. Lima, and R.R. Regal. 1997a. Do mosquito control treatments of wetlands affect red-winged blackbirds (*Agelaius phoeniceus*) growth, reproduction, or behavior? Env. Toxicol. Chem. 16:1014-1019.

Hassan-Williams, C., and T.H. Bonner. 2010. Texas freshwater fishes. Texas State University-San Marcos, Biology Department / Aquatic Station. http://www.bio.txstate.edu/~tbonner/txfishes/index.htm

Hershey, A.E., L. Shannon, R. Axler, C. Ernst, and P. Mickelson 1995. Effects of methoprene and *Bti* (*Bacillus thuringiensis* var. *israelensis*) on non-target insects. Hydrobiologia 308:219-227.

Hershey, A.E., A.R. Lima, G.J. Niemi, and R.R. Regal. 1998. Effects of *Bacillus thuringiensis israelensis* (*Bti*) on nontarget macroinvertebrates in Minnesota wetlands. Ecol. Appl. 8:41-60.

Hurst, T.P., B.H. Kay, P.A. Ryan, and M.D. Brown. 2007. Sublethal effects of mosquito larvicides on swimming performance of larvivorous fish *Melanotaenia duboulayi* (Atheriniformes: Melanotaeniidae). J. Econ. Entomol. 100:61-65.

Jackson, J.K., B.W. Sweeney, T.L. Bott, J.D. Newbold, and L.A. Kaplan. 1994. Transport of *Bacillus thuringiensis* var. *israelensis* and its effect on drift and benthic densities of nontarget macroinvertebrates in the Susquehanna River, northern Pennsylvania. Can. J. Fish. Aquat. Sci. 51:295-314.

Jackson, J.K., R.J. Horwitz, and B.W. Sweeney. 2002. Effects of *Bacillus thuringiensis israelensis* on black flies and nontarget macroinvertebrates and fish in a large river. Trans. Amer. Fish. Soc. 131:910-930.

Jansen W, G. Bauer, and E. Zahner-Meike. 2001. Glochidial mortality in freshwater mussels. In G, Bauer and K. Wachtler, eds., Ecology and Evolution of the Freshwater Mussels Unionoida. Springer-Verlag, Berlin. pp 185–211.

Joose, E., and H.A. Verhoef. 1987. Developments in ecophysiological research on soil invertebrates. Adv. Ecol. Res. 16: 175-248

Kleinow, K.M., J.W. Nichols, W.L. Hayton, J.M. McKim, and M.G. Barron. 2008. Toxicokinetics in fish. In R.T. Di Guilo and D.E. Hinton, eds., The Toxicology of Fishes. CRC Press, Boca Raton, FL. Pp 55-152.

Kreger, M.D., I. Estevez, J.S. Hatfield, and G.F. Gee. 2004. Effects of rearing treatment on the behavior of captive whooping cranes (*Grus americana*). Applied Animal Behaviour Science 89:243-261.

Kreger, M.D., J.S. Hatfield, I. Estevez, G.F. Gee, and D.A. Clugston. 2005. The effects of captive rearing on the behavior of newly-released whooping cranes (*Grus americana*). Applied Animal Behaviour Science 93:165-178.

Lacey, L. A., and M.S. Mulla. 1990. Safety of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* to nontarget organisms in the aquatic environment. In M. Laird, L.A. Lacey, and E.W.S.O., eds, Safety of Microbial Insecticides. 259 pp. CRC Press Inc.; Boca Raton; USA.

Liber, K., K.L. Schmude, and D.M. Rau. 1998. Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms. Ecotoxicology 7:343-354.

Lickliter, R., and J.W. Ness. 1990. Domestication and comparative psychology: status and strategy. J. Compar. Psychol. 104:211–218.

McIntyre, J. 1988. The common loon. Sprit of northern lakes. University of Minnesota Press, Minneapolis, MN, USA.

Merritt, R.W., E.D. Walker, M.A. Wilzbach, K.W. Cummins, and W.T. Morgan. 1989. A broad evaluation of B.t.i. for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. Journal of the American Mosquito Control Association 5: 397-415.

Mitchell, C.D., and M.W. Eichholz. 2010. Trumpeter Swan (*Cygnus buccinator*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/105

Molloy, D., ed. 1982. Biological control of black flies (Diptera: Simuliidae) with *Bacillus thuringiensis* var. *israelensis* (serotype 14): a review with recommendations for laboratory and field protocol. Miscellaneous Publications of the Entomological Society of America 12:1-30.

Molloy, D., and H. Jamnback. 1981. Field evaluation of *Bacillus thuringiensis* var. *israelensis* as a black fly biocontrol agent and its effect on nontarget stream insects. J. Econ. Entomol. 74:314-318.

Niemi, G.J., A.E. Hershey, L. Shannon, J.M. Hanowski, A. Lima, R.P. Axler, and R.R. Regal. 1999. Ecological effects of mosquito control on zooplankton, insects, and birds. Env. Toxicol. Chem 18:549-559.

Pennsylvania's Black Fly Suppression Program. 2008. Department of Environmental Protection, PA. http://www.depweb.state.pa.us/blackfly/cwp/view.asp?a=3&Q=505536 [Accessed 26 October 2008].

Pistrang, L.A., and J.F. Burger. 1984. Effect of *Bacillus thuringiensis* var. *israelensis* on a genetically-defined population of black flies (Diptera: Simuliidae) and associated insects in a montane New Hampshire stream. Canadian Entomologist 116:975-981.

Poulin, B., G. Lefebvre, and L. Paz. 2010. Red flag for green spray: adverse trophic effects of *Bti* on breeding birds. J. Appl. Ecol. 47:884-889.

Price, E.O. 1984. Behavioral aspects of animal domestication. Quarterly Review of Biology 59:1–32.

Price, E.O. 1998. Behavioral genetics and the process of animal domestication. In: T. Grandin, ed., Genetics and the Behavior of Domestic Animals, Academic Press, New York, pp. 31–65.

Rasmussen, C.O. 2008. Black flies drive off nesting loons. Mazina'igan Fall 2008:5. Great Lakes Indian Fish & Wildlife Commission, Odanah, WI.

Read, N. 2002. Long-term study of nontarget effects of mosquito larvicides. Status Report of the Metropolitan Mosquito Control District February, 2002.

Rowe, J.W. 1992. Dietary habits of the Blanding's Turtle (*Emydoidea blandingi*) in Northeastern Illinois. J. Herpetol. 26:111-114.

Sample, B. E., R. J. Cooper, and R. C. Whitmore. 1993. Dietary shifts among songbirds from a diflubenzuron-treated forest. Condor 95:616-624.

Saxena, P. 1978. Digestive physiology of *Bradynopyga geminata* (Odonata: Libellulidae) Cellular and Molecular Life Sciences 34:1579-1580.

Smith, R.N., S.L. Cain, S.H. Anderson, J.R. Dunk, and E.S. Williams. 1998. Blackfly-induced mortality of nestling red-tailed hawks. Auk 115:368-375.

Urbanek, R.P., and T.A. Bookhout. 1992. Nesting of greater sandhill cranes on Seney National Wildlife Refuge. In D.A. Wood, ed., Proceedings of the 1988 North American Crane Workshop. State of Florida Game and Fresh Water Fish Commission Nongame Wildlife Program Technical Report #12. Pp 161-172.

Waller, D.L. 1992. Evaluation of the toxicity of *Bti* (*Bacillus thuringiensis* var. *israelensis*) to the bivalve mollusk *Obliquaria reflexa*. Report to the Metropolitan Mosquito Control District, St. Paul, Minnesota, June 23, 1992.

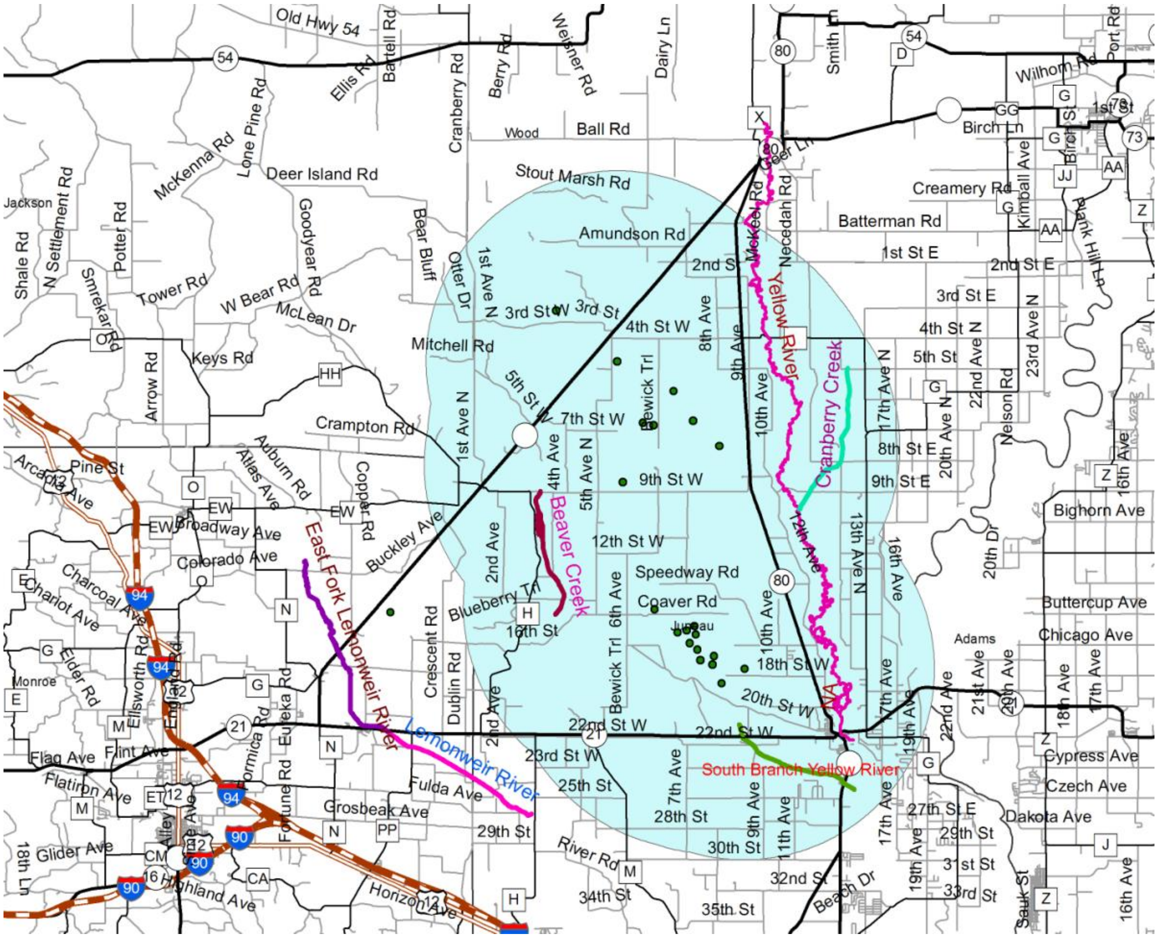
Watters, G.T. 2006. A brief look at freshwater mussel biology. In J.L. Farris and J.H. Van Hassel, eds., *Freshwater Bivalve Ecotoxicology*. CRC Press, Boca Raton, FL. pp. 51-64.

Watts, B.D. 1995. Yellow-crowned Night-Heron (*Nyctanassa violacea*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/161

Yonoe, C. M. 1925. The hydrogen ion concentration in the gut of certain lamellibranchs and gastropods**.** J. Mar. Biol. Ass. U.K. 13:938-952.

Attachment A

Map of *S. annulus* and *S. johannseni* breeding sites and potential areas of treatment.



Attachment B

Results of macroinverebrate sampling and analysis of community indicators by the University of Wisconsin – Stevens Point

Key to Sample #'s:

20100405-29-01 = pre-application upstream
20100406-29-01 = post-application upstream
20100405-29-02 = pre-application downstream
20100406-29-02 = post-application downstream

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***
Sample: 20100405-29-01 (yyyymmdd-Co-Field#) Reference
Site: NO
Waterbody: YELLOW RIVER Master Waterbody #: 1352800
Project: USFWS YELLOW RIVER Bti APPLI Storet Station:
Collector: GRAY, ELMER Time Sampling: #
Reps: 1
Sampling Device:
Taxonomist: DIMICK, JEFFREY
Sorter: WANGBERG, LYNN % Sample Sorted: 7
SWIMS ID: Name: Key:
0
==

Location-Legal: S 0 T 0N R 0 County: JUNEAU
Lat/Long: N44deg 12min 54.8sec W 90deg 7min 16.9sec
Method:
Description: PRETREATMENT ABOVE Bti
4TH ST. BRIDGE, 150 M ABOVE

==

	HBI	Width ft	Depth ft	Temp øC	DO mg/l	Measured Vel fps	Est Velocity fps
Habitat							
General							
Rep #1	5.520						
Rep #2	0.000						
Rep #3	0.000						
Rep #4	0.000						
Rep #5	0.000						

Aquatic Vegetation 0 % of Total Stream Channel at Sampling Site
==

Substrate at Rep Location %:

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Substrate Sampled %: (Same as Above NO)

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

==

NP=Not Present Pollutant Sources:

Water Quality Indicators:		I=Insignificant		Livestock Pasturing	*
Turbidity	*	S=Significant		Barnyard Runoff	*
Chlorine/Toxic Scour	*			Cropland Runoff	*
Macrophytes	*	Factors Affecting Habitat:		Tile Drains	*
Filamentous Algae	*	Sludge Deposits	*	Septic Systems	*
Planktonic Algae	*	Silt and Sediment	*	Streambank Erosion	*
Slimes	*	Channel Ditching	*	Urban Runoff	*
Iron Bacteria	*	Impoundments	*	Construction Runoff	*
Perceived Water Quality		Low Flows	*	Point Source (Spec)	*
at Site:		Wetlands	*	Other (Specify)	*
Comments:		Comments:		Comments:	

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

SAMPLE ID# 20100405-29-01

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
PLECOPTERA							
NEMOURIDAE							
SHIPSA							
ROTUNDA		*1	2.00	01040401	1	0	0
PERLODIDAE							
ISOPERLA							
UNIDENTIFIED		*1		01060400	1	0	0
PTERONARCYIDAE							
PTERONARCYS							
UNIDENTIFIED		*1	0.00	01070100	1	0	0
COLEOPTERA							
ELMIDAE							
MACRONYCHUS							
GLABRATUS		*2	4.00	07020301	1	0	0
DIPTERA							
SIMULIIDAE							
SIMULIUM							
UNIDENTIFIED (LARVAE, IMMATURE)		*3		08110200	88	0	0
ANATINUM		*3		08110230	7	0	0
EMARGINATUM		*3		08110233	32	0	0
LONGISTYLATUM		*3		08110243	1	0	0
PUPAE (S. EMARGINATUM)		*4		08110245	9	0	0
TANYPODINAE							
CONCHAPELOPIA							
UNIDENTIFIED		*5	6.00	08270700	1	0	0
ORTHOCLADIINAE							
RHEOCRICOTOPUS							
ROBACKI		*5	6.00	08304010	3	0	0
ORTHOCLADIUS (ORTHOCLADIUS)							
OLIVERI		*5	6.00	08305811	1	0	0
CRICOTOPUS (CRICOTOPUS)							
BICINCTUS		*5	6.00	08307503	1	0	0
CHIRONOMINAE							
MICROTENDIPES							
RYDALENSIS GROUP (PINDER, REISS 1983)		*5	6.00	08333703	2	0	0
RHEOTANYTARSUS							
UNIDENTIFIED		*5	6.00	08335600	11	0	0
TANYTARSUS							
UNIDENTIFIED		*5	6.00	08336700	3	0	0

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

SAMPLE ID# 20100405-29-01

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
	*** TOTALS: ***				163	0	0
	*** BIOTIC INDEX: ***				5.520		

Taxonomic Key Code Ref:
*1 Hilsenhoff 1995
*2 Hilsenhoff, Schmude 1992
*3 Hilsenhoff, unpublished
*4 Adler, Currie 2008
*5 Epler 2001

Species Status Codes:

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

Sample: 20100406-29-01 (yyyymmdd-Co-Field#) Reference Site: NO
Waterbody: YELLOW RIVER Master Waterbody #: 1352800
Project: USFWS YELLOW RIVER Bti APPLICA Storet Station:
Collector: GRAY, ELMER Time Sampling: # Reps: 1
Sampling Device:
Taxonomist: DIMICK, JEFFREY
Sorter: WANGBERG, LYNN % Sample Sorted: 7
SWIMS ID: Name: Key: 0

===

Location-Legal: S 0 T 0N R 0 County: JUNEAU
Lat/Long: N44deg 12min 54.8sec W 90deg 7min 16.9sec
Method:
Description: POST-TREATMENT ABOVE Bti
4TH ST. BRIDGE, 150 M ABOVE
24 H AFTER TREATMENT

===

	HBI	Width ft	Depth ft	Temp øC	DO mg/l	Measured Vel fps	Est Velocity fps	Habitat General
Rep #1	5.391							
Rep #2	0.000							
Rep #3	0.000							
Rep #4	0.000							
Rep #5	0.000							

Aquatic Vegetation 0 % of Total Stream Channel at Sampling Site

===

Substrate at Rep Location %:

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Substrate Sampled %: (Same as Above NO)

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

===

NP=Not Present
I=Insignificant
S=Significant

Water Quality Indicators:		Factors Affecting Habitat:		Pollutant Sources:	
Turbidity	*	Sludge Deposits	*	Livestock Pasturing	*
Chlorine/Toxic Scour	*			Barnyard Runoff	*
Macrophytes	*			Cropland Runoff	*
Filamentous Algae	*			Tile Drains	*
				Septic Systems	*

Planktonic Algae	*	Silt and Sediment	*	Streambank Erosion	*
Slimes	*	Channel Ditching	*	Urban Runoff	*
Iron Bacteria	*	Impoundments	*	Construction Runoff	*
Perceived Water Quality at Site:		Low Flows	*	Point Source (Spec)	*
		Wetlands	*	Other (Specify)	*
Comments:		Comments:		Comments:	

SAMPLE ID# 20100406-29-01

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
PLECOPTERA							
NEMOURIDAE							
PROSTOIA							
COMPLETA		*1	1.00	01040301	1	0	0
SHIPSA							
ROTUNDA		*1	2.00	01040401	3	0	0
PTERONARCYIDAE							
PTERONARCYS							
UNIDENTIFIED		*1	0.00	01070100	1	0	0
DIPTERA							
ATHERICIDAE							
ATHERIX							
VARIEGATA		*1	2.00	08010101	1	0	0
SIMULIIDAE							
SIMULIUM							
UNIDENTIFIED (LARVAE, IMMATURE)		*2		08110200	213	0	0
ANATINUM		*2		08110230	34	0	0
EMARGINATUM		*2		08110233	91	0	0
AESTIVUM		*2		08110236	2	0	0
LONGISTYLATUM		*2		08110243	5	0	0
PUPAE (S. EMARGINATUM)		*3		08110245	1	0	0
CHIRONOMIDAE (LARVAE, DAMAGED)		*1		08250000	1	0	0
TANYPODINAE							
THIENEMANNIMYIA "COMPLEX" (EPLER 2001)							
UNIDENTIFIED		4	6.00	08274500	2	0	0
ORTHOCLADIINAE							
PARAKIEFFERIELLA							
UNIDENTIFIED		*4		08302900	1	0	0
RHEOCRICOTOPUS							
ROBACKI		*4	6.00	08304010	3	0	0
THIENEMANNIELLA							
UNIDENTIFIED		*4	6.00	08304700	1	0	0
ORTHOCLADIUS (ORTHOCLADIUS)							
OLIVERI		*4	6.00	08305811	2	0	0
CRICOTOPUS (CRICOTOPUS)							
BICINCTUS		*4	6.00	08307503	1	0	0
CRICOTOPUS (ISOCLADIUS)							
UNIDENTIFIED		*4	7.00	08307600	1	0	0
CHIRONOMINAE (LARVAE, DAMAGED)		*4		08330000	1	0	0
MICROTENDIPES							
RYDALENSIS GROUP (PINDER, REISS 1983)		*4	6.00	08333703	5	0	0
RHEOTANYTARSUS							
UNIDENTIFIED		*4	6.00	08335600	13	0	0
STEMPELLINELLA							
UNIDENTIFIED		*4	4.00	08336100	1	0	0
TANYTARSUS							

UNIDENTIFIED	*4	6.00	08336700	11	0	0

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

SAMPLE ID# 20100406-29-01 PAGE 3

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
LUMBRICULIDA							
LUMBRICULIDAE							
STYLODRILUS							
HERINGIANUS		*5		44010201	1	0	0
BASOMMATOPHORA							
PHYSIDAE							
PHYSA							
UNIDENTIFIED		*6		51030200	1	0	0
*** TOTALS: ***					397	0	0
*** BIOTIC INDEX: ***					5.391		

Taxonomic Key Code Ref:
*1 Hilsenhoff 1995
*2 Hilsenhoff, unpublished
*3 Adler, Currie 2008
*4 Epler 2001
*5 Brinkhurst, Gelder 1991
*6 Brown 1991

Species Status Codes:

```
         *** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***
Sample: 20100405-29-02 (yyyymmdd-Co-Field#)              Reference Site:
NO
Waterbody: YELLOW RIVER                 Master Waterbody #: 1352800
Project: USFWS YELLOW RIVER Bti APPLI   Storet Station:
Collector: GRAY, ELMER                  Time Sampling:              # Reps:
1
Sampling Device:
Taxonomist: DIMICK, JEFFREY
Sorter: WANGBERG, LYNN                    % Sample Sorted: 7
SWIMS ID:                    Name:                              Key:
0
=============================================================================
==
Location-Legal:       S 0 T 0N R 0    County: JUNEAU
      Lat/Long: N44deg 12min 36.0sec  W 90deg  7min 20.1sec
        Method:
   Description: PRETREATMENT BELOW Bti
                4TH ST. BRIDGE, 250 M BELOW

=============================================================================
==
               Width  Depth  Temp  DO   Measured Est Velocity
         HBI    ft     ft     øC  mg/l  Vel fps     fps            Habitat
General
Rep #1   5.227
Rep #2   0.000
Rep #3   0.000
Rep #4   0.000
Rep #5   0.000
Aquatic Vegetation   0 % of Total Stream Channel at Sampling Site
=============================================================================
==
Substrate at Rep Location %:
       Bedrock Boulders Rubble Gravel Sand Silt Clay Detritus Muck Debris/Veg
Rep #1   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #2   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #3   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #4   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #5   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Substrate Sampled %:  (Same as Above  NO )
       Bedrock Boulders Rubble Gravel Sand Silt Clay Detritus Muck Debris/Veg
Rep #1   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #2   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #3   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #4   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
Rep #5   0.0      0.0     0.0    0.0   0.0  0.0  0.0    0.0    0.0    0.0
=============================================================================
==
                               NP=Not Present        Pollutant Sources:
Water Quality Indicators:       I=Insignificant    Livestock Pasturing     *
Turbidity             *         S=Significant      Barnyard Runoff         *
Chlorine/Toxic Scour  *                            Cropland Runoff         *
Macrophytes           *  Factors Affecting Habitat: Tile Drains            *
```

Filamentous Algae	*	Sludge Deposits	*	Septic Systems	*
Planktonic Algae	*	Silt and Sediment	*	Streambank Erosion	*
Slimes	*	Channel Ditching	*	Urban Runoff	*
Iron Bacteria	*	Impoundments	*	Construction Runoff	*
Perceived Water Quality at Site:		Low Flows	*	Point Source (Spec)	*
		Wetlands	*	Other (Specify)	*
Comments:		Comments:		Comments:	

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

SAMPLE ID# 20100405-29-02

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
PLECOPTERA							
NEMOURIDAE							
SHIPSA							
ROTUNDA		*1	2.00	01040401	3	0	0
DIPTERA							
SIMULIIDAE (LARVAE, DAMAGED)		*1		08110000	1	0	0
SIMULIUM							
UNIDENTIFIED (LARVAE, IMMATURE)		*2		08110200	93	0	0
ANATINUM		*2		08110230	14	0	0
EMARGINATUM		*2		08110233	19	0	0
AESTIVUM		*2		08110236	3	0	0
PUPAE (S. EMARGINATUM)		*3		08110245	5	0	0
TANYPODINAE							
CONCHAPELOPIA							
UNIDENTIFIED		*4	6.00	08270700	2	0	0
ORTHOCLADIINAE							
PARAMETRIOCNEMUS							
UNIDENTIFIED		*4	5.00	08303000	1	0	0
CHIRONOMINAE							
MICROTENDIPES							
RYDALENSIS GROUP (PINDER, REISS 1983)		*4	6.00	08333703	1	0	0
RHEOTANYTARSUS							
UNIDENTIFIED		*4	6.00	08335600	10	0	0
STEMPELLINELLA							
UNIDENTIFIED		*4	4.00	08336100	2	0	0
TANYTARSUS							
UNIDENTIFIED		*4	6.00	08336700	3	0	0
*** TOTALS: ***					157	0	0
*** BIOTIC INDEX: ***					5.227		

Taxonomic Key Code Ref: Species Status Codes:

*1 Hilsenhoff 1995
*2 Hilsenhoff, unpublished
*3 Adler, Currie 2008
*4 Epler 2001

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

Sample: 20100406-29-02 (yyyymmdd-Co-Field#)   Reference Site: NO

Waterbody: YELLOW RIVER   Master Waterbody #: 1352800

Project: USFWS YELLOW RIVER Bti APPLICA Storet Station:

Collector: GRAY, ELMER   Time Sampling: # Reps: 1

Sampling Device:

Taxonomist: DIMICK, JEFFREY

Sorter: THOMAS, NATHAN   % Sample Sorted: 13

SWIMS ID: Name: Key: 0

==

Location-Legal: S 0 T 0N R 0   County: JUNEAU

Lat/Long: N44deg 12min 36.0sec W 90deg 7min 12.1sec

Method:

Description: POST-TREATMENT BELOW Bti
4TH ST. BRIDGE, 250 M BELOW
24 H AFTER TREATMENT

==

	HBI	Width ft	Depth ft	Temp øC	DO mg/l	Measured Vel fps	Est Velocity fps	Habitat General
Rep #1	5.462							
Rep #2	0.000							
Rep #3	0.000							
Rep #4	0.000							
Rep #5	0.000							

Aquatic Vegetation 0 % of Total Stream Channel at Sampling Site

==

Substrate at Rep Location %:

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Substrate Sampled %: (Same as Above NO)

	Bedrock	Boulders	Rubble	Gravel	Sand	Silt	Clay	Detritus	Muck	Debris/Veg
Rep #1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rep #5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

==

NP=Not Present   Pollutant Sources:

Water Quality Indicators: I=Insignificant   Livestock Pasturing *

Turbidity *   S=Significant   Barnyard Runoff *

Chlorine/Toxic Scour *   Cropland Runoff *

Macrophytes *   Factors Affecting Habitat: Tile Drains *

Filamentous Algae	*	Sludge Deposits	*	Septic Systems	*
Planktonic Algae	*	Silt and Sediment	*	Streambank Erosion	*
Slimes	*	Channel Ditching	*	Urban Runoff	*
Iron Bacteria	*	Impoundments	*	Construction Runoff	*
Perceived Water Quality		Low Flows	*	Point Source (Spec)	*
at Site:		Wetlands	*	Other (Specify)	*
Comments:		Comments:		Comments:	

*** USFWS YELLOW RIVER Bti BIOTIC INDEX REPORT ***

SAMPLE ID# 20100406-29-02 PAGE 2

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
PLECOPTERA							
NEMOURIDAE							
SHIPSA							
ROTUNDA		*1	2.00	01040401	3	0	0
TRICHOPTERA							
LIMNEPHILIDAE							
PYCNOPSYCHE							
UNIDENTIFIED		*1	4.00	04081300	1	0	0
COLEOPTERA							
ELMIDAE							
ANCYRONYX							
VARIEGATUS		*2	6.00	07020101	1	0	0
DIPTERA							
SIMULIIDAE							
SIMULIUM							
UNIDENTIFIED (LARVAE, IMMATURE)		*3		08110200	82	0	0
ANATINUM		*3		08110230	11	0	0
EMARGINATUM		*3		08110233	29	0	0
LONGISTYLATUM		*3		08110243	3	0	0
PUPAE (S. EMARGINATUM)		*4		08110245	6	0	0
TANYPODINAE							
CONCHAPELOPIA							
UNIDENTIFIED		*5	6.00	08270700	1	0	0
ORTHOCLADIINAE (LARVAE, DAMAGED)		*5		08300000	1	0	0
PARAKIEFFERIELLA							
UNIDENTIFIED		*5		08302900	1	0	0
RHEOCRICOTOPUS							
ROBACKI		*5	6.00	08304010	4	0	0
RHEOSMITTIA							
UNIDENTIFIED		*5		08304100	2	0	0
CRICOTOPUS/ORTHOCLADIUS (FERRINGTON ET AL. 2008)							
UNIDENTIFIED		*5		08306700	1	0	0
CHIRONOMINAE							
MICROTENDIPES							
RYDALENSIS GROUP (PINDER, REISS 1983)		*5	6.00	08333703	1	0	0
RHEOTANYTARSUS							
UNIDENTIFIED		*5	6.00	08335600	10	0	0
TANYTARSUS							
UNIDENTIFIED			6.00	08336700	5	0	0

*** TAXA ***	SPECIES STATUS CODE	TAXA KEY USED	TOL VAL	ORGANISM ID	ORGANISM COUNT REP1	REP2	REP3
	*** TOTALS: ***				162	0	0
	*** BIOTIC INDEX: ***				5.462		

Taxonomic Key Code Ref:
*1 Hilsenhoff 1995
*2 Hilsenhoff, Schmude 1992
*3 Hilsenhoff, unpublished
*4 Adler, Currie 2008
*5 Epler 2001

Species Status Codes:

Page No. 1
11/12/10

** District/Sample Number/Rep 9/20100405-29-01/1

Rank		Family	Genus	Species
1	Name	SIMULIIDAE	SIMULIUM	EMARGINATUM
	Count	128	128	32
	% of Total	83%	83%	21%
2	Name	CHIRONOMIDAE	RHEOTANYTARSUS	**UNIDENTIFIED**
	Count	22	11	11
	% of Total	14%	7%	7%
3	Name	NEMOURIDAE	RHEOCRICOTOPUS	ANATINUM
	Count	1	3	7
	% of Total	1%	2%	5%
4	Name	PERLODIDAE	TANYTARSUS	ROBACKI
	Count	1	3	3
	% of Total	1%	2%	2%
5	Name	PTERONARCYIDAE	MICROTENDIPES	**UNIDENTIFIED**
	Count	1	2	3
	% of Total	1%	1%	2%
5	Name	ELMIDAE	MICROTENDIPES	
	Count	1	2	0
	% of Total	1%	1%	0%
5	Name		SHIPSA	
	Count	0	1	0
	% of Total	0%	1%	0%
5	Name		ISOPERLA	
	Count	0	1	0
	% of Total	0%	1%	0%
5	Name		PTERONARCYS	
	Count	0	1	0
	% of Total	0%	1%	0%
5	Name		MACRONYCHUS	
	Count	0	1	0
	% of Total	0%	1%	0%
5	Name		CONCHAPELOPIA	
	Count	0	1	0
	% of Total	0%	1%	0%
5	Name		ORTHOCLADIUS	
	Count	0	1	0

	% of Total	0%	1%	0%
5	Name		CRICOTOPUS	
	Count	0	1	0
	% of Total	0%	1%	0%

Rank		Family	Genus	Species
** District/Sample Number/Rep 9/20100405-29-02/1				
1	Name	SIMULIIDAE	SIMULIUM	EMARGINATUM
	Count	130	129	19
	% of Total	86%	85%	13%
2	Name	CHIRONOMIDAE	RHEOTANYTARSUS	ANATINUM
	Count	19	10	14
	% of Total	13%	7%	9%
3	Name	NEMOURIDAE	SHIPSA	**UNIDENTIFIED**
	Count	3	3	10
	% of Total	2%	2%	7%
4	Name		TANYTARSUS	ROTUNDA
	Count	0	3	3
	% of Total	0%	2%	2%
5	Name		CONCHAPELOPIA	AESTIVUM
	Count	0	2	3
	% of Total	0%	1%	2%
5	Name		STEMPELLINELLA	**UNIDENTIFIED**
	Count	0	2	3
	% of Total	0%	1%	2%

Rank		Family	Genus	Species
** District/Sample Number/Rep 9/20100406-29-01/1				
1	Name	SIMULIIDAE	SIMULIUM	EMARGINATUM
	Count	345	345	91
	% of Total	87%	87%	23%
2	Name	CHIRONOMIDAE	RHEOTANYTARSUS	ANATINUM
	Count	43	13	34
	% of Total	11%	3%	9%
3	Name	NEMOURIDAE	TANYTARSUS	**UNIDENTIFIED**
	Count	4	11	13
	% of Total	1%	3%	3%
4	Name	PTERONARCYIDAE	MICROTENDIPES	**UNIDENTIFIED**
	Count	1	5	11
	% of Total	0%	1%	3%
5	Name	ATHERICIDAE	SHIPSA	LONGISTYLATUM
	Count	1	3	5
	% of Total	0%	1%	1%
5	Name	LUMBRICULIDAE	RHEOCRICOTOPUS	RYDALENSIS GROUP
	Count	1	3	5
	% of Total	0%	1%	1%
5	Name	PHYSIDAE		
	Count	1	0	
	% of Total	0%	0%	

Rank		Family	Genus	Species
** District/Sample Number/Rep 9/20100406-29-02/1				
1	Name	SIMULIIDAE	SIMULIUM	EMARGINATUM
	Count	125	125	29
	% of Total	80%	80%	19%
2	Name	CHIRONOMIDAE	RHEOTANYTARSUS	ANATINUM
	Count	26	10	11
	% of Total	17%	6%	7%
3	Name	NEMOURIDAE	TANYTARSUS	**UNIDENTIFIED**
	Count	3	5	10
	% of Total	2%	3%	6%
4	Name	LIMNEPHILIDAE	RHEOCRICOTOPUS	**UNIDENTIFIED**
	Count	1	4	5
	% of Total	1%	3%	3%
5	Name	ELMIDAE	SHIPSA	ROBACKI
	Count	1	3	4
	% of Total	1%	2%	3%